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Diffuse Low-Grade Gliomas in Adults

Natural History, Interaction with the Brain, and New Individualized Therapeutic Strategies

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Foreword

When it is all said and done, this will be the definitive source on diffuse low-grade gliomas and will serve as a comprehensive textbook for all clinicians and health-care providers who take care of patients with this disorder. What makes this book so unusual is the individualized personal approach to this problem on a patient-by-patient basis. In other words, one size does not fit all when it comes to diffuse low-grade gliomas. The approach the authors have taken in defining this entity and the options for management are quite unique and innovative, to say the least. We have come full circle in the way in which we approach this disease from a "wait-and-see" attitude to a more proactive individualized treatment regime based upon the dynamics of the lesion as seen preoperatively and the individualized approach to surgery and therapy which has influenced the outcome of this disease over the past decade.

The book is organized in a very logical and informative fashion, starting off with several chapters on epidemiology and pathological classification. There has been a significant evolution of our thought processes on the gliomagenesis of these lesions as well as its molecular profile, and therefore to truly understand how best to manage a patient with a diffuse low-grade glioma, one has to know the distinct patterns that exist in molecular classification for each patient. In other words, it is simply not acceptable anymore to classify a tumor based on histology without knowing the genotypic expression profile of the tumor. Further depth is developed in chapters devoted to the cellular origin of this lesion and the possible relationship this tumor has with stem cells or progenitors from different regions of the brain. While some of the chapters involving genomics do overlap, I particularly enjoyed reading the different ways in which this information is expressed and interpreted. Other particularly useful contributions include the role of proteomic classification, including a bioinformatic approach linking classification to outcome, as well as chapters involving cell cultures and animal models. To date, there have been very few cell cultures established for low-grade gliomas and, for that matter, appropriate animal models. However, it is clear that xenografts do exist, as do genetically engineered mouse models based upon certain types of phenotypic backgrounds. Overall, I found the basic science components of this textbook to be extraordinarily revealing, very thorough, and quite easy to read and apply to a given patient's tumor.

The clinical aspects of this entity are further evolved in chapters on imaging, clinical presentation, and quality of life issues. The imaging is particularly valuable in that all aspects are covered, from anatomical imaging with

MRI to physiological imaging involving MRI and metabolic imaging with PET scanning. All of this information is woven together in subsequent chapters that develop predictive models based upon growth rates and invasion along white matter tracts to understand what the natural history of this disease will be likely to demonstrate. A particularly important contribution is that by Dr. Soffietti who explains in great detail various prognostic factors that influence the natural history and therefore define the risk of the disease as it affects the patient. This is done in such a way that incorporates all of the molecular biology, imaging, and clinical factors to enable the clinician to predict outcome and progression for a given patient's diffuse low-grade glioma.

Subsequent contributions apply to the functional assessment of various brain regions involved with these types of tumors. We all realize that language and cognition are so important to understand in terms of deciding how best to manage a patient and what to expect with the treatment strategy that we select for that given individual. Thus, bringing together some of the functional imaging modalities that we have, such as fMRI and MEG along with connectivity maps, allows us to be able to predict how language and cognition could be affected with surgery as well as various treatment options.

Clearly, surgery remains the most important treatment modality for this lesion, and thus, the chapters by Duffau and colleagues on surgery and functional considerations are extremely valuable in terms of planning any operative procedure with a lesion of this type. Dr. Duffau has one of the greatest experiences in the world in operating on diffuse low-grade gliomas, and he explains in great detail how to consider not only the anatomy but the function as mapped intraoperatively and how this influences the outcome of the patient. Great insight is given to how stimulation mapping can be used to decrease permanent deficits, making surgery for some of these complicated lesions quite safe. As he states so nicely, the surgeon has to adapt the surgical strategy to the anatomy and function of the brain done under awake conditions to maximize the extent of resection while minimizing morbidity. Another chapter is devoted to the oncological considerations of extensive resections and how important this is in not only affecting progression free survival but overall survival and malignant transformation.

Following surgery, adjuvant modalities are necessary in certain circumstances when residual disease remains. Excellent contributions are made in the areas of chemotherapy and radiation and how and when some of these modalities should be used. Several innovative strategies are also described, such as the use of chemotherapy performed preoperatively to reduce the volume of the mass, thus making so-called inoperable lesions quite operable. In fact, even patients with gliomatosis may become surgical candidates if they respond appropriately to chemotherapy. In addition, in the postoperative setting, one has to consider the use of functional rehabilitation as a means to improve the outcome of the patient and expedite relearning of functions that may be temporarily lost or compromised. This brings into consideration the concept of neuroplasticity and reorganization, which we know exists and, through a number of writings from Dr. Duffau, has shown all of us that areas thought to be functional can lose their functionality over time with chronically based lesions in so-called eloquent areas.

When reading this textbook, one cannot ignore the fact that we as clinicians must find new endpoints and look at different strategies for how this disease progresses and how it should be managed. We all recognize that there have to be better endpoints than time to progression or malignant transformation. We also have to consider clinical issues, such as the time it takes to return to a normal quality of life and functional status as equally important to the more standard endpoints used previously.

This is exactly why this book represents a more personalized approach to patients with diffuse low-grade gliomas. New strategies must be considered that take into consideration the concept of plasticity, which allows function to leave given areas and provides the surgeon the opportunity to remove what was once thought to be inoperable. The theme of this book, in terms of an individualized approach to a patient with a diffuse low-grade glioma, lets us think about different concepts such as allowing plasticity to help us shape the course of this disease and its treatment. We also need to think about the use of neoadjuvant therapy to shrink the tumor with chemotherapy and to first understand the growth kinetics of the lesion prior to just operating at the initial diagnosis. All of these factors contribute to the personalized approach, as explained throughout this textbook, to this lesion. I also like the concept of a supratotal resection, in which the goal with all of these functional considerations is to operate, not on the anatomy but to remove the lesion and a significant margin, as function permits, around the lesion. This will enable us to once again change the approach in an individualized way to the patient who has this maximal extent of resection. So far, the data seems quite convincing that patients who have these more aggressive supratotal resections might have a lower risk of malignant transformation, thus enabling surgery to change the natural history of the disease.

All in all, this is a major contribution that will be significant in the history of neurosurgery and neuro-oncology as it pertains to patients with diffuse low-grade gliomas. We cannot think in traditional terms any more about how to manage this disease. We have to employ the new strategies and concepts as described throughout this textbook to better individualize the approach to a given patient, therefore making more of an impact in the long run. I thoroughly enjoyed reading this book, and without a doubt, if you only have one reference source on diffuse low-grade gliomas, this must be it!

San Francisco, CA Mitchel S. Berger, MD

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1 Introduction: From the Inhibition of Dogmas to the Concept of Personalized Management in Diffuse Low-Grade Gliomas

Hugues Duffau

"opheléein ê mê blaptein" (first be useful, then do not be harmful)

Hippocrates

Abstract

Recent technical and conceptual advances in genetics, cognitive neurosciences, imaging, and treatments revolutionized the understanding of diffuse low-grade glioma, leading to the inhibition of dogmas and to the fundamental principle of *personalized management*.

Moreover, a better knowledge of brain processing enables now to take into consideration interaction between the disease (the glioma) and the host (the brain) and thus to modulate the dynamic therapeutic strategies with the goal *to increase the median survival as well as to improve the quality of life* - that is, to move toward a "functional neurooncology". In the era of "evidence-based medicine," it is crucial to not forget "individual-based medicine."

Keywords

Diffuse low-grade glioma • Personalized management • Individual-based medicine • Functional neurooncology

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Supratentorial "grade II glioma" (as defined by the current WHO classification) is a rare and complex entity in adults, which has been a matter of debate since many decades. This controversy is due to many factors.

First, for a long time, the natural history of this disease was poorly studied and thus poorly known. Indeed, in the traditional literature, the vast majority of authors considered WHO grade II glioma as a "stable" and "benign" brain tumor. As a consequence, the "wait-and-see attitude" was advocated during many years, in particular because this tumor usually involves young adults enjoying a "normal life." In other words, the classical view is to claim that these patients "are doing well," because they have generally no deficit on a standard neurological examination, even if they are in rule under antiepileptic drug(s) due to inaugural seizures in 80–90 % of cases.

In addition, medical and surgical neurooncologists also believed that it was almost impossible to remove this kind of tumors without generating functional deficits – especially when located in the so-called eloquent areas, as frequently observed. Above all, mainly based on the subjective estimation of the extent of resection by the neurosurgeon, it was argued that surgical removal had no impact on the natural course of this disease and that the most appropriate management was therefore to achieve only a biopsy with the aim of obtaining neuropathological examination – in order to decide whether a single follow-up could be considered or whether radiotherapy should be performed. This means that the treatment was selected almost exclusively on the basis of the morphological criteria according to the WHO classification (astrocytoma versus oligodendroglioma versus mixed glioma; grade II versus III).

Finally, the clinical results were evaluated in the majority of series on only few parameters, that is, the progression-free survival (PFS), overall survival (OS), and eventually Karnofsky performance score.

Interestingly, recent technical and conceptual advances in genetics, cognitive neurosciences, imaging, and treatments revolutionized our understanding of this pathology, leading to the fundamental principle of *personalized management*. Moreover, a better knowledge of brain processing enables now to take into consideration interaction between the disease (the glioma) and the host (the brain) and thus to modulate the therapeutic strategies with the goal *to increase the median survival as well as to improve the quality of life* – that is, to solve the classical dilemma opposing OS versus cerebral functions [1]. In this setting, the purpose of this book is to revisit the biology, behavior, and management of "WHO grade II glioma" patients, in order to open new avenues to the origins of this tumor, its natural history, as well as the present and future in the oncological and cognitive individualized therapeutic attitudes.

Indeed, based on a large amount of original data published in the last decade, it is time to inhibit the dogmas detailed in the first paragraph and to evolve toward a paradigmatic shift in the comprehension and the treatment of this disease. First, it will be demonstrated that this tumor is not stable but that it has a constant growth with, ultimately, a malignant transformation leading to neurological deficits and death usually in less than 10 years [2, 3]. Moreover, neurooncologists (especially neurosurgeons) have to understand that this glioma is not a "tumor mass" compressing the parenchyma but that this diffuse lesion is in fact an infiltrative and chronic disease of the brain, extending far away beyond the abnormalities visible on neuroimaging [4]. It was indeed previously thought that the abnormalities visible on neuroimaging (on CT scan initially and then on MRI, as hypersignal on FLAIR-weighted MRI) corresponded to the whole disease (associated with edema), leading to speak about "normal brain" around these signal abnormalities – which is totally wrong. This issue is crucial for the therapeutic (especially surgical) management [5]. It also explains why the term "diffuse low-grade glioma" (DLGG) will be preferred in this book, because in essence it excludes the well-delineated WHO grade I ("low-grade") gliomas, but it also prevents the use of the current WHO classification. Indeed, it was extensively reported that there was a high inter- and even intra-neuropathologists variability, both regarding the subtypes (e.g., the proportions of astrocytomas versus

oligodendrogliomas is reversed in France in comparison with the USA, due to a different interpretation of the same morphological criteria) as well as regarding the grading: the WHO classification does not officially recognize the existence of a continuum between the so-called grade II versus grade III, while in practice many gliomas have an "intermediate" behavior (for instance, with some "more aggressive" micro-foci in the core of a grade II glioma). Consequently, the name of DLGG will be used here, knowing the heterogeneity of this tumor even within each subtype of "astrocytomas" or "oligodendrogliomas," in order to move toward a revisited multimodal classification, more appropriate to the clinical practice [6].

From a functional point of view, it will be shown that these patients have generally neurocognitive disorders at the time of diagnosis and that neuropsychological assessment should be systematically performed before and after each treatment – the classical neurological examination being not sensitive enough to objectively evaluate DLGG patients [7]. This point is very important to tailor the management with the aim of optimizing both the survival of patients and their health-related quality of life (QoL), for example, to prevent the long-term negative impact of radiotherapy on higher functions by avoiding early irradiation [8]. To this end, new endpoints will be proposed, due to the fact that PFS is meaningless in DLGG before any treatment or after an incomplete surgical resection, since in essence all DLGGs are continuously growing (whereas this endpoint would be unambiguous after a "total resection" or could be also more or less properly defined under adjuvant treatment such as chemotherapy/radiotherapy) [9]. In this context, the classical radiological criteria, as initially proposed by Macdonald et al. [10] or more recently by the RANO group [11], are not appropriate to monitor DLGG kinetics. It will be shown that an objective and accurate 3D volumetric assessment should be performed for each examination on FLAIR-weighted MRI, with computation of an individual growth rate before and after each therapy [9]. New metabolic criteria will also be helpful to increase the sensitivity of this radiological monitoring.

Furthermore, on the lights of this better knowledge of natural history, original individualized therapeutic strategies will be proposed in DLGG. Regarding surgery, in the past decade, numerous evidences have supported the significant impact of maximal resection on the course of DLGG, by delaying the malignant transformation and therefore increasing the overall survival [12–14]. Moreover, noninvasive neuroimaging and intraoperative electrophysiological mapping (direct electrical stimulation of cortex as well as subcortical white matter pathways) methods have enabled a better comprehension of the dynamic functional anatomy in each patient, leading to maximize the surgical indications in "eloquent areas" while preserving or even improving the QoL – thanks to epilepsy control as well as optimization of cognition after a specific functional rehabilitation [12, 15]. In parallel, this new philosophy based on functional mapping-guided resection and not anymore on (anatomical/oncological) image-guided resection [16] has also allowed a significant increase of extent of resection and thus OS. In other words, stronger links between cognitive neurosciences and neurooncology have (at least partly) solved the classical dilemma in DLGG, which so far opposed OS versus QoL, by maximizing both oncological and functional outcomes – thanks to new surgical concepts which take into account cerebral plasticity and connectomics (i.e., the view of a brain organized in parallel distributed and interactive cortico-subcortical subnetworks) [17]. In particular, it was demonstrated that a reoperation allowing a more extensive resection after a partial removal (for functional reasons) some years before could be considered thanks to mechanisms of remapping which occurred in the meantime [18]. Moreover, new drugs of chemotherapy have increasingly been used in DLGG, since their tolerance was dramatically improved (e.g., temozolomide with oral administration) and their impact have begun to be demonstrated, both from an oncological point of view (possible tumor stabilization or even shrinkage) and from a functional point of view (e.g., possible control of intractable epilepsy). This is the reason why original

therapeutic strategies have recently been proposed, such as neoadjuvant chemotherapy in cases of inoperable DLGG (e.g., due to a bilateral invasion), allowing a shrink of the lesion and then opening the door to a subsequent surgery [19]. In the same state of mind, the actual benefit-to-risk ratio of radiotherapy was also redefined, with a current tendency to delay the irradiation.

Last but not the least, recent molecular data enabled a better understanding of the biology of DLGG, beginning to explain their heterogeneity in their behavior and prognosis, despite common clinical, radiological, and even neuropathological presentation – reinforcing the need to modulate the current WHO classification [20]. In particular, new insights into genomics and proteomics will be exposed in this book, with a discussion about their possible prognostic or predictive value. In the same vein, a better knowledge of the origin of DLGG may lead to develop new anticancer drugs, for instance, against progenitor cells. To this end, the elaboration of adapted animal models of DLGG would represent a great advance to study the mechanisms underlying genesis of this tumor, in order both to identify new therapeutic targets as well as to test future molecules. In addition, the use of biomathematical modeling could also enable a better approximation of the glioma "date of birth." This might lead to propose a screening of a subpopulation in which early detection of DLGG could be performed by MRI, with the goal to diagnose incidental DLGG and to switch toward an earlier and more efficient treatment in asymptomatic patients [21–23].

In summary, the current philosophy in DLGG patients is to anticipate a multimodal and long-term management at the individual level from the diagnosis to the malignant stage of the disease, with on-line therapeutic adaptation over time on the basis of regular functional feedback and radiological monitoring. Indeed, due to the better knowledge of the negative prognosis of DLGG, the "wait-and-see" attitude is not adapted anymore, even in patients enjoying a normal life. Although clinicians have to remember the classical "primum non nocere" principle, they also have to be aware about the fact that they can be harmful by inaction, that is, by observing the tumor evolution while doing nothing: their role is first of all to be useful by treating the disease. In this state of mind, the ultimate aim in DLGG is not (yet) to cure this tumor but nonetheless to be preventive by delaying malignant transformation as longer as possible while preserving an optimal QoL, that is, to move toward a "functional neurooncology" [24].

Of note, randomized trials are not adapted to this disease due to the long OS (thus with difficulties in the practical organization and above all leading to the fact that the final results are often disputable given the advances in diagnostic and therapeutic techniques made in the meantime) as well as to possible ethical issues. Indeed, one should remind that that there is a paradox to claim that the service must be patient centered while proposing that patients can be recruited into randomized clinical trials that will generate the evidence to move the discipline forward. Can the neurooncologist certify that there is no potential antagonism between the benefit for the patient now versus the benefit for the discipline later? This issue likely explains, at least in part, why no surgical trials have ever been built in DLGG. As a consequence, prospective collection of data should be more systematically achieved to implement large national and international data banks [25].

Last but not the least, the most important point will remain the honest, strong, and unique relationship between the medical and/or surgical oncologist and the patient, based on a clear and complete information which should be given since the beginning of the management as well as during the follow-up over years. In other words, in the era of "evidence-based medicine," it is crucial to not forget "individual-based medicine."

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Part I

Epidemiology and Classification

Epidemiology of Diffuse Low-Grade Gliomas

2

Luc Bauchet

Abstract

Diffuse low-grade gliomas (DLGGs) belong to primary central nervous system tumors (PCNSTs) and include diffuse astrocytomas (fibrillary astrocytoma, gemistocytic astrocytoma, protoplasmic astrocytoma), oligodendroglioma, and oligoastrocytoma.

Specific epidemiological publications of DLGG are very rare. However, it is possible to obtain epidemiological data concerning DLGG by selecting some publications referring to all PCNSTs, gliomas, or even to low-grade gliomas (LGGs). (We will show that the term "LGG" is not well appropriate.).

This work summarizes the definitions and descriptive epidemiological data for DLGG and PCNST. DLGGs account for approximately 15 % of all gliomas, and incidence rate is about 1/100,000 person-years or just a little less. Main prognostic factors (e.g., age, performance status, location, volume and growth rate of the tumor, extent of surgical resection, histology) are discussed, and it is shown how they influence survival. Recent literature proposes a lot of new spontaneous prognostic factors, but until now, just a few are validated. In the other hand, little data are available to define best combinations of the different therapeutic strategies (successive surgeries, chemotherapy, radiotherapy, and new treatments).

This work proposes new efficient methodology to evaluate medical care and quality of life. The developments of modern informatics technology will revolutionize our methods of recording data. Collaboration between all medical specialties (including epidemiology and biostatistics) and development of large databases are the keys of efficiency for the future.

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This chapter also summarizes knowledge of risk factors for DLGG and PCNST and proposes classical and new directions for searching etiologies for these tumors.

Keywords

Brain tumor • Database • Epidemiology • Glioma • Low-grade glioma • Neuro-epidemiology • Neuro-oncology • Neuropathology • Neurosurgery

Introduction

Referring to the World Health Organization (WHO) classification [1], diffuse low-grade gliomas (DLGGs) include diffuse astrocytomas (fibrillary astrocytoma, gemistocytic astrocytoma, protoplasmic astrocytoma), oligodendroglioma, and oligoastrocytoma with the corresponding ICD-O codes: 9420/3, 9411/3, 9410/3, 9450/3, and 9382/3, respectively.

Unfortunately, specific epidemiological publications for DLGG are very rare. However, it is possible to obtain epidemiological data concerning DLGG by selecting some articles referring to all primary central nervous system tumors (PCNSTs), to neuroepithelial tumors or gliomas, or even to low-grade gliomas (LGGs). These publications can come from registry works or from consortium or single institution studies. The main difficulties are that definitions referring histology or topography coding could vary with time, countries, and people involved in.

In the first part of this work, we will focus on PCNST generalities and definitions, then discuss the proportion of DLGG among PCNST, sex ratio, and median ages at diagnosis for DLGG, and show few surgical data for gliomas in France. We will finish the first part by explaining why the term of DLGG is more appropriate than the term of LGG.

The second part will present data concerning incidence, survival, and prevalence for DLGG.

The third part will introduce prognostic factors for DLGG, knowing that they will be detailed in other chapters of this book.

The last part will introduce what could be new methodologies: (1) for improving clinical epidemiology (to have best evaluation of oncological care on survival and on quality of life for DLGG patients); (2) for looking for etiologies for these tumors, because until now, except in very few cases, the causes of DLGG are not known; and (3) for early detection of DLGGs.

PCNST and DLGG: Generalities

PCNST Generalities and Definitions

PCNSTs represent a complex heterogeneous group of pathological entities that may be benign, malignant, or of unpredictable evolution [1–9]. These tumors represent a major public health problem [10]. The term "primary brain tumor" has been defined in numerous ways in the literature. The primary difficulty in building a tumor registry and/or a database is to define the type of tumor to be recorded.

Recent publications [11–18], the classification system of the World Health Organization [1, 5], and the European recommendations for coding tumors of the brain and central nervous system (CNS) [19] include all primary benign and malignant tumors located in the CNS, including the envelopes of the CNS and the beginning of the nerves localized in the skull and spine. PCNSTs include tumors of neuroepithelial tissue (gliomas and all other neuroepithelial tumors), tumors of the meninges (meningiomas, mesenchymal tumors, and other tumors of the meninges), tumors of the cranial and paraspinal nerves, lymphomas and hematopoietic neoplasms, and others.

By definition, this excludes metastatic tumors. But even now, there are still discrepancies

concerning the inclusion of lymphoma, pituitary and pineal glands, and olfactory tumors of the nasal cavity, in the different databases and registries. It is important to notice that some institutions account for primary malignant tumors, only.

Coding systems have been introduced as an indispensable interface between pathologists and cancer registries. The *International Classification of Diseases for Oncology* (*ICD-O*) was established more than 30 years ago. It assures that histopathologically stratified population-based incidence and mortality data become available for epidemiological and oncological studies. The histology (morphology) code is increasingly complemented by genetic characterization of human neoplasms. The ICD-O histology codes have been adopted by the systematized nomenclature of medicine (SNOMED), issued by the College of American Pathologists. ICD-0-3 and SNOMED codes are available in Louis et al. [1] and in Rigau et al. [17]. Glioma morphology codes are shown here (see Table 2.1).

The ICD-O topography codes largely correspond to those of the tenth edition of the *International Statistical Classification of Diseases*, *Injuries and Causes of Death* (*ICD-10*)

Table 2.1 Histological repartition of the 18,627 glioma cases with clinical and surgical data from French Brain Tumor DataBase, 2004–2009 [21]

	ICD-O	ADICAP	N	M	F	m	Med	CRYO	Reported surgery		
									T	R %	B %
Tumors of neuroepithelial tissue											
Glioma NOS	9380/3	N7R0	358	218	140	48,83	55,0	41	312	*31,4 %*	*68,6 %*
Astrocytic tumors											
Astrocytoma NOS	9400/3	N7S0	251	146	105	42,95	45,0	39	211	*40,8 %*	*59,2 %*
Pilocytic astrocytoma	9421/1	N0S8	938	486	452	16,64	13,0	263	688	*86,6 %*	*13,4 %*
Pilomyxoid astrocytoma	*9425/3*	*(0001)*	5	1	4	8,80	7,0	1	3	*33,3 %*	*66,7 %*
Subependymal Giant cell astrocytoma	9384/1	N0T2/3	73	35	38	18,12	17,0	18	51	*94,2 %*	*5,8 %*
Pleomorphic xantho-astrocytoma	9424/3	N7S9	72	38	34	34,11	31,0	19	43	*86,0 %*	*14,0 %*
Fibrillary astrocytoma	9420/3	N7S2	147	88	59	40,19	45,0	50	113	*34,5 %*	*65,5 %*
Gemistocytic astrocytoma	9411/3	N7S4	82	53	29	48,42	49,0	25	67	*53,7 %*	*46,3 %*
Protoplasmic astrocytoma	9410/3	N7S6	18	8	10	46,05	43,5	2	15	*46,7 %*	*53,3 %*
Anaplastic astrocytoma	9401/3	N7T6	516	289	227	55,94	59,0	145	439	*31,7 %*	*68,3 %*
Glioblastoma	9440/3	N7X0	9,652	5,712	3,940	61,90	63,0	2,695	6,751	*63,2 %*	*36,8 %*
Giant cell glioblastoma	9441/3	N7X2	172	98	74	55,33	58,5	58	132	*70,5 %*	*29,5 %*
Gliosarcoma	9442/3	N7X4	112	73	39	58,29	59,0	34	78	*93,6 %*	*6,4 %*
Gliomatosis cerebri	9381/3	N7R9	62	37	25	47,80	50,0	14	35	*20,0 %*	*80,0 %*
			12,100	7,064	5,036	56,49	61,0	3,363	8,626		
				58,5 %	*41,5 %*			*27,3 %*		*61,8 %*	*38,2 %*
Oligodendroglial tumors											
Oligodendroglioma	9450/3	N7V0	1,781	1,014	767	43,96	43,0	487	1,547	*56,9 %*	*43,1 %*
Anaplastic oligodendroglioma	9451/3	N7V4	1621	911	710	53,18	55,0	514	1,455	*62,4 %*	*37,6 %*
			3,402	1,925	1,477	48,35	49,0	1,001	3,002		
				56,6 %	*43,4 %*			*29,4 %*		*59,6 %*	*40,4 %*
Oligoastrocytic tumors											
Oligoastrocytic tumors NOS	9382/3	N7R4	49	24	25	41,08	44,0	9	30	*63,3 %*	*36,7 %*
Oligoastrocytoma	9382/3	N7V2	558	309	249	44,00	42,0	148	460	*51,5 %*	*48,5 %*
Anaplastic oligoastrocytoma	9382/3	N7V3	1,055	601	454	53,63	56,0	276	907	*54,6 %*	*45,4 %*
			1,662	934	728	50,02	52,0	433	1,397		
				56,2 %	*43,8 %*			*26,1 %*		*53,8 %*	*46,2 %*
Ependymal tumors											
Subependymoma	9383/1	N0W6	94	69	25	51,49	52,5	26	67	*91,0 %*	*9,0 %*
Myxopapillary ependymoma	9394/1	N7W2	157	98	59	39,57	38,0	32	101	*97,0 %*	*3,0 %*
Ependymoma, NOS	9391/3	N7W0	601	328	273	42,13	44,0	137	397	*93,7 %*	*6,3 %*
Cellular ependymoma	9391/3	N7W1	33	19	14	34,91	37,0	11	17	*94,1 %*	*5,9 %*
Papillary ependymoma	9393/3	N7W4	23	14	9	40,69	36,0	6	13	*100,0 %*	*0,0 %*
Clear cell ependymoma	9391/3	N7W5	34	20	14	30,11	21,5	4	28	*89,3 %*	*10,7 %*
Ependymoma, anaplastic	9392/3	N7W8	149	86	63	25,90	13,0	61	113	*91,2 %*	*8,8 %*
Tanicytic ependymoma	*9391/3*	*(0002)*	14	10	4	40,28	43,5	3	10	*60,0 %*	*40,0 %*
			1,105	644	461	39,74	41,0	280	746		
				56,6 %	*43,4 %*			*25,2 %*		*91,4 %*	*8,6 %*
Total			18,627	10,785	7,842	53,43	57,0	5,118	14,083		
Gliomas				*57,9 %*	*42,1 %*			*27,5 %*		*62,2 %*	*37,8 %*

Nomenclature, ICD-O (see Louis et al. 2007 [1]); ADICAP, French nomenclature (available at: http://ssr-anapath.googlecode.com/files/Adicap_v5-03.pdf)

The italicized ICD-O numbers are provisional codes proposed for the 4th edition of ICD-O

Abbreviations: *T* total, *B* biopsy, *R* resection, *M* male, *F* female, *N* number, *Med* median age at diagnosis, *m* mean age at diagnosis, *CRYO* cryopreservation

of the WHO. ICD-O-3 topography codes include brain (C71.0–C71.9), meninges (C70.0–C70.9), spinal cord, cauda equina, cranial nerves, and other parts of the central nervous system (C72.0–C72.9) [20]. Now, many registries use these codes, but some registries still record malignant tumors only. Some registries (e.g., Central Brain Tumor Registry of the United States – CBTRUS) include more topography codes as pituitary and pineal glands (C75.1–C75.3) and olfactory tumors of the nasal cavity [C30.0 (9522–9523)].

Another challenge for registries and databases is to record all cases of defined tumors. So the proportion of each type and subtype of tumors could vary from institution to other institution.

Proportion of DLGGs Among PCNSTs and Gliomas

For example, we present the proportion of each major type of PCNST and the distribution of DLGG in the French Brain Tumor DataBase (FBTDB) [21] (Fig. 2.1a–c). We point out (1) FBTDB records cases with histological confirmation only, and usually, registries record the cases with and without histological confirmation, and (2) the distribution of the different subtypes of PCNST is similar in France and in the USA, except for oligodendroglial tumors (see [17]). Two main reasons may explain this difference: (1) the US data were collected between 2002 and 2006 [14], while the French data were collected between 2004 and 2009. Indeed, most studies (e.g., [22, 23]) have reported a recent increase of oligodendroglial tumors in comparison to astrocytic tumors, and (2) French neuropathologists are more influenced by the classification proposed by Daumas-Duport et al. [24] than American neuropathologists.

We could see here the difficulties to compare data from different institutions when histological diagnosis is not completely reproducible [25].

An additional difficulty to know the overall proportion of DLGG is that (1) some institutions use terminology such as "low-grade gliomas" (LGG, see below) and (2) when some information is not detailed enough, registries include some cases in astrocytoma not otherwise specified (NOS) or in glioma NOS, even in unspecified neoplasm.

However, we can consider that the overall DLGG represents about 15 % of all gliomas.

Sex Ratio, Median Age at Diagnosis, and Few Surgical Data for DLGG

The number of males and females and median age at diagnosis (MAD) for DLGG in FBTDB [21] are shown in Table 2.1. Sex ratios, MAD in CBTRUS [14, 16], and FBTDB are shown in Table 2.2. Sex ratio (male/female) for all DLGG is very similar in CBTRUS (1.33) and in FBTDB (1.32). It is the same for MAD for each subtype of DLGG. One can hypothesize that these similarities across the Atlantic could argue for genetic components of these tumors. To be rigorous about classification system, we can notice that (1) gemistocytic astrocytoma are not included in DLGG in CBTRUS (because gemistocytic astrocytomas are more aggressive tumors, CBTRUS counts gemistocytic astrocytomas with anaplastic astrocytomas) and (2) CBTRUS does not separate oligoastrocytomas from anaplastic oligoastrocytomas, because these tumors have the same SNOMED code (9382/3) (see Table 2.1).

Another important thing to notice when one speaks of MAD is how diagnosis is defined. When surgery is performed, registries and the large majority of databases use usually the date of the histological diagnosis. If we refer to clinical studies, some of them specify the age of radiological diagnosis or, sometimes, the age at first symptom. For example, the "Reseau d'étude des gliomes" (REG, a French group that studies DLGG) considered several starting points for their analyses: the date of available radiological diagnosis (which did not differ significantly from the date of clinical onset) and the date of first treatment. Among 1,091 patients (collected at adult age), it was noted that median age (MA) at discovery was 37 years old (range, 4–75) and the MA at first treatment was 44 years old (range, 18–76) (Capelle et al., in revision).

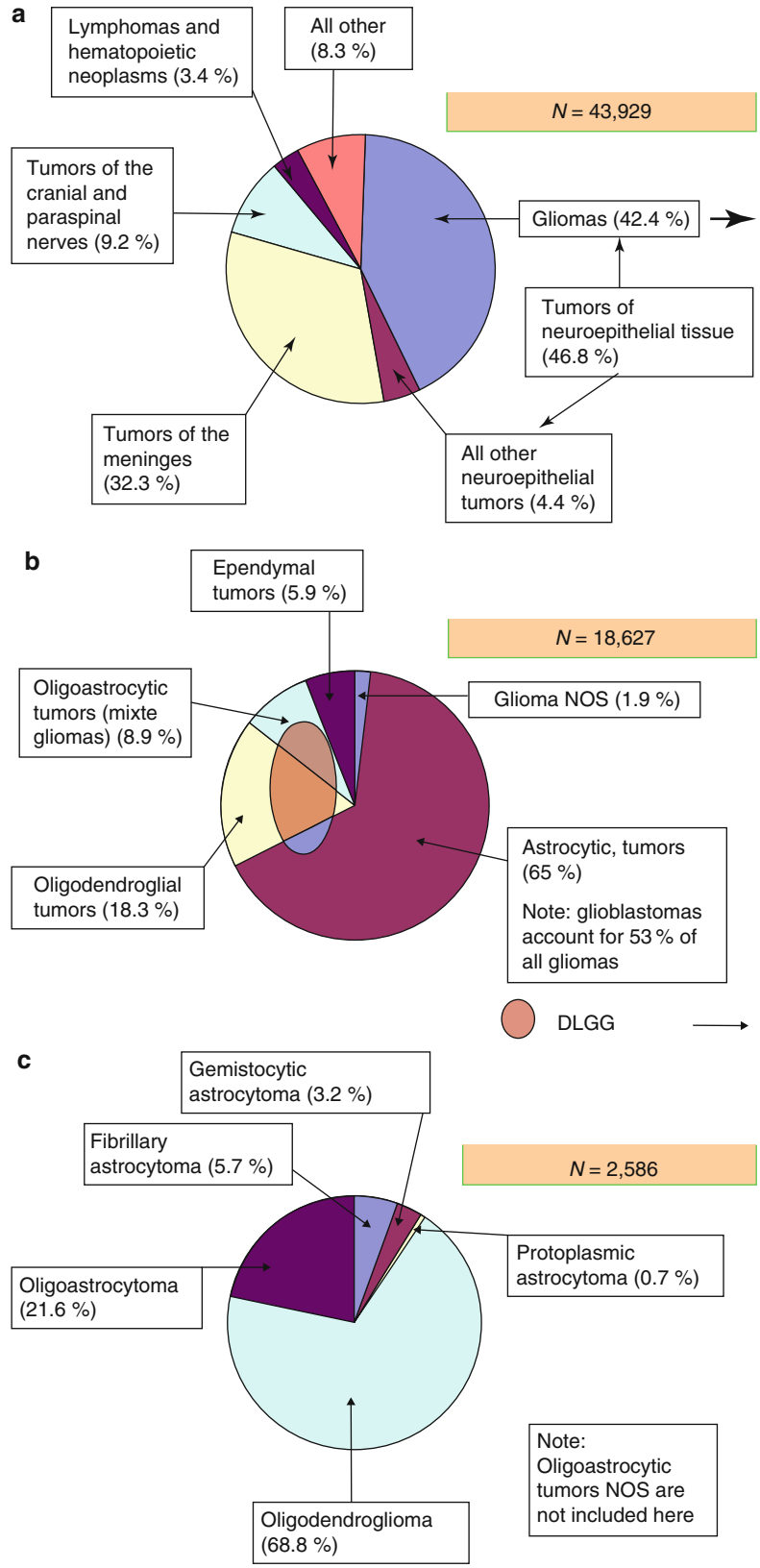

Fig. 2.1 (**a**) Distribution of all primary central nervous system tumors by main histological types. (**b**) Distribution of all gliomas (all sites including supratentorial, infratentorial, and spinal cord) by main histological subtypes. (**c**) Distribution of all diffuse low-grade gliomas (all sites including supratentorial, infratentorial, and spinal cord) by all histological subtypes. *Abbreviations*: *DLGG* diffuse low-grade glioma, *NOS* not otherwise specified (Data adapted from French Brain Tumor DataBase (2004–2009) [21])

Table 2.2 Sex ratio and median age at diagnosis in CBTRUS [14, 16] and FBTDB [21]

	Sex ratio (male/female)		Median age at diagnosis	
	CBTRUS 2012	FBTDB 2012	CBTRUS 2009	FBTDB 2012
Protoplasmic and fibrillary astrocytoma	1.4	1.4	47	45
Oligodendroglioma	1.2	1.3	41	43
Mixed glioma	1.3[a]	1.3[b]	42[a]	42[b]

[a]CBTRUS does not differentiate oligoastrocytoma and anaplastic oligoastrocytoma (same SNOMED code)
[b]Here, FBTDB specifies oligoastrocytoma only

Surgical data concerning DLGG are rare in population studies. Table 2.1 shows the percentages of resection versus biopsy for 14,083 gliomas and all subtypes (included DLGG) (FBTDB, collected data from 2004 to 2009). Of the 54 institutions that participated, the proportion of resection versus biopsy varied considerably from one institution to another (data not shown). We can also notice that 27 % at least of all glioma tumors were cryopreserved (see [17, 21]). This is very important for future biological studies.

The Term of DLGG Is More Appropriate than the Term of LGG

For many years, epidemiological studies have investigated all gliomas or even all PCNST. More recently, the term "low-grade glioma (LGG)" has been introduced. LGG is probably used as a practical definition being quite simply in opposition to the term "high-grade glioma (HGG)." LGGs are slow-growing, intrinsic lesions that contain glioma cells. Referring to the WHO classification of tumors of the central nervous system by Louis et al. [1], LGG may be defined by grade I (GI) and grade II (GII) gliomas and includes subependymal giant cell astrocytoma (GI), pilocytic astrocytoma (GI), pilomyxoid astrocytoma (GII), diffuse astrocytoma (GII), pleomorphic xanthoastrocytoma (GII), oligodendroglioma (GII), oligoastrocytoma (GII), subependymoma (GI), myxopapillary ependymoma (GI), and ependymoma (GII). Some authors include gangliomas, desmoplastic gangliomas [26], and even dysembryoplastic neuroepithelial tumors (DNETs) [27]. However, many studies on LGG exclude ependymomas and refer to astrocytic and/or oligodendrocytic tumors only. Furthermore, some pediatric studies mainly focus on pilocytic astrocytic tumors, and some adult studies often focus on GII astrocytic and/or oligodendrocytic tumors only.

Here, the term "diffuse low-grade gliomas (DLGG)" includes grade II gliomas for diffuse astrocytomas, oligodendrogliomas, and oligoastrocytomas (mixed gliomas). This term is much more precise because in essence it excludes well-delineated grade I and II gliomas and ependymomas with a different natural history. One benefit of this term is also to ignore the difficulties to differentiate an oligoastrocytoma from an astrocytoma or an oligodendroglioma. Moreover, even if heterogeneities are present in the GII glioma group of diffuse astrocytomas, oligodendrogliomas, and oligoastrocytomas, these tumors are often studied together. Usually they concern middle-aged adults in professional activities. Main clinical presentation is epilepsy with or without mild cognitive disorders. Focal deficit and/or raised intracranial pressure are possible but very less frequent. And the majority of these tumors have typical imaging characteristics on MRI as non-enhancing infiltrative mass lesions arising in the white matter. Frequently they extend to the cortical surface [28].

Incidence, Survival, and Prevalence for DLGG

Epidemiological data for DLGG are patchy. In 2012, we can consider that we have (1) good incidence data in some countries, (2) some interesting elements regarding survival, and (3) little item on the prevalence.

Incidence

CBTRUS data [14] give incidence rates (per 100,000 person-years, age adjusted to the 2000 US standard population): glioma overall, 5.97; protoplasmic and fibrillary astrocytoma, 0.10; oligodendroglioma, 0.31; mixed glioma, 0.19; male/female, 0.13/0.08, 0.34/0.27, and 0.24/0.16; and white/black, 0.12/0.04, 0.34/0.13, and 0.21/0.08 for the same histology, respectively. Concerning mixed glioma, the ICD-O-3 histology code (9382/3) grouped oligoastrocytoma (GII) and anaplastic oligoastrocytoma (GIII), and it is not possible to determine the incidence rate for oligoastrocytoma grade II specifically. In France, oligoastrocytoma accounts for 1/3 and anaplastic oligoastrocytoma accounts for approximately 2/3 of all mixed gliomas [17, 21]. Indeed, most studies (e.g., [22, 23, 29]) have reported a recent increase of oligodendroglial tumors in comparison to astrocytic tumors.

Gemistocytic astrocytoma (9,411/3) is included in GGII in the WHO classification [1], but because it is more prone to malignant progression, CBTRUS counts gemistocytic astrocytoma with anaplastic astrocytoma. Gemistocytic astrocytoma is a rare subtype of gliomas: only 82 gemistocytic astrocytomas are recorded among 18,627 gliomas in the FBTDB [21].

In some other registries, it is possible to have incidence rate (/100,000 person-years) for all DLGG: for example, incidence rate is 0.71 in the Gironde Registry [18] (Gironde is an area in southwestern France where about 1,400,000 inhabitants were recorded as living in 2007), 0.63 in Zurich area registry, Switzerland [30], and 1.22 in the Austrian Brain Tumour Registry [13].

It is important to note, as already mentioned, that when some information is not detailed enough, registries include some cases in astrocytoma NOS or in glioma NOS, even in unspecified neoplasm. Therefore, the incidence of DLGG is probably a little underestimated in registries (see below, chapter and tables about survival).

In occidental world, the incidence rate for all DLGG is between 0.5 and 1.3/100,000 person-years. So, we can hypothesis that *the value of 1/100,000 person-years or a little bit less could be considered as a good approximation*.

One point also important to notice when speaking about incidence is the referring population. Indeed, the age groups vary from one population to another. The process of standardization (or adjustment) of rates is a classic epidemiological method that removes the confounding effect of variables that we know – or think – to differ in populations we wish to compare. In practice, age is the factor that is most frequently adjusted for. Age standardization is particularly used in comparative incidence or mortality studies, since the age structure has an important impact on a population's overall incidence and mortality. Consider an example [18]. From 2000 to 2007, a total of 1,907 new PCNSTs were registered among the 1,407,500 inhabitants of Gironde, corresponding to an overall crude rate of 17.6/100,000, unchanged when standardizing on the French population, because age groups are the same in Gironde and in French population. To enable international comparisons, standardized rates were calculated as follows: 17.5/100,000 (reference population, Europe), 15.9/100,000 (reference population, US; used by Surveillance Epidemiology and End Results and the Central Brain Tumor Registry of the United States), and 12.1/100,000 (reference population, world; used by the International Agency for Research on Cancer for the calculation of cancer in the five continents).

To our knowledge, publications that give specific incidence of DLGG using world population as reference are exceptional. In the Canton of Zurich, Switzerland (population 1.16 million), from 1980 to 1994, 987 astrocytic and oligodendroglial tumors were diagnosed, of which 122 (12.4 %) were DLGG. The incidence rates adjusted to the World Standard Population, per million population per year, were 2.28 for low-grade diffuse astrocytomas, 0.89 for oligoastrocytomas, and 2.45 for oligodendrogliomas (total, 0.56/100,000 person-years) [31].

Survival

US survival rates for DLGG, and selected glioma subtypes (i.e., anaplastic oligodendroglioma,

anaplastic astrocytoma, glioblastoma, astrocytoma NOS, and glioma malignant NOS) for comparison, are presented in Table 2.3 (adapted from CBTRUS 2012 [16]). The estimated 5- and 10-year relative survival rates for protoplasmic and fibrillary astrocytoma and oligodendrogliomas are 47.58/35.36 % and 79.25/62.62 %, respectively. Three points could be noted: (1) the estimated 5- and 10-year relative survival rates for mixed gliomas (included oligoastrocytomas and anaplastic oligoastrocytomas) are 58.35/45.52 %, respectively; (2) the number of astrocytoma NOS and glioma NOS is particularly high; and (3) survival of astrocytoma NOS and glioma NOS is not so poor as glioblastoma survival.

In the population-based study of the Canton of Zurich, Switzerland ($N = 122$ DLGG), the survival rate (mean follow-up 7.5 ± 4.8 years) was highest for patients with oligodendroglioma (78 % at 5 years, 51 % at 10 years), followed by those with oligoastrocytoma (70 % at 5 years, 49 % at 10 years) and fibrillary astrocytoma (65 % at 5 years, 31 % at 10 years). Survival of patients with gemistocytic astrocytoma was poor, with survival rates of 16 % at 5 years and 0 % at 10 years [31].

Elsewhere in Europe, data from cancer registries are not specific for DLGG and vary with periods of time and regions. For example, the Danish registry, one of the oldest registries, compared the overall survival of patients with oligodendroglial tumors (oligodendrogliomas and anaplastic oligodendrogliomas) during the periods 1943–1977 and 1978–2002. The median survival increased from 1.4 years (95 % confidence interval [CI], 1.0–1.6) to 3.4 years (95 % CI, 2.6–4.2) during the period of study [32]. More recently, EUROCARE group (included data from 39 cancer registries located in different regions of Europe) showed that estimates of 5-year relative survival rates (95 % CI) for patients with oligodendrogliomas/anaplastic oligodendrogliomas, alive in 2000–2002, were 74.1 (64.4–81.8)/35.1 (21.2–49.5) in Northern Europe, 65.8 (57.5–73.0)/35.5 (24.4–46.9) in UK and Ireland, 75.5 (61.8–85.2)/29.7 (13.4–48.3) in

Table 2.3 One-, two-, three-, four-, five-, and ten-year relative survival rates[a,b] for diffuse low-grade gliomas, anaplastic oligodendroglioma, anaplastic astrocytoma, glioblastoma, astrocytoma NOS, and glioma malignant NOS, SEER 17 Registries, 1995–2008[c]

Histology	*N*	1 year (%)	2 years (%)	3 years (%)	4 years (%)	5 years (%)	10 years (%)
Protoplasmic and fibrillary astrocytoma	718	75.39	61.53	55.95	50.58	47.58	35.36
Oligodendroglioma	2,631	94.15	89.95	85.96	82.32	79.25	62.62
Mixed glioma	1,425	87.42	75.56	68.54	63.00	58.35	45.52
Anaplastic astrocytoma	3,107	60.98	43.02	34.70	30.26	27.00	19.01
Anaplastic oligodendroglioma	1,058	80.30	66.16	58.58	52.85	48.42	33.21
Glioblastoma	21,910	35.20	13.17	7.54	5.50	4.70	2.32
Astrocytoma, NOS	3,571	70.08	59.78	53.88	49.79	46.82	36.20
Glioma malignant, NOS	3,096	60.69	49.11	45.72	43.22	41.76	36.40

Adapted from 2012 CBTRUS report [16]

Abbreviations: *SEER* Survival, Epidemiology and End Results, *N* number of cases, *NOS* not otherwise specified

[a]The cohort analysis of survival rates was utilized for calculating the survival estimates presented in this table. Long-term cohort-based survival estimates reflect the survival experience of individuals diagnosed over the time period, and they may not necessarily reflect the long-term survival outlook of newly diagnosed cases

[b]Rates are an estimate of the percentage of patients alive at 1, 2, 3, 4, 5, and 10 years, respectively

[c]Estimated by CBTRUS using Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) (SEER*Stat Database: Incidence – SEER 17 Regs Research Data + Hurricane Katrina Impacted Louisiana Cases, Nov 2010 Sub (1973–2008 varying) – Linked To County Attributes – Total U.S., 1969–2009 Counties, National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2011 (updated 28 Oct 2011), based on the Nov 2010 submission)

Central Europe, 47.8 (32.4–62.0)/6.1 (1.3–16.6) in Eastern Europe, 63.8 (51.4–74.1)/33.3 (14.7–53.6) in Southern Europe, and 67.2 (62.5–71.6)/31.5 (25.0–38.3) in all cases [33].

In the work by Crocetti et al. [34], data from 76 cancer registries out of the 89 accepting to participate in the RARECARE project were considered. The estimated 5-year relative survival was 14.5 % for astrocytic tumors (42.6 % for low-grade astrocytomas, 4.9 % for high-grade astrocytomas, and 17.5 % for gliomas NOS) and 54.5 % for oligodendroglial tumors (64.9 % for low grade and 29.6 % high grade).

Prevalence

Prevalence rates are ideally suited to provide an overall estimate of cancer survivorship and direction for health planning as they reflect the complex relationships between incidence, survival, and population demographics and hence provide valuable information to the research and medical community. But prevalence data for PCNSTs are limited and very difficult to obtain. In theory, this would imply that the registration of cases is (and has been) exhaustive for many years (to account for long survivors) and that the histological classification systems have not changed over this long period. In 2001, Davis et al. showed that the prevalence rate for all PCNST was 130.8 per 100,000 with approximately 350,000 individuals estimated to be living with this diagnosis in the USA in 2000. The prevalence rate for primary malignant tumors was 29.5 per 100,000, the prevalence rate for primary benign tumors was 97.5 per 100,000, and 3.8/100,000 for primary borderline tumors [35]. The same group published new prevalence data in 2010. On the basis of the sum of nonmalignant and averaged malignant estimates, the overall prevalence rate of individuals with a PCNST (as defined by CBTRUS) was estimated to be 209.0 per 100,000 in 2004 and 221.8 per 100,000 in 2010. The female prevalence rate (264.8 per 100,000) was higher than that in males (158.7 per 100,000). The average prevalence rate for malignant tumors (42.5 per 100,000) was lower than the prevalence for nonmalignant tumors (166.5 per 100,000) [36]. In Europe, Crocetti et al. [34] recently published that the estimated prevalence rate for all astrocytic tumors of CNS was 20.4/100,000 and the estimated prevalence rate for oligodendroglial tumors of CNS was 2.7/100,000, with incidence rate (per 100,000 person-years, age standardized on European population) of 4.4 and 0.4, respectively.

If until now, no specific prevalence data for DLGG are available in large population, we could use a crude approximation. If the average duration of disease and the population of patients are stationary, the prevalence can be estimated by the incidence (I) and the mean duration of disease (D) with the following equation: $P \approx I \times D$. Given the survival data (see previous and following paragraphs), a crude approximation of the DLGG mean duration could be about 10 years. An estimation of the incidence rate for DLGG was little less than 1/100,000 person-years (see above). So according to this crude approximation (and with $I \approx 0.9$/100,000 person-years), the approximate value of the prevalence rate for DLGG would be about 9/100,000. It is important to notice that the prevalence rate for DLGG is probably higher than the prevalence rate for glioblastoma.

Prognostic Factors for DLGG

Due to the lack of class I evidence concerning the impact of the available treatments for DLGG and the difficulties to make large clinical trials on DLGG (limited number of patients and long survival times), the knowledge of spontaneous prognostic factors is crucial for analyzing the effects of the different therapeutic strategies performed on different populations. Excepted for age, sex, and race, little is known for these factors in population-based studies, so most of the prognostic factors come from clinical studies.

Age, Sex, and Race

Age is one of the most important spontaneous prognostic factors for DLGG. CBTRUS 2012

data [16] are shown in Table 2.4. In the study by Claus and Black [29] entitled "survival rates and patterns of care for patients diagnosed with supratentorial low-grade gliomas (*data from the SEER Program, 1973–2001*)," improved survival was significantly associated with female gender (hazard ratio [HR], 0.84; 95 % CI, 0.74–0.95), younger age, white race (HR, 0.70; 95 % CI, 0.54–0.93), histology, and later year of diagnosis. In Europe, Crocetti et al. [34] noted that for glial tumors, 5-year survival was slightly higher for women (20.7 %; 95 % CI, 19.6–21.9) than for men (18.7 %; 95 % CI, 17.8–19.7). Sant et al. [33] found also slightly better survival for women than men only for malignant PCNSTs. For many cancers, women survive longer than men, and this has been attributed to lower prevalence of comorbidities in women as well as better performance status (allowing full application of effective surgical and adjuvant treatments) as well as to better "resistance" to disease [37].

Clinical Status

The clinical and neurological status, before and/or after an oncological treatment, classically influence survival [38, 39]. The presence of a neurological deficit increases with age, tumor extension, and mass effect [40]. At time of diagnosis, the existence of epilepsy is inversely linked to the presence of a deficit and consequently carries a favorable prognostic value when isolated [41, 42].

Tumor Location, Size, and Growth Rates

DLGGs are commonly located in or close to eloquent areas, i.e., those areas of the brain involved in motor, language, visuospatial, and memory functions [39, 43, 44]. Larger tumors and tumors crossing the midline correlate with a shorter survival [41]. Growth rates are inversely correlated

Table 2.4 One-, two-, three-, four-, five-, and ten-year relative survival rates[a,b] for protoplasmic and fibrillary astrocytoma and oligodendroglioma by age groups, SEER 17 Registries, 1995–2008[c]

	Age group	*N*	1 year (%)	2 years (%)	3 years (%)	4 years (%)	5 years (%)	10 years
Protoplasmic and fibrillary astrocytoma	0–14	104	93.8	85.7	85.7	84.1	84.1	82.2
	0–19	127	94.2	85.9	85.9	84.7	84.7	80.2
	20–44	281	92.3	82.6	73.4	65.6	59.7	42.0
	45–54	92	73.5	55.4	52.5	43.6	41.8	[d]
	55–64	107	55.3	27.4	21.3	[d]	[d]	[d]
	65–74	66	30.9	[d]	[d]	[d]	[d]	[d]
	75+	45	[d]	[d]	[d]	[d]	[d]	[d]
Oligodendroglioma	0–14	114	97.4	96.5	94.5	94.5	94.5	90.8
	0–19	199	97.5	95.4	93.0	93.0	92.4	89.4
	20–44	1 390	98.2	95.8	92.6	88.6	85.2	67.4
	45–54	572	94.1	88.7	84.5	81.1	77.6	56.7
	55–64	281	86.7	78.4	71.7	67.8	64.4	47.9
	65–74	116	78.7	69.5	60.1	53.0	49.7	32.6
	75+	73	60.5	45.4	40.1	35.6	34.0	[d]

From 2012 CBTRUS report [16]

Abbreviations: SEER Survival, Epidemiology, and End Results, *N* number of cases, *NOS* not otherwise specified

[a]The cohort analysis of survival rates was utilized for calculating the survival estimates presented in this table. Long-term cohort-based survival estimates reflect the survival experience of individuals diagnosed over the time period, and they may not necessarily reflect the long-term survival outlook of newly diagnosed cases

[b]Rates are an estimate of the percentage of patients alive at 1, 2, 3, 4, 5, and 10 years, respectively

[c]Estimated by CBTRUS using Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) (SEER*Stat Database: Incidence – SEER 17 Regs Research Data + Hurricane Katrina Impacted Louisiana Cases, Nov 2010 Sub (1973–2008 varying) – Linked To County Attributes – Total U.S., 1969–2009 Counties, National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released Apr 2011 (updated 28 Oct 2011), based on the Nov 2010 submission).

[d]Too few cases to report/estimate

with survival [45, 46]. It is important to note that DLGGs show continuously linear growth before anaplastic transformation. Very slow progression is possible, but these tumors always grow. The average slope is 4 mm of mean diameter per year approximately before and/or after surgical resection (without adjuvant therapy) [47, 48].

Prognostic Scores

In a recursive partitioning analysis, Bauman et al. [49] identified four prognostic groups of patients with statistically different median survivals (MS): (1) [KPS <70 and age > 40y; MS, 12m], (2) [KPS ≥70, age >40y, and enhancement present; MS, 46m], (3) [KPS <70 and age 18–40y or KPS ≥70 and age >40y, no enhancement; MS, 87m], and (4) [KPS ≥70 and age 18–40y; MS, 128m], with the following abbreviations – KPS, Karnofsky performance status; y, years; and m, months.

In 22,844 and 22,845 EORTC trials, Pignatti et al. [41] showed that age > 40 years, astrocytoma histology subtype, largest diameter of the tumor >6 cm, tumor crossing the midline, and presence of neurologic deficit before surgery were unfavorable prognostic factors for survival. The total number of unfavorable factors can be used to determine the prognostic score.

In the University of California at San Francisco LGG prognostic scoring system, patients were assigned a prognostic score based upon the sum of points assigned to the presence of each of the four following factors: (1) location of tumor in presumed eloquent cortex, (2) Karnofsky Performance Scale (KPS) Score ≤80, (3) age >50 years, and (4) maximum diameter >4 cm [50, 51]. Survival estimates according to this DLGG score were applied significantly in 537 patients and are exposed in Tables 2.5 and 2.6.

Imaging and Biological Prognostic Factors

Besides these clinical prognostic factors, imaging prognostic factors (conventional, diffusion, perfusion, spectroscopy MR, and PET imaging) as well as molecular and genetic prognostic factors (i.e., p53, PDGF, 1p/19q codeletion, IDH1/2, methylation) are being identified. They will be presented elsewhere in this book, but it is important to mention that they have to be validated in large population studies.

Table 2.5 Hemispheric diffuse low-grade glioma scoring system (UCSF)

	Yes/no
Age > 50 years	1/0
KPS ≤ 80	1/0
Eloquent cortex location (presumed)	1/0
Maximum diameter > 4 cm	1/0
Score	0–4

Adapted from Chang et al. [50, 51]
Note: gemistocytic astrocytomas were excluded
Abbreviation: *KPS* Karnofsky performance status

Therapeutic Prognostic Factors

Among available treatments for DLGG, only large resection seems to show improving survival and delaying tumor progression, in clinical studies [39, 52]. The EORTC study 22,845 revealed an advantage for immediate postoperative radiotherapy in terms of progression-free survival (5.3 vs. 3.4 years), but not for overall survival [53]. The role of chemotherapy for DLGG remains to be defined. But some interesting results have been published with chemotherapy using either PCV or temozolomide. Chemotherapy could be used for unresectable DLGG, at progression, or now as neoadjuvant chemotherapy followed by surgical resection [54]. No study clearly evaluates the impact of multistep treatments in large population. Treatments for DLGG will be discussed later in this book.

About clinical epidemiology for DLGG, three points need to be underlined. First, endpoints in DLGG studies remain to be better defined [55]. Given the continuous growth of DLGG on MRI [47, 48], progression-free survival is poorly defined. Time to anaplastic transformation would be better to use, but this criteria is not often used in the literature [56] (see Chap. 29). Second, given the long time survival of DLGG patients,

Table 2.6 Survival estimates (cumulative overall survival probabilities) according to the UCSF hemispheric diffuse low-grade glioma score in the combined construction and validation sets ($N=537$)

	0 year		2.5 years		5 years		10 years		12.5 years	
DLGG score	*P*	NR	*P*	NR	*P*	NR	*P*	NR	*P*	NR
All patients	1	537	0.92	423	0.80	250	0.62	74	0.44	25
Score 0	1	81	1.0	72	0.98	56	0.97	30	0.85	15
Score 1	1	139	0.98	119	0.90	72	0.77	19	0.40	3
Score 2	1	204	0.95	164	0.81	96	0.52	21	0.35	7
Score 3	1	93	0.76	56	0.53	21	0.29	4	NA	NA
Score 4	1	20	0.68	12	0.46	5	NA	NA	NA	NA

Adapted from Chang et al. [51]
Note: gemistocytic astrocytomas were excluded
Abbreviations: *NA* not applicable, *NR* number of patients at risk, *P* probability

the evaluation of quality of life is very important to analyze after any treatment. Third, databases and even registries will have to record prognostic factors, treatments, and quality of life throughout the duration of the disease for evaluating the best oncological care.

Prognostic Factors: Conclusion

Now we have a lot of candidates for spontaneous (clinical, radiological, and biological) prognostic factors, but we just have validated a few in population studies. On the other hand, we have just few suspected therapeutic prognostic factors. So, future will include lot of works to validate these spontaneous prognostic factors and to determine efficient prognostic therapeutic factors. We will probably need new strategies and new systems for evaluating medical care of patients.

New Methodologies for Clinical and Analytical Epidemiology for DLGG and PCNST

New Methodology for Clinical Epidemiology for PCNST

As mentioned, the number of candidates to be prognostic factors is very important. On the other hand, surgery, radiotherapy, and chemotherapy have prognostic impacts, but the application criteria remain also to be actually determined. Moreover, given the long survival of DLGG patients, treatments are now applied to different stages of the disease (slow progression without transformation, phase with microfocis of transformation, and phase with fast anaplastic evolution). Again, there is no therapeutic approach formally evaluated to preset the order and duration of treatments. Finally, given the quality of life often kept for a long part of the evolution of the disease, the study of the quality of life after each treatment is a major factor to consider and study also (Chapter 14, Quality of Life in DLGG).

The methodology currently used in present tumor registries does not consider all these factors. The works performed in single institutions often involve selected patients with an insufficient number of patients to validate the various prognostic factors and the different therapeutic approaches.

One solution could be to build large clinical, biological, and therapeutic databases, with an attempt to complete registration for all patients. Regional or national databases involving all participating medical teams, epidemiologists, and biostatisticians are now possible [17, 21, 57, 58]. This supposes to note and register main clinical (i.e., symptoms, KPS, MMSE – mini mental status exam – and/or other neurocognitive evaluation), radiological (i.e., tumor location, enhancement, tumor volume, tumor growth rate, multimodal imaging data when available), surgical (i.e., extent of resection, residual volume of the tumor), histological, and biological (i.e., 1p–19q codeletion, IDH 1/2 mutation, and others

when available) factors, treatments performed, quality of life, and dates of anaplastic transformation and death. Of course, all of these factors are not always noted in medical notes, but it is in progress. As brain tumors (i.e., DLGG) are more frequently seen in specialized and/or academic institutions, most of these factors are collected now. Some strategies have already been proposed for DLGG [38, 59] but need to be evaluated at population level and need to be developed for more specific items.

To date, the registration of all these items across a region or country could seem unrealistic, but the development of computing systems (see complexity and efficiency of informatics systems of banks or plane companies) as well as cooperation between different medical specialties and biostatisticians can open the door to such strategies.

Often, the main limitation to the current registration of these data is the lack of time for clinicians to capture these elements in a computer database. The use of clinical research technicians to enter data into a computer system is a solution used by some major centers, and this allows the construction of local databases. However, current economic conditions do not facilitate an employment of clinical research technician in all departments.

Now, healthcare information systems are growing up rapidly. One old method, but still in application, is the "passive" computer archiving (only storage of medical notes, medical exams, and imaging without any computer requests available). More recently, computer requests are developed, mainly for economic purposes, but medical applications become a large avenue for future healthcare information systems. The computer interface systems have also made great progress, and advances in health information technology also include mobile computing systems (i.e., tablet PC and smartphone applications). The goal of future applications is that data from medical notes (operative report, pathology report, multidisciplinary meeting report, radiotherapy report, chemotherapy report, quality of life evaluation, etc.) and imaging are directly exported toward specific database without having to reenter data.

The difficulties in implementing such a system are not technical but involve confidentiality rules and sharing (or pooling) medical data between different actors of the healthcare system. The rules of confidentiality vary from countries to others. But many secured systems are now available. Second, many patients are now accustomed to sign consent forms for medical studies. Third, when we explain to brain tumor patients the interest to participate in medical research, very few patients refused to be involved. Fourth, in many countries now, patients own their medical data and it is possible to send medical data of patients to any doctor (or medical institution) chosen by the patient himself. So if patients sign a form allowing to send their medical data to a secure medical institution (database with all required authorization), hospital administrative authorities cannot refuse.

Until now, the sharing of medical data between different institutions is often difficult because of competition regarding scientific publications. But the system proposed here is not the discovery of a new potential prognostic factor or of a new technique: it is a validation across a population of items already published in clinical series (i.e., original research or clinical trials).

Moreover, the evaluation of medical practices and the definition of quality criteria are increasingly required by government authorities. The new methodology presented here is designed both for validation of prognostic factors and/or therapeutic strategies as well as for evaluation of quality of care. But for efficiency, this has to be managed by group of specialists (neurosurgeons, neuro-oncologists, neuropathologists, etc., with help of epidemiologists and biostatisticians) and not by technicians disconnected of neuro-oncology.

Risk Factors and Methods to Investigate Causes of DLGG

Because DLGG tumors are rare, it is difficult for any single clinical series to assemble a large number of cases enough to explore etiological risk factors. These tumors are often combined

with other glial tumors in analyses for epidemiological studies, which makes it difficult to disentangle the association between the risk factors under study and the association with either DLGG or glioma. However, DLGGs have age, gender, ethnic distributions, and clinical characteristics different from other glial tumors, suggesting potentially different risk factors (see second paragraph of this chapter).

The identification of 1p/19q loss in ≈70 % of oligodendrogliomas [60, 61] and its correlation with improved survival and the fact that specific genes are involved in the biology of oligodendroglial tumors [62] suggest that the etiology of oligodendroglioma may differ from the etiology of other gliomas. On the other hand, IDH1/2 mutations are the only known genetic alteration with a high prevalence (80 % of cases) in all WHO grade II diffuse gliomas, and their frequency does not change during the progression from diffuse astrocytoma (WHO grade II) to anaplastic astrocytoma (WHO grade III) and secondary glioblastoma (WHO grade IV). Similarly, oligodendroglioma (WHO grade II) shows a frequency of IDH1 mutation similar to that in anaplastic oligodendroglioma (WHO grade III) [63].

The high frequency of IDH1/2 mutations in oligodendrogliomas, astrocytomas, and secondary glioblastomas derived thereof suggests these tumors share a common progenitor cell population, and the absence of this molecular marker in primary glioblastomas suggests a different cell of origin [64].

Known risk factors for glioma include inherited genetic syndromes [65, 66] and exposure to high-dose ionizing radiation [67, 68]. A family history of brain tumors (about 5 % [69]) and mutagen sensitivity have previously been associated with an increased risk of glioma, while allergies/asthma and chicken pox have been associated with a decreased risk of glioma [3].

A summary of possible risk factors that have been investigated for glioma is available in the review by Bondy et al. [3]. Smoking, alcohol consumption, dental X-rays, and head injury are not believed to be associated with the risk of glioma. Other nongenetic risk factors that have been investigated include cellular telephone use [70–72], diet [73, 74], anti-inflammatory drug use [75, 76], pesticides [77–79], exogenous hormones [80], and other lifestyle factors [81]. All these factors are still debated as possible risk factor (or not) for gliomas and do not give enough information for DLGG. Only, very few works studied environmental risk factors for LGG (i.e., [82]), and results were mainly inconclusive. Recently, McCarthy et al. [60] studied risk factors for oligodendroglial tumors (329 oligodendrogliomas, 146 anaplastic oligodendroglioma, and 142 mixed gliomas). Data came from seven case–control studies (five US and two Scandinavian) and were pooled. Results for oligodendrogliomas and anaplastic oligodendrogliomas (for comparison) are shown in Table 2.7.

Asthma was associated with a decreased risk of oligodendroglioma (OR = 0.5; 95 % CI, 0.3–0.9). Significant heterogeneity between Scandinavian sites and US sites was found for family history of other cancers, while the Scandinavian sites found a significantly increased risk in those with oligodendroglioma (OR = 4.0; 95 % CI, 1.7–9.6) compared with the US sites for which no association was found (OR = 1.0; 95 % CI, 0.7–1.3). For variables that were reported by only US study sites (Table 2.8), having had chickenpox was associated with a decreased risk of oligodendroglioma (OR = 0.6; 95 % CI, 0.4–0.9) and compared with the use of a public water source, use of bottled water was inversely associated with oligodendroglioma (OR = 0.4; 95 % CI, 0.2–0.9). Finally in this study, no significant associations were noted for ever regular alcohol drinking, diabetes, antidepressant use, anti-inflammatory use, solvent exposure, paint exposure, pesticide exposure, or farm exposures. Only history of seizures was associated with oligodendroglioma and anaplastic oligodendroglioma, but as seizures are symptoms, it is difficult to conclude at a risk factor. One surprising result is that medical X-rays to the head and neck were associated with a decreased risk of these tumors. No explanation is already available.

With respect to environmental exposures, future studies should pay greater attention to whether or not suspect agents can cross the blood–brain barrier or whether they can reach the brain by other routes [3].

Table 2.7 Adjusted odds ratios (ORs) for data on selected exposures collected at five US and two Scandinavian (Sweden and Denmark) sites for oligodendroglioma (OGD) and anaplastic oligodendroglioma (AO) compared with frequency-matched controls

Exposure	Oligodendroglioma, adj. OR[a] (95 % CI)	Anaplastic oligodendroglioma, adj. OR[a] (95 % CI)	Number OGD/AO	Number controls	Study sites excluded[b]
Ever smoker	0.9[c] (0.7, 1.2)	0.9 (0.7, 1.4)	328/146	1,255	None
Family history of brain tumor	1.6 (0.9, 3.1)	**2.2[d] (1.1, 4.5)**	271/122	995	2, 6
Family history of cancer	1.1[e] (0.8, 1.4)	1.1 (0.7, 1.5)	324/144	1,230	None
Asthma	**0.5[d] (0.3, 0.9)**	**0.3[d] (0.1, 0.9)**	174/92	674	1, 3, 4.1
Allergies[f]	1.1[e] (0.8, 1.6)	0.6 (0.4, 1.1)	245/97	880	1, 4.1
Asthma and/or allergies[f]	0.9 (0.6, 1.2)	**0.6[d] (0.4, 0.9)**	222/122	869	3, 4.1
Eczema	0.6 (0.3, 1.3)	0.4 (0.1, 1.3)	136/72	541	1, 3, 4.1, 4.2
History of seizures	**6.7[c,d] (4.3, 10.6)**	**8.7[d] (5.0, 15.2)**	248/130	1,036	3
Antihistamine use	1.0 (0.6, 1.4)	1.2 (0.7, 1.9)	229/123	866	2, 4.1, 7
Dominant hand					
Left vs. right	1.2 (0.6, 2.3)	1.1 (0.4, 2.8)	122/64	494	1, 3, 4.1, 4.2
Both vs. right	1.1 (0.3, 3.4)	0.9 (0.1, 7.5)			
Radiation treatment	1.1 (0.5, 2.6)	1.3 (0.5, 3.4)	327/146	1,247	None
Dental X-rays	0.8 (0.5, 1.2)	1.3 (0.5, 2.9)	212/86	772	1, 4.1, 4.3
Medical X-rays to the head and neck	**0.7[d] (0.5, 1.0)**	**0.6[d] (0.4, 1.0)**	179/80	731	1, 3, 4.3
Any trauma to the head	1.3 (1.0, 1.8)	1.0 (0.6, 1.5)	267/110	1,037	5, 7

Adapted from McCarthy et al. [60]

[a]Unconditional logistic regression, adjusting for age group, gender, and site. 95 % CI: 95 % confidence intervals

[b]1 = MD Anderson; 2 = NCI; 3 = NIOSH; 4.1 = UCSF series 1; 4.2 = UCSF series 2; 4.3 = UCSF series 3; 5 = UIC/Duke; 6 = Sweden; 7 = Denmark

[c]*P* value for interaction term with site was <0.05 among US sites with available data. For oligodendroglioma, site-specific adj. OR (95 % CI) for smoking ranged from MD Anderson, 0.4 (0.2, 0.9), to Duke/UIC, 2.3 (1.0, 5.3); for history of seizures ranged from NIH, 1.2 (0.4, 3.9), to Duke/UIC, 35.2 (4.0, 311.2); for anaplastic oligodendroglioma, site-specific adj. OR (95 % CI) for medical X-rays ranged from UCSF, 0.4 (0.1, 0.9), to NIH: 2.6 (0.7, 10.4)

[d]$P < 0.05$

[e]*P* value for test for heterogeneity was <0.05 between US and Scandinavian sites. For oligodendroglioma, site-specific adj. OR (95 % CI) for family history of other cancers for Scandinavian and US sites, respectively, was 4.0 (1.7, 9.6) and 1.0 (0.7, 1.3); for allergies for Scandinavian and US sites, respectively, was 4.6 (1.3, 15.5) and 0.9 (0.6, 1.3)

[f]Allergies for the Denmark and Sweden data only includes hay fever

Studies of syndromes, familial aggregation (see [69, 83]), linkage, and mutagen sensitivity in adults suggest genetic susceptibility to gliomas. Although the genetic syndromes caused by rare inherited mutations and associated with higher risk of brain tumor account for few cases, they provide an important starting point for identifying candidate genes and pathways for gliomagenesis. Syndromes, including gliomas or medulloblastoma, with gene names and chromosome location, are neurofibromatosis 1 (NF1) (17q11) and NF2 (22q12), tuberous sclerosis 1 (TSC1) (9q34) and TSC2 (16p13), retinoblastoma 1 (RB1) (13q14), Li–Fraumeni (TP53) (17p13), and Turcot syndrome and multiple hamartoma (the adenomatous polyposis coli gene APC, 5q21; the human mut-L homolog 1 gene hMLH1, 3p21.3; hMSH2, 2p22-21; the postmeiotic segregation increased 2 gene PMS2, 7p22; and the phosphatase and tensin homolog gene PTEN, 10q23.3) [3]. Inherited predispositions to glioma were recently reviewed [65, 66]. The roles of more common variants in many of these genes (and related pathways) in sporadic gliomas (i.e., DLGG) are unknown to date.

The priorities recently recommended by the Brain Tumor Epidemiology Consortium (BTEC) [84] emphasized the need for expanding research in genetics and molecular epidemiology. Gu et al.

Table 2.8 Adjusted odds ratios (ORs) for data on select exposures collected only at 5 US sites for oligodendroglioma (OGD) and anaplastic oligodendroglioma (AO) compared with frequency-matched controls

Exposure	Oligodendroglioma, adj. OR[a] (95 % CI)	Anaplastic oligodendroglioma, adj. OR[a] (95 % CI)	Number OGD/AO	Number controls	Study sites excluded[b]
Ever regular alcohol drinker	0.8 (0.6, 1.2)	0.7 (0.5, 1.2)	287/120	1,092	None
Diabetes I or II	0.8 (0.4, 1.9)	0.7 (0.2, 2.0)	192/108	754	3, 4.1
Chicken pox	**0.6**[c] (**0.4**, **0.9**)	**0.5**[c] (**0.3**, **0.9**)	172/100	731	2, 3
Antidepressant use	0.9 (0.6, 1.3)	0.8 (0.5, 1.4)	177/104	745	2, 3
Anti-inflammatory use	0.9 (0.6, 1.4)	0.9 (0.5, 1.4)	148/98	586	2, 3, 4.1
Solvent exposure	0.9 (0.7, 1.3)	1.2 (0.7, 2.0)	203/83	733	4.1, 4.2, 4.3
Paint exposure	1.4 (1.0, 2.0)	1.4 (0.8, 2.4)	225/100	834	4.1, 4.2
Pesticide exposure	1.1 (0.7, 1.6)	1.6 (0.8, 3.2)	223/99	826	4.1, 4.2
Farm exposures	0.7 (0.5, 1.1)	0.8 (0.5, 1.4)	200/81	760	1, 2
Water source					
Private vs. public	1.0 (0.7, 1.6)	1.6 (0.8, 3.1)	199/83	727	4.1, 4.2, 4.3
Bottled vs. public	**0.4**[c] (**0.2**, **0.9**)	0.5 (0.2, 1.3)			

Adapted from McCarthy et al. [60]

[a]Unconditional logistic regression, adjusting for age group, gender, race, site, and interview year. 95 % CI: 95 % confidence intervals

[b]1 = MD Anderson; 2 = NCI; 3 = NIOSH; 4.1 = UCSF series 1; 4.2 = UCSF series 2; 4.3 = UCSF series 3; 5 = UIC/Duke

[c]*P* value < 0.05

[85] reviewed the literature to identify molecular epidemiologic case–control studies of PCNSTs that were hypothesis-driven and focused on four hypothesized candidate pathways: DNA repair, cell cycle, metabolism, and inflammation. They summarized the results in terms of genetic associations of single-nucleotide polymorphisms of these pathways (this chapter could not review all pathways and molecular epidemiologic studies for gliomas, but lectors are encouraged to read this paper). In this article, authors advise that large collections of glioma families for more extensive linkage analyses are being collected in the international GLIOGENE study of familial glioma [86]. They also discussed future research directions based on available evidence and technologies and concluded that high-resolution whole-genome approach with significantly large sample size could rapidly improve our understanding of the genetic etiology of PCNSTs.

Two recent genome-wide association studies reported that single-nucleotide polymorphisms (SNPs) in (or near) *TERT* (5p15), *CCDC26* (8q24), *CDKN2A/B* (9p21), *PHLDB1* (11q23), and *RTEL1* (20q13) are associated with infiltrating glioma [87, 88]. Jenkins et al. [89] showed that 8q24 polymorphisms were associated with gliomas containing an oligodendroglial component but not with GBM. Conversely, the 5p15, 9p21, and 20q13 regions were associated with GBM risk, but were not strongly associated with oligodendroglial tumors. This pattern supports the current model of glioma initiation and progression. For example, since 8q24 associations are seen for risk of oligodendroglioma regardless of 1p/19q deletion, as well as potentially with non-GBM astrocytoma, they predict that 8q24 SNPs may be associated with gliomas containing IDH1/2 mutations [90]. Indeed, it can be hypothesized that 8q24 alterations may facilitate the acquisition of IDH mutations or may interact with IDH mutation to facilitate tumorigenesis [91]. Jenkins et al. also predict that the 8q24 SNPs will be associated with secondary GBM and/or the CpG island methylator phenotype [92]. Unfortunately, case number was limited and therefore statistical power was insufficient to generate meaningful data for grade 2 astrocytoma and secondary GBM.

A group from Europe [93] analyzed a total of 1,458 tagging single-nucleotide polymorphisms (SNPs) that were selected to cover DNA repair

genes, in 81 grade II and grade III gliomas samples, collected in Sweden and Denmark. The statistically significant genetic variants from the first dataset ($P<0.05$) were taken forward for confirmation in a second dataset of 72 grade II and III gliomas from northern UK. In this dataset, eight gene variants mapping to five different DNA repair genes (ATM, NEIL1, NEIL2, ERCC6, and RPA4) were associated with survival. Finally, these eight genetic variants were adjusted for treatment, malignancy grade, and patient age and gender, leaving one variant, rs4253079, mapped to ERCC6, with a significant association to survival (OR 0.184, 95 % CI 0.054–0.63, $P=0.007$). This is the first report describing a prognostic value of DNA repair genes variants in grade II and III gliomas. The authors concluded in a possible novel association between rs4253079 and survival in this group of patients with low-grade and anaplastic gliomas, which needs confirmation in larger datasets.

Genetic causes of glioma were recently reviewed by Melin [94]. The causes of glioma have until recently been unknown for most cases, partly due to lack of statistically powered studies enabling subclassification of glioma subtypes. The novel chromosomal loci associated with different glioma subtypes have provided us with an additional understanding of causes of glioma. All low-penetrant genes contribute with a modest increased risk and cannot by themselves be used for risk prediction. Nevertheless, they could provide a tool to understand the underlying biology of glioma progression and to be used in future studies of gene–environment studies of specific glioma subtypes [94].

As demonstrated in this chapter, a lot of work has still to be done for understanding glioma causes and for evaluating all therapeutic strategies. As PCNSTs include more than 140 histological types and subtypes, with probably (1) different biological mechanisms involved in tumorigenesis and (2) different risk factors, the French neuro-oncology community decided to record and analyze each histological type and subtype specifically. French Brain Tumor DataBase (FBTDB) was born in 2004 [11]. This work aims of prospectively recording all PCNST cases in France, for which histological diagnosis is available. The objectives are (a) to create a national database and network to perform epidemiological studies, (b) to implement clinical and basic research protocols, and (c) to harmonize the healthcare of patients affected by PCNST.

Since 2006, more than 9,000 new cases of PCNST per year are recorded by FBTDB (French population in 2008, 64,000,000 inhabitants; crude rate of newly diagnosed and histologically confirmed PCNST = 14.06/100,000 person-years). This is very close to the estimated incidence of newly diagnosed and histologically confirmed PCNST in France (14.08/100,000 person-years, overall crude rate of 17.6/100,000, unchanged when standardizing on the French population, and 79.3 % of overall tumors were histologically confirmed, data from Tumor Registry of Gironde [18]).

A work in progress of FBTDB is to study the distribution of DLGG and the distribution of diffuse grade III gliomas (DGIIIG) on the metropolitan French territory (years 2006–2009). Even if in this study the authors were mainly interested by the distribution of DLGG, they decided to study also the distribution of DGIIIG because histological grading of gliomas could be debated sometimes by some pathologists [25]. Preliminary results seem to show heterogeneity in the distribution of DLGG and the distribution of DGIIIG among the French territory, with a higher number of cases in the northern, eastern, and some central areas of France for both glioma groups. Even if small discrepancies exist between the two distributions (DLGG/DGIIIG), they are globally comparable. If this heterogeneity in the geographic distribution of DLGG and DGIIIG is confirmed, the authors will compare environmental, genetic, and functional factors between areas with high incidence and areas with low incidence. This could be another way to look for risk factors of DLGGs.

Early Detection of DLGGs

Before concluding this chapter about epidemiology of DLGGs, the author would like to comment the impact and the ways for considering the early detection (or screening) of these specific tumors.

DLGGs usually affect young adult and have major adverse economic and social impacts. Prevalence of DLGG is probably underestimated as patients have long history of illness after the occurrence of symptoms. It is however important to underline that treatment, especially surgical resection, may significantly increase the overall survival in DLGG by delaying anaplastic transformation and may also improve the quality of life (QoL) – a crucial point nevertheless not discussed in many publications. Many recent reports have indeed demonstrated the actual impact of surgery on the natural history of DLGG [39, 52, 95]. Such demonstration has been possible by performing an objective evaluation of the extent of resection (EOR) on postoperative MRI, which was not done in "historical series," explaining the controversy about the role of surgery in the classical literature. Interestingly, all recent studies with postsurgical MRI observed a significant relationship between the EOR and overall survival (for a recent review, see [95]). This is the reason why it was recently proposed to operate asymptomatic patient with a DLGG [96]. Indeed, it was recently demonstrated that all incidental DLGGs were progressive tumor, with a constant radiological growth (median velocity of diametric expansion around 3.5 mm/year, i.e., very close to the growth rate of symptomatic DLGG) [97]. The same authors showed that incidental DLGGs differ significantly from symptomatic DLGGs: they have smaller initial tumor volumes ($P<0.001$), lower incidence of contrast enhancement ($P=0.009$), and are more likely to undergo gross total surgical removal ($P<0.001$). So if this strategy and this result are confirmed in larger study, we could argue that effective treatment does exist at the early phase of the illness.

In future, early detection of DLGG could be performed by three different ways, at least. First, MRI is an easy exam considered without risk. Abnormality of signal on FLAIR or T2-weighted images is easy to detect in fast exams. Combining screening of some neurological illness and/or neurovascular illness by MRI could be a realistic first approach. Then, multimodal MRI and consecutive MRIs have good diagnostic power and may suggest specific treatments and/or follow-up. Secondly, if progress in knowledge of risk factors of DLGG occurs, it will be possible to define groups of patients with increase of risk of DLGG. And it could be proposed to these patients to have MRI. We can notice that Gerin et al. [98] recently proposed a model for estimating date of birth of DLGG. Within the assumptions of this model, the authors have identified two types of tumor: the first corresponds to very slowly growing DLGG that appears during adolescence, and the second type corresponds to slowly growing DLGG that appears later, during early adulthood. All these tumors become detectable around a mean patient age of 30 year old. So, this represents the first step for defining one subgroup of the population, in which the DLGG screening could be done. Thirdly, many researches on biological markers (in blood, urine, saliva, etc.) for gliomas are ongoing now. For example, we can expect that progress in nanotechnology will help to detect very small level of such biological markers.

Conclusion

In large clinical series or in databases in which cases are histologically confirmed, DLGGs account for approximately 15 % of all gliomas. In occidental population, incidence rate for DLGG can be estimated about 1/100,000 person-years, if we consider that some astrocytoma and glioma NOS (high number in registries) are probably DLGG. Prevalence rate for DLGG is probably equal or higher than prevalence rate for glioblastoma but remains to be defined more precisely. Until now, causes of DLGG are mainly unknown. Some spontaneous prognostic factors have been identified (i.e., age, KPS, volume, and location of the tumor). Extent of resection has been increasingly shown to correlate with improved outcome, as well as with better seizure control and reduced histological upgrading rates. Many biological and radiological factors are candidates to be prognostic factors now, but evaluations on large population studies are required. Therapeutic strategies using several successive surgeries (spread over time), chemotherapy, radiotherapy, and

combination of different treatments are not currently codified. Therefore, many evaluations are also required. To move forward quickly and effectively in the knowledge of biological processes, identifying the causes, assessment of oncological care, and quality of life after any treatment, the medical and scientific community must significantly change its current practice of recording data. The creation or development of large database (including clinical, radiological, biological, and genetic data) involving most major medical centers and different specialties (neurosurgery, neurology, neuro-oncology, neuropathology, neuroradiology, biology, epidemiology, and biostatistics) and the use of modern informatics technology will yield major results in the relatively near future.

Knowledge of molecular biology and genetics will develop. That will make possible to better understand the genesis of DLGG and other PCNST. But the observation remains a simple and effective method for searching causes of DLGG and other PCNST. The study of the spatial and temporal distribution of DLGG (and other TPSNC) cases on large area will allow for searching environmental, genetic, and functional differences in populations with high and low incidence. By this easy way, it could be possible to discover factors and/or their interactions responsible for the initiation and development of DLGG and/or others PCNST.

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Histological Classification

3

Valérie Rigau

Abstract

Glial tumors are difficult to classify. The lack of reproducibility between pathologists is proven, with a difference of interpretation between reaction cells and tumor cells, and between astrocytes and oligodendrocytes. This drawback does not exist in any other organ, and so far no immunohistochemical marker can make the distinction. This chapter on neuro-oncopathology reviews the history of the different histological classifications used over time to try to perform the grading of gliomas, with special emphasis on the WHO classification which is considered as the gold standard but which has however several limitations. Finally, we discuss our experience with diffuse low-grade gliomas with regard to the proposal of a new intermediate grade.

Keywords

Diffuse low-grade glioma • Histological classification

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Introduction

The current clinical practice of neuro-oncology is dependent on accurate tumor classification. No variable predicts prognosis more precisely, and classification is also the basis on which neuro-oncologists apply therapies in a relatively uniform way for all patients with a given tumor type. So, therefore, treatment of brain tumors is yet dictated by histological diagnosis.

The Several Glioma Classifications

History of the Classification of Gliomas from Virchow Until the First WHO Classification in 1979

Knowledge of the earlier history of the classification of gliomas until the first WHO classification of brain tumors in 1979 is essential for understanding the actual difficulties in the histological typing of the common forms of gliomas in adult patients.

The WHO classification of gliomas is based upon the presumed cell of origin and the degree of malignancy (grading). Two contradictory systems are used. In their "embryonal remains" theory, Bailey and Cushing [1] suggested that gliomas were derived from transformation of normal glial cells during their development. In the Kernohan's hypothesis [2], it was stated that gliomas developed from adult cells which could be dedifferentiated.

The Descriptive Period from Virchow to the Classification of Bailey and Cushing (1946–1924)

The root of the problem lies in the complex structure of the brain parenchyma, the indistinct aspect of the glial cells, and the early history of the classification of brain tumors. During this early period, brain tumors have been described and named according to their resemblance to their normal counterpart. But this was at a time when knowledge of the histology of the brain was rudimentary. Virchow [3] first identified the neuroglia in 1846, the term glia reflecting the indistinct appearance of these cells on routine stain. Five years later, Virchow [4] described the gliomas and individualized them from the "other sarcomas" of the brain. The advent of methods of argentic impregnation first allowed identifying the astrocytes. These cells were first identified under this name by Stroebe in 1886 [5]. In 1893, Andriezen [6] described two types of astrocytes: the protoplasmic astrocytes of the cortex and the fibrous astrocytes of the white matter. Von Lenhossek [7] first described the astrocytomas in 1895, under the name of "astroma," and thereafter gliomas were considered to be astrocytic tumors. It was only in 1917 that del Rio Hortega [8] discovered the oligodendrocytes and oligodendrogliomas, first described in 1926 by Bailey and Cushing [1] in their classification of the tumors of the "glioma group." It is noteworthy that astrocytomas had been described 22 years before the discovery of oligodendrocytes and that oligodendrogliomas were individualized more than 35 years after the description of astrocytomas.

The "Modern Period": From the Histogenetic/Histoprognostic Classification of Gliomas of Bailey and Cushing to the First WHO Classification (1924–1974)

To quote Scherer [9], "until then, authors considered histogenetic and other theoretical discussion as accessory to the facts described; Ribbert and his followers group arbitrarily chosen facts around hypothetical system." However, the classification of gliomas that Bailey and Cushing [1] proposed in 1926 in their monography "A classification of the tumors of the glioma group on a histogenetic basis with a correlated study of prognosis" has opened the modern area of the histoprognostic classification of brain tumors. Their classification, which was contemporary with the development of neurosurgery, had the great merit to be the first which had both biological and clinical significance.

The classification of Bailey and Cushing was based upon the theory of embryonal remains. For them, the behavior of a neoplasm is dictated by the predominant cell type. Adopting the point of view that "the histogenesis of the brain furnishes

the indispensable background for an understanding of its tumors," Bailey and Cushing attempted to classify gliomas according to their resemblance with one of the 20 cell types that they had identified in their own pyramidal scheme of cytogenesis of the glia. This led them to identify 14 main histological types. Their classification of the gliomas group comprised all tumors derived from the primitive "medullary epithelium," including the medulloblastoma, those that are still included in the generic category of gliomas being the "spongioblastoma multiforme" (glioblastoma), "spongioblastoma unipolare" (pilocytic astrocytoma), astroblastoma, fibrillary and protoplasmic astrocytoma, oligodendroglioma, and ependymoma.

Bailey and Cushing reasoned that the cells of astrocytomas microscopically closely resembled astrocytes and those of oligodendrogliomas histologically mostly mimicked oligodendrocytes. As these tumors became more malignant, they resembled less differentiated precursor cells. Hence, malignant astrocytomas were called "astroblastomas." Some of these concepts were confirmed during the latter half of the twentieth century. For instance, both at the ultrastructural and immunohistochemical levels, many astrocytomas are comprised of cells that exhibit astrocytic differentiation. Whether the cell of origin of a glial tumor can be inferred from its differentiation, however, is unclear. Indeed, the specific cells of origin for gliomas remain unfamiliar.

Astrocytomas According to Bailey and Cushing

In the classification of Bailey and Cushing, astrocytomas represented the ultimate stage of differentiation of the spongioblast, the tumor cells being thus considered to reproduce the cytological features of the normal mature astrocytes. Thanks to the new specific gold chloride impregnation method for astrocytes, two types of astrocytes were described in 1911 by Cajal [10]: the protoplasmic astrocytes of the cortex and the fibrillary astrocytes of the white matter. Bailey and Cushing have also distinguished two subtypes of astrocytomas: the protoplasmic and the fibrillary astrocytomas. In their monography, these tumors were described as follows:

- Protoplasmic astrocytomas: "They may consist wholly of protoplasmic astrocytes, with only the a few small vascular channels. No neuroglial fibrillae can be demonstrated but the stellar shape of the cells may be plainly seen when impregnated by Cajal's gold sublimate method, which shows the whole extent of their branching processes. Amitotic division is common but mitoses are rarely seen. Many nerve fibres with altered myelin sheets may be seen passing through these soft tumors."
- Fibrillary astrocytoma (Fig. 3.1): "The term astrocytoma fibrillare is the one we prefer to give to a comparatively common lesion composed wholly of fibrillary cells, though a small and variable number of protoplasmic astrocytes may be present. … The appearance of the tumor under low magnification shows a feltwork of neuroglia-fibrillae, between which are scattered widely separated nuclei. Mitotic figures are not found and amitotic division is rare. A few blood-vessels are present but they are infrequent… The nature of the neoplastic cells is beautifully shown by Cajal's gold sublimate method (for astrocytes). Sometime calcified plaques may be found, through which run neuroglial fibrillae."

In addition to the concept that differentiated tumoral astrocytes may look like normal mature astrocytes, the whole cell population of these tumors was implicitly considered by them to be tumoral. As further denounced by Scherer [9], Bailey and Cushing neglected the works of the "ancient" authors who previously emphasized that most gliomas are not solid but infiltrative tumors. To quote Scherer again, "No exact method of determining the predominant cell type was indicated, it seems to correspond to a simple impression." In fact the "predominant cell type" easily becomes the one which is easily recognizable on routine or on available specific stain (or later, for which a specific antibody was available), that is, the astrocytes.

Another problem is that Bailey and Cushing fail to recognize astrogliosis. Still in 1932, Rand

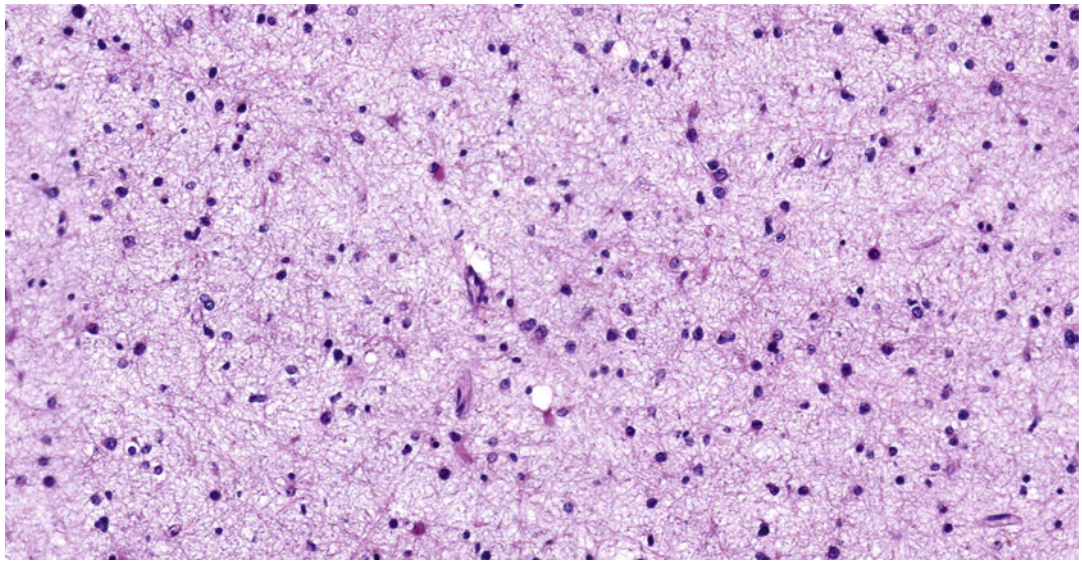

Fig. 3.1 Low-grade astrocytoma: a moderately cellular tumor composed of uniform fibrillary astrocytes

and Courville [11] claimed that gliosis occurred only as the result of tissue destruction. Biggart in 1949 [12] described the various forms of astrogliosis in non-tumoral processes and illustrated one example of reactive gliosis which, on gold sublimate, was strikingly similar to the example of fibrillary astrocytoma illustrated in the monography of Bailey and Cushing. However, Biggart [12] stated that "whether or not a gliosis can occur, not in response to actual destruction of the nervous tissue is a matter of debate."

Astrocytoma Versus Oligodendroglioma?

Elvidge, Penfield, and Cone [13], who for the first time in 1937 regrouped the protoplasmic and fibrillary astrocytoma under the term of "astrocytoma diffusum," emphasized that "a striking characteristic of these tumors is that nerve cells are not destroyed but may be displaced." Scherer [9] as well stated that "in all astrocytomas nerve cells and fibre are perfectly preserved, including in the mist of the tumor." Bergstrand [14] observed that astrocytomas contain a large number of nuclei suggesting oligodendrocytes, which do not stain positively with methods for astrocytes but which sometimes show a specific oligodendroglioma impregnation. For that reason he doubted the fact that the name astrocytoma was justified. Moreover, Singer and Seiler [15] went as far as denying the existence of astrocytomas. These latter authors not only suspected that the tumoral cell population of astrocytomas was oligodendroglial but also considered the fiber network of astrocytomas as merely reactive stroma.

Scherer [9], in his paper "Cerebral Astrocytomas and Their Derivatives" (1940), reported that "only a minority of the tumor elements stains specifically with gold sublimate impregnation…. the remainder being oligodendroglial-like." Commenting the observations of Singer and Seller, he said, "it is true that the cortical parts of astrocytomas are generally very poor in fibers, even though their white mater parts are highly fibrillary. This has a considerable theoretical importance, in so far as it shows that these neoplasms follow the same rule as reactive processes." Unfortunately, Scherer finally claimed that "This lend further support to the idea that astrocytomas grow by diffuse proliferation of preexistent elements and not by progressive infiltration" and in a further publication that "This behaviour cannot be explained by tumor cells coming from the white and invading the grey matter or vice

versa, but only by neoplastic proliferation of the local elements which maintain their fibrillary or afibrillar character."

Even if it is unclear whether Scherer played a major role or not in the "rehabilitation" of (diffuse) astrocytomas, the fact is that about 10 years later, Kernohan et al. [2] and Ringuertz [16] – who simultaneously proposed a grading system for astrocytomas – adhered to the idea of Bailey and Cushing that tumoral astrocytes can show a normal appearance. According to Kernohan, astrocytomas grade 1 "are made up of normal or almost normal astrocytes, but with an increase number," and according to Ringuertz, in astrocytomas (equivalent to the grade 1 and 2 of Kernohan), "all or nearly all cells are normal astrocytes."

Meanwhile, Kernohan et al. [2] estimated the prognosis of glial tumors based upon the extent of observed anaplastic features (i.e., mitoses, endothelial proliferation, cellular atypia, necrosis). Such a classification was proven to be of prognostic value, especially for astrocytomas. The Kernohan system continues to be used in modified forms. Although the term low-grade glioma (LGG) is widely used, it is not explicitly defined in either system. LGG describes a spectrum of primary brain tumors composed of cells that histologically resemble one or more differentiated types of macroglial cells (diffuse and fibrillary astrocytes, oligodendrocytes, ependymal cells) without evidence of anaplasia. In the Kernohan scheme, LGGs encompass grade 1 and 2 tumors. LGGs have been referred to as "benign" gliomas, but this is a misnomer. Although these tumors have a more favorable natural history than high-grade gliomas (HGGs), LGGs are only occasionally associated with prolonged (>10 years) survival and frequently develop characteristics similar to more aggressive brain tumors.

Gemistocytic Astrocytoma

This subtype of "astrocytoma" (from the Greek gemistos which means "filled up") was described by Elvidge et al. [13] in 1937. In their paper "The Gliomas of the Nervous System," these authors explained that they gave this name to these tumors by analogy with the "gemästete Zell" (or "plump astrocytes") that Nissl [17] had described in 1899. The authors indicated that "This form of astrocyte occurs within the brain as a result of chronic pathological conditions such as are produced by arteriosclerosis within the brain. The nucleus becomes large and the cell body enlarges and is filled with homogeneous cytoplasm….In pathological reactions, there is an easy transition of plump cells into giant cells astrocytes with multiple nuclei." Therefore, in this paper the problems that the individualization of gemistocytic astrocytomas will pose were anticipated, as they emphasized: "It must be remembered a few plump cell … may appear scattered through a glioblastoma multiforme. They may occur as any as occasional form in any astrocytoma so that it is difficult to be sure whether the cells are really neoplastic or whether they are merely astrocytes reacting in this way to the abnormal conditions provided by the neoplasm."

The Concept of Primary Anaplasia Versus "Secondary Glioblastomas" and Anaplastic Astrocytomas

According to Zülch [18], the term of anaplasia was first coined by Von Hansemann [19] in 1898 and was defined by this author by the low level of differentiation of a tumor cell in comparison with the mother cell. However in 1928, Ewing [20] developed under the term of anaplasia, the concept according to which neoplasm arises by a process of transformation of normal mature cells and progress by a process of dedifferentiation. To the best of our knowledge, Cox in 1933 [21] was the first to adopt this concept for the classification of gliomas. In the following years, a growing number of pathologists will adhere to the concept of gliomas descending from a given normal mature glial cell. The success of this theory may mostly be explained by the fact that it led to a simplification of the initially complicated embryogenetic scheme of Bailey and Cushing. Also, as anaplasia is by essence a cytologic concept, this approach in the classification of gliomas remained perfectly compatible with the yet

largely adopted principle of classifying gliomas based upon cytological features. The major impacts of the concept of anaplasia will be the notion of secondary gliomas, the advent of systems of grading for astrocytomas that led to the individualization of anaplastic astrocytoma, and overall the filiation that could be established between astrocytomas, anaplastic astrocytomas, and glioblastomas.

Grading of Astrocytomas and Anaplastic Astrocytomas

The four-part grading system of astrocytomas of Kernohan et al. [2] and the three-part grading system that Ringertz [16] proposed some months later (1950) were in their principle based upon the relative proportion of anaplastic versus differentiated tumor cells. The other criteria included the cell density, degree, and pattern of angiogenesis, as well as presence or absence of necrosis. In fact, from these earlier grading systems until to date, the "place" of anaplastic astrocytomas in between the low-grade diffused astrocytomas and glioblastoma remained difficult to determine. In the system of Kernohan grade 3 (that comprised 50–75 % normal looking-like astrocytes) and grade 4 (with a marked anaplastic transformation), a marked vascular proliferation and necrosis were present.

Oligodendrogliomas

In 1921 del Rio Hortega [22] first considered the possibility of participation of oligodendroglia cells in the formation of gliomas. Oligodendrogliomas were described for the first time in 1926 by Bailey and Cushing. In their classification of gliomas, these tumors were considered as reproducing the differentiated oligodendrocytes, these latter descending from "medulloblasts" as previously suggested by Hortega.

In their monography, Bailey and Cushing described the oligodendrogliomas as follows:

> A cellular tumor of peculiar type, in which fibrillary astrocytes occasionally occur, is sometime encountered. The neoplastic cells have spherical nuclei, with a heavy chromatin network surrounded by a ring of cytoplasm which stains very feebly by ordinary staining methods. Between the cells is an indefinite material which stain neither for neurofibrillae, neuroglia, nor for connective tissue. This material may give the growth somewhat the appearance of the cross-section of a plant. No mitotic figures can be found. These tumors are prone to be calcified.

The example illustrated in this monography corresponded to the solid tumor tissue of a "classical" oligodendroglioma. Following this earlier description, oligodendrogliomas will long been considered as a monomorphous tumor recognizable at first glance. However, 6 years later, thanks to a modified method of silver impregnation by del Rio Hortega, Bailey in collaboration with Bucy [23] could prove (on the basis of 9 cases) that these tumors are indeed oligodendroglial. Bailey nonetheless acknowledged that, even with this method, in most cases, only a few tumor cells stained positively. One may find, as anticipated in this chapter, the problems that will raise the individualization of oligo-astrocytoma and their distinction from "pure" oligodendroglioma. From the detailed description given the oligodendrogliomas in this study, one may retain that:

1. "Swollen tumor cells (tumor cells with a fried egg appearance) are present in every tumor but the number of these cells varies in the different case…they are, as a rule fewer in those specimens that were fixed immediately." Elvidge et al. [13] had previously described an "acute swelling" of the oligodendrocyte in acute degenerative lesion. Thus, their presence in these tumors provided an additional argument that they are oligodendroglial. However, Bailey indicated that typically "the cytoplasm is very indistinct, is finely granular and eosinophilic …."
2. The aspect of perineuronal satellitosis of the tumor cells in the cortex "near the tumor" was also emphasized; nonetheless this feature has long been considered a hallmark of diffuse astrocytomas.
3. "The vascularity of the tissue varies considerably, in a few cases an extensive thick network of capillaries is present … many specimen show a marked proliferation of the endothelial lining of the blood vessels."

4. "In the loose area that contains very few cells and is quite avascular, the tumor cells are not oligodendroglia but are typical astrocytes. It would seem that here we have the possibility of a mixed oligodendroglioma and astrocytoma."
5. "There are numerous cells in all theses tumors which it is practically impossible to classify. They have characteristics of both oligodendroglia and astrocytes… numerous other cells might be either unipolar spongioblast or unipolar oligodendroglia such as Hortega has depicted. The presence of all these transitional cells indicates clearly the close kinship of the oligodendroglia and astrocytes."

Until the 1950s, that is, during the period of research when the methods of metallic impregnation were still systematically used, many studies focused on the identification and origin of the transitional cells of oligodendroglioma. It was the subject of several projects, and they culminated with the works of Ravens et al. [24]. These authors, in the introduction of their paper "The cytology of oligodendrogliomas," stated that "some authors consider oligodendrogliomas relatively simple to recognize histologically. Yet when studied in greater detail it is found that there are many different types of oligodendrogliomas. Some of these tumors have been the subject of disagreement among neuropathologists, and have resulted in confusion when cited in the literature." Part of the "ambiguous cells" identified by these authors would now be described as minigemistocytes or gliofibrillary oligodendrocytes.

Oligo-astrocytomas

Oligo-astrocytomas were initially described by Cooper in 1935 [25]. *According to the description of this author* "the main characteristic of these tumors is the intermingling of oligocytes and astrocytes in a seemingly haphazard fashion. Neither type of cell predominates except in localised area, but whatever their relative proportions, each cell maintains its individual and reaction to stains. Hyalinisation of the astrocytes is invariably apparent. Like oligocytomas and astrocytomas, the mixed tumours are relatively avascular." As shown in this chapter, the individualization of these tumors has been purely arbitrary from the origin. Cooper claimed that these neoplasms "form a distinct group between the two extremes, the 'pure' astrocytomas and the pure oligodendrogliomas … In pure oligocytomas astrocytes are invariably present, their long processes extending between and around the oligocytes. In the pure astrocytomas, the main characteristic is the large size of the tumour cell and the extensive ramifications of its processes, the individual cells are more separated than those of oligocytomas owing to the process." He explained the existence of these tumors as follows: "At the moment, the writer is of the opinion that astrocytes can be transformed into oligocytes by the swelling or hyalinisation of the cytoplasm with consequent withdrawal of the processes."

The Sainte Anne's Hospital Classification

The classification of gliomas according to Sainte Anne's hospital was proposed following a study on stereotactic biopsies correlated with imaging [26].This work helped to define the spatial structure of gliomas (pure infiltrating, pure solid, or mixed gliomas) to specify the mode of growth of gliomas and to redefine the diagnostic criteria for oligodendrogliomas. The correlation between histology and imaging was used to study the representativeness of samples, the CT or MRI providing the macroscopy of the lesion.

Two distinct components can be observed in gliomas. On one hand, there is the solid tumor tissue that is formed only by tumor cells. This component is accompanied by a microangiogenesis, reflecting the contrast enhancement on imaging. On the other hand, there are isolated tumor cells, infiltrating a morphologically and functionally intact parenchyma, and therefore without neoformed vessels, or contrast enhancement on imaging. For example, pilocytic astrocytomas are composed entirely of tumor tissue, while glioblastoma is composed of tumor tissue and isolated tumor cells. Oligodendrogliomas and oligo-astrocytomas may also have a mixed

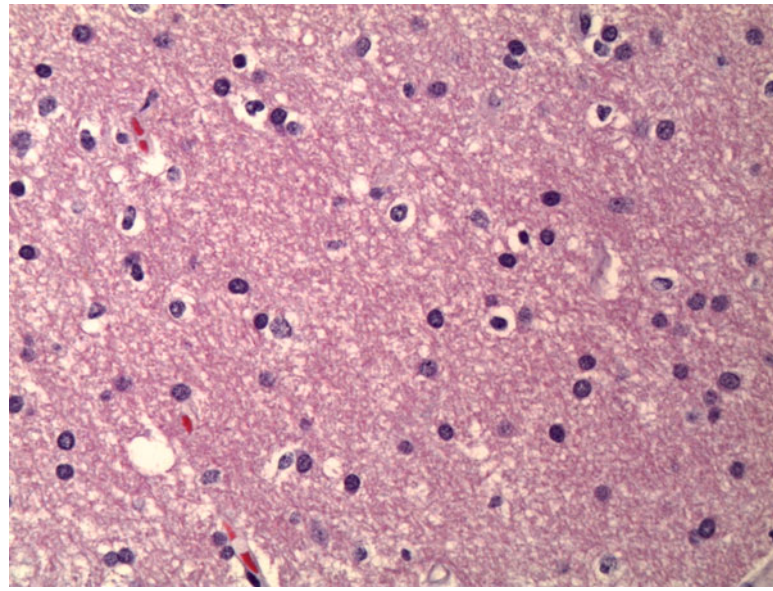

Fig. 3.2 Low-grade oligodendroglioma: diffusely infiltrating glioma composed of monomorphic cells with round nuclei and perinuclear halos

structure, but earlier in their evolution, they are most often consisted of isolated tumor cells.

Astrocytic gliomas are highly angiogenic, in contrast to oligodendrogliomas and oligo-astrocytomas that can grow for years in the form of isolated tumor cells without causing contrast enhancement. In oligodendrogliomas, angiogenesis is a late phenomenon characterizing the transition from grade A to grade B.

According to the classification of Sainte Anne's hospital, there are four categories among the infiltrating gliomas: oligodendrogliomas or oligo-astrocytomas grade A or B, glioblastomas, and malignant glioneuronal tumors (TGNM) [27]. Oligodendrogliomas may be pure infiltrating or mixed structure, solid, and infiltrative. The nuclear characteristic of oligodendrocytes is critical to their identification. They contain rounded nuclei with a net nuclear membrane and chromatin clumps giving them an appearance "in bud" (Fig. 3.2). These aspects are particularly evident on smears. In the purely infiltrating zone, diagnosis is sometimes difficult when the tumor is localized in the white matter, due to the induction of astrocytic gliosis immunoreactive with an anti-gliofilament (GFAP). This is the reason why these tumors are often interpreted, according to the WHO classification (see below), as grade II fibrillary astrocytoma or grade III anaplastic astrocytoma in the presence of significant mitotic activity. However, the diagnosis of oligodendroglioma becomes easier in the cortex where the tumor cells tend to cluster around neurons (perineuronal satellitosis).

Oligodendrogliomas of mixed structure (solid and infiltrative) are usually easy to diagnose when the component of solid tumor tissue has the appearance of a typical honeycomb, with clear halo, and perinuclear endocrine vascularization. In these forms, the calcifications are more frequent (and multinodular contrast enhancement can be often observed). The grading of oligodendrogliomas according to Sainte Anne's classification is mixed, both histological and neuroimaging based. Indeed, it is based on two criteria, that is, the endothelial cell hyperplasia and contrast enhancement in imaging. Two grades of malignancy are defined as:

- Grade A, characterized by the absence of endothelial hyperplasia and contrast enhancement (median age 11)
- Grade B which includes endothelial hyperplasia and/or contrast uptake (median survival of 3.5 years)

The endothelial hyperplasia or microangiogenesis is defined by the presence in at least one field of the microscope (objective 10) of vessels whose endothelial cells have nuclei that are touching.

The oligo-astrocytomas have the same characteristics as oligodendrogliomas on imaging. At histology, the oligodendroglial component, as defined above, joins a component of astrocyte gemistocytic appearance. The astrocytic component is thoroughly intertwined with the oligodendroglial component and does not form distinct foci.

Although the classification of the Sainte Anne hospital seems easier to apply with better reproducibility, its use has never gone beyond the borders.

The Sainte Anne hospital classification poses different problems [28]. By definition, it requires imaging and therefore it requires a change in the management of samples. It is not an insurmountable problem, but something to be acquired by neurosurgeons and pathologists can take time. For oligodendrogliomas, some obstacles appear. In some cases it may be difficult to distinguish grade A or grade B, due to the difficulty of assessing microangiogenesis on histology and absence of contrast enhancement on MRI (shots too early after gadolinium injection). These difficulties are the result of the microangiogenesis process which is a progressive phenomenon. It imposes standardized imaging procedures and caution in the interpretation of certain forms where microangiogenesis is *a minima* on histology with contrast enhancement still absent on MRI: it is typically a transition grade, from grade A to grade B.

The Sainte Anne hospital classification is ideally suited for hemispheric tumors, but it is more difficult to use for tumors of the brain stem. One reason could be the frequent poor quality and small size samples making it difficult to assess nuclear characterizations of oligodendrogliomas. Another reason could be a different embryological origin for some of these tumors. The Sainte Anne hospital classification is not recognized by the international scientific community. If the correspondence with the grading of WHO classification can easily be achieved in some cases (e.g., grade B oligodendrogliomas, oligodendrogliomas grade III on WHO classification), it is difficult or impossible in others (e.g., grade A oligodendroglioma vs. anaplastic astrocytoma if there are few mitoses). Finally, it is necessary to test its reproducibility between pathologists from different centers.

We should begin to think of gliomas in a different conceptual framework. Taken together, these data suggest that it is time to reconsider how the glioma classification should evolve in the next few years.

The Current Classification: The 2007 WHO Classification

The international classification of human tumors published by the World Health Organization (WHO) was initiated through a resolution of the World Health Assembly in 1957. Its goal was to establish a classification and grading of human tumors, which was accepted and used worldwide. Without clearly defined histopathological and clinical diagnostic criteria, epidemiological and clinical trials could not be conducted beyond institutional and national boundaries.

The most widely used current classification of human gliomas is that of the World Health Organization, revised in 2007 [29]. The 2007 WHO system divides still diffuse gliomas into astrocytic tumors, oligodendrogliomas, and oligo-astrocytomas. These are then graded into histological degrees of malignancy. Oligodendrogliomas and oligo-astrocytomas are divided into grade II and anaplastic grade III lesions. The astrocytomas include grade II, grade III, and grade IV lesions (with grade IV known as glioblastoma).

Of note, even today, in the case of anaplastic oligodendroglioma, for which existing treatment can be highly effective, histological examination does not provide a good method of distinguishing chemosensitive from chemoresistant tumors.

Thus, from the histological classification of Bailey and Cushing [1], the first edition of WHO classification edited by Zülch and published in 1979 [18] has inherited a definition of histologic categories based on the subjective notion of differentiation and intrinsic histopronostic value conferred to each histologic type. From the classification of Kernohan et al. [2], it took the histological value attributed to anaplastic

components. In this first classification, glioblastoma belonged to the group of poorly differentiated and embryonic tumors, coming from astrocytoma tumors or oligodendroglial tumors.

The second edition, edited by Kleihues et al., reflected the advances brought by the introduction of immunohistochemistry into the diagnostic pathology. The 1993 WHO classification [30] was a compromise between Bailey's classification and Kernohan's classification, the St Anne-Mayo histological astrocytoma grading [31] and Smith oligodendroglioma classification. The histological type always depends on the predominant cytological type. The grading from 1 to 4 depends on the cytological type (pilocytic grade 1), the presence of anaplasia, the differentiation, and some criteria which are not specific: mitosis and cellular density.

Glioblastoma rejoined the group of astrocytomas, and astrocytoma grade II was defined by the exclusive presence of nuclear atypia.

The third edition, edited by Kleihues and Cavenee, published in 2000 [32], incorporated genetic profiles, epidemiology, clinical symptoms, imaging, prognosis, and predictive factors. The 2000 WHO classification is a compromise between Bailey's classification and Kernohan's classification, the St Anne-Mayo histological astrocytoma grading, and Smith oligodendroglioma classification. In this new classification, we can find the data of the molecular biology and the cytogenetic. About grade II, the 2000 WHO classification tolerates one mitosis.

The classification of oligodendrogliomas and oligo-astrocytomas has evolved in parallel to that of astrocytomas. In 1979, these tumors were classified as grade II or grade III if they contain or not foci of anaplasia. Then in the 1993 classification, inspired by the grading of Smith, WHO introduced a set of very specific histological criteria to differentiate oligodendrogliomas versus oligo-astrocytomas (grade II) and anaplastic oligodendrogliomas versus oligo-astrocytomas (grade III). These criteria were preserved in the 2000 classification and also in the last one in 2007.

Paradoxically, these rare successes draw attention to the essential limitation of current glioma classification schemes. First, responding tumors may be histologically indistinguishable from nonresponding ones.

Specimens too tiny for exact diagnosis are encountered not only in cases of needle biopsies. Gliomas are heterogenous tumor by definition; samples for diagnosis need to be big enough to have a good assessment to the totality of the tumor. From the point of view of pathologists, the resection sample needs to be totally included in order not to underestimate the grade by forgetting anaplasia focus.

The classification of gliomas remains controversial, and some studies made with this third edition have shown that the inter- and the intraobserver reproducibility of the WHO system of typing and grading gliomas was unsatisfactory [33].

The 2007 WHO classification of tumors of the central nervous system is the fourth edition. It lists several new entities and histological variants. As in the previous edition, the classification is accompanied by clinicopathological characteristics of each tumor type.

The working group differentiated clinicopathological entities, variants of entities, and histological patterns. A new entity has to be characterized by distinctive morphology, location, age distribution, and biological behavior and not simply by an unusual histopathological pattern.

Concerning glioma, only a few new entities are identified in the new variants. Angiocentric glioma is a grade I glioma which occurs predominantly in children and young adults. The pilomyxoid astrocytoma is a glioma, and it is assigned as a grade II glioma, with a typical location in the hypothalamic/chiasmatic region. There is small cell glioblastoma and glioblastoma with oligodendroglial component. However, there is nothing more about low-grade glioma.

Neoplasms designated "grade II" are generally infiltrative in nature, and despite low-level proliferative activity, they often recur. Grade II tumors tend to progress to higher grades of malignancy, for example, low-grade diffuse astrocytomas that transform into anaplastic astrocytomas and glioblastomas. Similar transformation occurs in oligodendrogliomas and oligo-astrocytomas.

The designation of grade III is generally reserved for lesions with histological evidence of malignancy, including nuclear atypia and brisk mitotic activity.

A diffusely infiltrative astrocytic tumor with only cytological atypia alone is considered as grade II, tumor showing anaplasia and mitotic activity as grade III, and tumor additionally showing microvascular proliferation and/or necrosis as WHO grade IV.

The distinction between grade II and grade III glioma may be sometimes very difficult. In the WHO classification, the finding of a solitary mitosis in an ample specimen does not confer grade III behavior, but one mitosis in a stereotaxic biopsy is synonymous with grade III. The separation of grade II from grade III may be more reliable by determination of Ki-67 labeling indices, even if the immunohistochemistry is not detailed in the diagnostic criteria.

For the vascularization, the WHO classification accepts an apparent multilayering of endothelium and also a glomeruloid microvascular proliferation. Necrosis may be of any type and perinecrotic palisading need not be present.

Limitations of the WHO Classification

The third and the fourth classifications were based on the consensus of an international working group of 25 pathologists and geneticists and their collaboration with 50 other contributors. However, the WHO classification could be problematic. It is not reproducible, it does not make difference between tumoral cells and infiltrated residual parenchyma, and it takes in account neither the tumoral heterogeneity nor the clinical data and radiological data.

For instance, in a study focusing on stereotaxic biopsy, the observed diagnostic discordances between neuropathologist experts represent until 64 % for anaplastic astrocytomas. Moreover, similar discordances were observed when the same observer was asked to reinterpret the same histological preparations a few weeks later. In a study focusing on exercise samples, the interobserver discordance reached 48 % [33]. This non-reproducibility is due to the difficulty to distinguish the nature of tumoral cells (astrocyte vs. oligodendrocyte), mainly because of the absence of reliable staining available in immunohistochemistry recognizing tumoral oligodendrocyte. The problem is even greater for the mixed gliomas. However, the group of combined oligoastrocytic tumors is defined in a very subjective way. What percentage of each component is necessary to make the diagnosis?

It also rests on some elements which are too subjective concerning the grading, as the notion of anaplasia or cell density.

The WHO classification does not distinguish tumor cells from the residual brain parenchyma infiltrated by the tumor and considers the tumor as homogeneous. However, homogeneous tumor developed in a functional area should destroy it and would be accompanied by clinical deficit, which is not the case for most infiltrating low-grade gliomas. Although the WHO recognizes that gliomas are heterogeneous tumors, this data is not considered in the interpretation of samples. Thus, the validity of the diagnosis is entirely dependent on the representativeness of samples. But, deprived of the correlation with imaging, the pathologist cannot be certain of having benefited from a representative sample. The WHO classification does not include data from the clinical and imaging. In particular, it is applied equally to adults and children, while the tumors are different with benign tumors which predominate in the pediatric population (high frequency of pilocytic astrocytomas, gangliogliomas, or dysembryoplastic neuroepithelial tumors).

In summary, analysis of a brain tumor, its rank, and its grading remains difficult. In fact the brain is one of the few organs in which it is difficult to say if you are in the presence of tumor cells or reactive cells, due to the complexity of this tissue. It is not always easy to affirm whether this cell is either an oligodendrocyte or an astrocyte. Therefore, it is easily understandable that there is a low reproducibility between several pathologists in the analysis of tumors.

This is also the dilemma for tumor/reaction or for astrocyte/oligodendrocyte;

immunohistochemistry does not help us despite all the hopes placed on it. The WHO classification which is internationally recognized does not use the same criteria for astrocytomas and for oligodendrogliomas. Mitoses are tolerated up to five in oligodendrogliomas, while two mitoses sign astrocytoma anaplasia. The presence of endothelial hyperplasia passed a low-grade oligodendroglioma to an anaplastic oligodendroglioma, while in astrocytomas the presence of endothelial hyperplasia means a grade IV. Similarly, the presence of necrosis means that the tumor should be quoted grade III oligodendroglioma, whereas astrocytomas should be quoted grade IV. This could possibly be explained by the fact that there is no grade IV oligodendrogliomas, but then what about oligo-astrocytomas: must the neuropathologist speak about oligoastrocytoma grade III with necrosis or glioblastoma with oligodendroglial component? This classification, despite our good intentions, often leaves us, pathologists, at a standoff. The classifications bring only what they can, and thus, they remain imperfect and unsatisfactory. This is the reason why histology alone is insufficient for the interpretation of these tumors: the clinical and imaging should be considered and discussed again by the pathologist, in addition to histological data, in multidisciplinary meeting.

Microfoci of Increased Cell Density and Angiogenesis: Toward an "Intermediate" Grade

In our experience, in about 30 % of cases, it is found heterogeneous households on a background of diffuse low-grade glioma. In all cases, these heterogeneous households are synonymous with an increase of cell density, which appears to be an early marker of these foci. The presence of cytonuclear atypia is more variable. These foci are either small (less than 1 cm) and are called microfocus or near the size of a centimeter and are called macrofocus. In microfoci, associated with an increased cell density (Fig. 3.3), we can find cytonuclear atypical more pronounced than what we are supposed to find in low grade. In the macrofoci, vascular hyperplasia with endothelial hyperplasia can be found associated with hypercellularity. These foci are either unique or multiple often located in the center of the resected specimen. The criteria are insufficient to speak of gliomas WHO grade III. The diagnosis of diffuse low-grade glioma is made, but the presence of these foci is stated in the neuropathological report.

Immunohistochemical study is usually performed on the block with these foci and compared with immunohistochemical study on blocks with uniform grade II. It is not uncommon to see

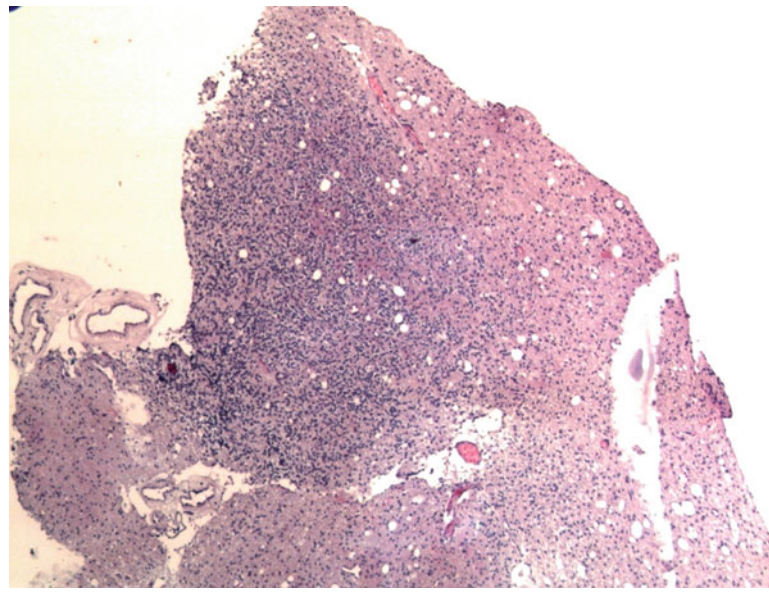

Fig. 3.3 Microfoci with an increased cell density

these foci with a higher Ki-67. It is now established that the grade II gliomas are precancerous lesions that make the bed of grade III, as already established in other organs. The main limitation of the WHO classification is to want to quote each case in a grade II or grade III box. It seems logical and obvious that there is a continuum between the boxes, so the intermediate-term glioma should be used in the future. The presence of these foci of increased cell density may correspond to a grade II becoming more aggressive. It is interesting to note that some move more toward the anaplasia (atypia) while others more toward neoangiogenesis.

These cases are monitored more closely by MRI, to calculate very accurately the kinetics of the tumor (see Chaps. 17 and 18. Interestingly, the presence of foci of anaplasia has also been suspected by some teams of nuclear medicine by using special techniques (amino acid methionine positron emission tomography with fluoroethyl or tyrosine).

As a consequence, it is time to introduce in the WHO classification the term of "intermediate diffuse glioma" for those cases in which the presence of these foci may lead to a faster evolution toward anaplasia.

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Is Gliomatosis Cerebri a Diffuse Low-Grade Glioma?

4

Catherine Godfraind

Abstract

Before addressing the issue about whether gliomatosis cerebri (GC) belongs to the group of diffuse low-grade gliomas (DLGG), I would like to examine what is known about GC, and in particular the similarities and differences between them and DLGG. In 1897, Rossolimo was the first to report the lesion that later Nevin labeled "gliomatosis cerebri." In the most recent WHO classification, GC is considered as an extensively infiltrating glioma of astrocytic differentiation. Diagnosis of GC is challenging, not only because its clinical and radiological symptoms are frequently nonspecific, but also because GC tends to mimic other neurological conditions. GC shares with DLGG glial cell lineage, epidemiology, and some radiological features. In a subset of GC, as indeed of DLGG, *IDH1* mutation was recognized as an early genetic alteration. Identification of this mutation made it clear that GC constitutes a heterogeneous group of tumors. In addition, this mutation establishes a genetic link between GC and DLGG. On the other hand, on a clinical basis, these two entities differ considerably for their infiltrative behavior. It is more likely that there are yet unidentified factors, not shared between GC and DLGG, that would favor the former to be more invasive, and the latter to be more expanding locally. The candidate role of *VEGF* and of *HGF/c-Met* pathway in tumor invasion is discussed. We also took into due consideration how *IDH1* mutation may protect tumor cells from anoxia. Finally, there appears to be clinical and radiological evidence showing that both GC and DLGG evolve from a less malignant tumor at the time of first diagnosis to a more malignant neoplasm, which is the concept of low-grade tumors. Although many issues still deserve clarification, and evidence is lacking for others, we suggest that GC be included in the group of DLGG.

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History

Grigory Ivanovich Rossolimo (1860–1928) is a Russian neurologist, contemporary to Bekhterev and Pavlov who created the first European institution dedicated to child neurology and psychology. He also developed techniques to minimize brain lesions during surgical interventions, ending his career as professor of neuropathology at the University of Moscow [1, 2]. In 1897, he described as a "multiple sclerose und gliose" a diffuse lesion of the brain, labeled in 1938 by Nevin, "gliomatosis cerebri" (GC) [3, 4]. His understanding of the physiopathology of this diffuse lesion was shared by some colleagues, whereas others considered it the result of a "blastomatous malformation", that is, a tumoral proliferation arising from multiple embryonic rests [3]. Until the identification of *IDH1* mutation in GC, the pathogenesis of this lesion remained the object of debate; however, knowing these early interpretations provides an explanation of some of the names found in the literature, such as "glioblastosis diffusa cerebri, diffuse glioblastosis cerebri, and glioblastomatosis." Other names are related to morphological aspects and include "central diffuse schwannosis, gliomatous hypertrophy," or later "diffuse cerebral astrocytoma" [5]. Interestingly, in all the initial reports, and namely in the one by Scheinker and Evans, important anatomical features of GC were already established: the enlargement of the affected regions, the absence of demarcation between normal and tumoral parenchyma, and the relative preservation of the underlining anatomical structures [3].

In 1979, the World Health Organization (WHO) classification of tumors of the central nervous system recognized GC as a separate tumor entity within the group of undifferentiated and embryonic tumors [6]. In 1993, WHO moved GC to the group of neuroepithelial tumors, among the "tumor of uncertain origin." Finally, in 2007, GC was included among "astrocytic tumors" [7]. Such changes testify of the poor knowledge of this rare lesion that was still reported, at the beginning of this millennium, as difficult to diagnose without postmortem examination, and in 2011 as a "diagnostic challenge" [8]. Indeed, the diffusely infiltrative component of the tumor, essential for its diagnosis, was tricky to assess *intravitam* before the advances in neuroradiological techniques. In addition, the clinical symptoms, frequently nonspecific, account for its arduous clinical assessment.

To date, the natural history of GC is better understood. The recent identification of *IDH1* mutation, as an early genetic anomaly occurring in the development of a subset of GC, allows us to recognize that GC constitutes a heterogeneous group of tumors, as well as to grasp the discrepancies found in the literature regarding them. Finally, one can now consider "gliomatosis cerebri" as a label applied to a group of tumor defined by the World Health Organization, and also a term suggestive of an infiltrative behavior developed following an earlier diagnosis of diffuse glioma.

Definition

Gliomatosis cerebri has been divided into primary and secondary. The former corresponds to the tumor defined by the WHO classification. The latter refers to a highly infiltrative tumor behavior developed on the setting of an otherwise diffuse glioma.

Primary Gliomatosis Cerebri

According to the latest WHO classification, GC is an extensively infiltrative glioma involving at least three contiguous cerebral lobes [7]. This definition requires defining the nature of the lesion and its extension, in order to assess the diagnosis of GC. This can be achieved either by a postmortem

investigation or *intravitam* by combining histological and radiological examination. It was only in 1987 that the first antemortem diagnosis of GC was achieved using this latter approach [9].

Primary GC is further subdivided into type I and type II [10]. Type I corresponds to a diffusely infiltrating tumor devoid of any circumscribed tumor mass at the time of the diagnosis. In type 2, on the contrary, a tumor mass is present. We and others, observe evolution of GC from type I to type II [11–13].

The glial cell constituting GC has similar differentiation as in DLGG. Astrocyte is the most frequent cell type encounter, accounting for 36 % of the GC cases in the largest series. This explains why WHO classified GC among astrocytic tumor. However, GC composed of cell with an oligodendroglial or an oligoastrocytic differentiation has also been described, in 18 and 6 % of the cases, respectively [14–20]. Finally, in 40 % of the reviewed GC, the cell type was either not mentioned, or was of undetermined nature [14]. In the author's lab, this undetermined group is not considered, and GC is labeled either as having an astrocytic, an oligodendroglial, or an oligoastrocytic differentiation.

Like DLGG, GC most frequently involves the cerebral hemispheres (76 %) [10]. Although, GC differs from diffuse gliomas by its extensively infiltrative behavior with a frequent bilateral extension. The deep gray nuclei are affected in up to 43 % of the cases [10]. Infratentorial extension to the brain stem (52 %) and cerebellum (29 %) is common [10]. Less often, the tumor spreads to the spinal cord (<10 %) [10, 21]. Interestingly, leptomeningeal involvement occurs during the evolution of GC in up to 17 % of the cases [10, 22, 23]. A similar evolution was also reported in diffuse glioma where it was detected most frequently microscopically at post-mortem (27 % of the studied cases [24]) than clinically suspected or diagnosed (2–7 % [25, 26]). Still more rare, is the infiltration of cranial nerves that we also observed in an unusual case of fetal GC [27–29].

Secondary Gliomatosis Cerebri

Secondary GC corresponds to a highly infiltrative behavior observed during the evolution of any primary diffuse glioma of any WHO grade. We suggest to include in this group also the extremely infiltrative outcome observed in about 30 % of glioblastomas treated with bevacizumab, as anti-angiogenic molecule [30–32].

Epidemiology

GC is a much rarer tumor than DLGG. It is not mentioned by the Central Brain Tumor Registry of the United States (http://www.cbtrus.org/). By the beginning of this millennium, around 200 cases were reported, and to date, about 300 are recorded [33]. Among them, approximately one quarter is pediatric, GC being reported also in the newborn [34]. The peak of incidence is slightly older than that of diffuse astrocytomas, ranging between 40 and 50 years [7, 23] with a younger age at onset for male compared to female (median of 39 years versus 45, respectively) [14]. The elderly are also affected [35] with the oldest reported patient being 85 years old [36]. As for most primary brain tumors, there is a slightly male prevalence with a sex ratio of 1.31 [14].

Clinical Presentation

A certain delay may occur between first symptoms and diagnosis of GC. This partly owns to the lack of specificity of the presentation. The most frequent symptoms encountered, according to the literature, include corticospinal tract deficits (58 %), behavioral changes or frank dementia (44 %), headache (39 %), seizures (38 %), cranial nerve deficits (37 %), increased intracranial pressure (34 %), and spinocerebellar signs (33 %) [10].

Among adults, we observed a correlation with the mutational status for *IDH1*. Indeed, *IDH1*-mutated tumors are more frequently associated with epilepsy, while the non-mutated ones are more commonly linked to neurological deficits [37]. In diffuse gliomas, a similar link between epilepsia and *IDH1-2* mutations has also been reported [38]. In children, seizures occur more often as the initial symptom than in adult [10, 34, 39, 40].

Finally, delays in diagnosis may also happen because GC can mimic other neurological diseases. Indeed, some were initially diagnoses as CNS inflammation including acute disseminated encephalomyelitis [41–43], cranial mononeuritis multiplex [44], neuro-Behçet [45], Rasmussen [46], paraneoplastic limbic encephalitis [47], and infectious lesions [48]. Others were incorrectly considered as vascular pathologies [49–52]. Depression [53], pseudotumor cerebri [54], or neurodegenerative diseases including Creutzfeldt-Jakob disease [53], multisystemic atrophy [36], or Parkinson's disease [55] were also erroneously diagnosed. Chemotherapy-induced leukoencephalopathy [56] and hemimegalencephaly were also given as the initial diagnosis [40].

Association

Disease associations can sometimes generate additional clinical challenge. This is exemplified by the reported case of a male patient diagnosed with multiple sclerosis [57]. Six years later, he developed personality changes, a rare symptom in MS but classical for GC. The RMI changes were not conclusive. Only another three years later, the diagnosis of GC was made when a tumor mass became obvious. Other clinical situations are less puzzling as when GC is associated with cutaneous lesions including multiple pilomatricomas [58], epidermal nevus syndrome [59], or pseudohermaphroditism [34]. In two genetically inherited disorders, the constitutional mismatch repair-deficiency disorder (CMMR-D) and neurofibromatosis, GC has been observed [60–63]. Interestingly to notice is the fact that these syndromes also shared a cutaneous manifestation: *café au lait* macules. Finally, associations with other tumors have been reported: breast cancer [64], pituitary adenoma [65], hematologic malignancies [56, 66], and Ollier disease [67].

Radiology

Radiological features of GC are also relatively nonspecific, accounting for the possible delay in diagnosis [68]. They include anatomical patterns reflecting the tumoral infiltration of the brain parenchyma. Among them, the lesion should be widespread, as already stated. It is frequently bilateral and asymmetrical, but symmetrical involvement of the white matter has been observed and should not rule out the diagnosis (Fig. 4.3b) [8, 69]. Enlargement of the anatomical structures occurs, resulting sometimes in a slight asymmetry of the lateral ventricles [14]. Finally, the anatomical structures should be preserved.

Cranial CT scan is often of modest help to demonstrate these features. Subtle clues may be observed, such as disappearance of the border between gray and white matter, asymmetrical hypodensities, edema, and asymmetry of the lateral ventricles. Unfortunately CT scan may appear normal at times [33].

MRI is more useful, and at the same time it may help in the choice of the target for biopsy (Fig. 4.1). GC appears as infiltrative lesions that are iso-intense or hypo-intense on T1-weighted sequences and hyperintense on T2-weighted and FLAIR sequences. With the two latter sequences, the respect of the underlining anatomical structures can be fully appreciated (Fig. 4.3b). Mass or uptake of gadolinium on T1-weighted images is not seen in case of GC type 1 but well in type 2.

In MR spectroscopy, uptake of myoinositol, a marker of glial cells, is commonly reported as increased [40, 70, 71]. It is considered as specific of GC, if associated to the absence of a pick of choline [71]. Choline is a marker of the membrane metabolism and thus of the cell proliferation. For others, a pick of choline is a classical finding [40, 70, 72, 73]. In our opinion, such apparent discrepancy only reflects change in density of tumor cell from case to case, as would the variation in level of the of *N*-acetyl-aspartate decrease, a marker of neuronal cell integrity [40, 70]. Finally, lactate, a marker of anaerobic metabolism, has been reported as increased [72].

Histology

Macroscopic Examination

As for DLGG, the gross anatomical structure of the brain is frequently well preserved. However, the parenchyma may appear focally swollen and firm with some blurring of the boundaries

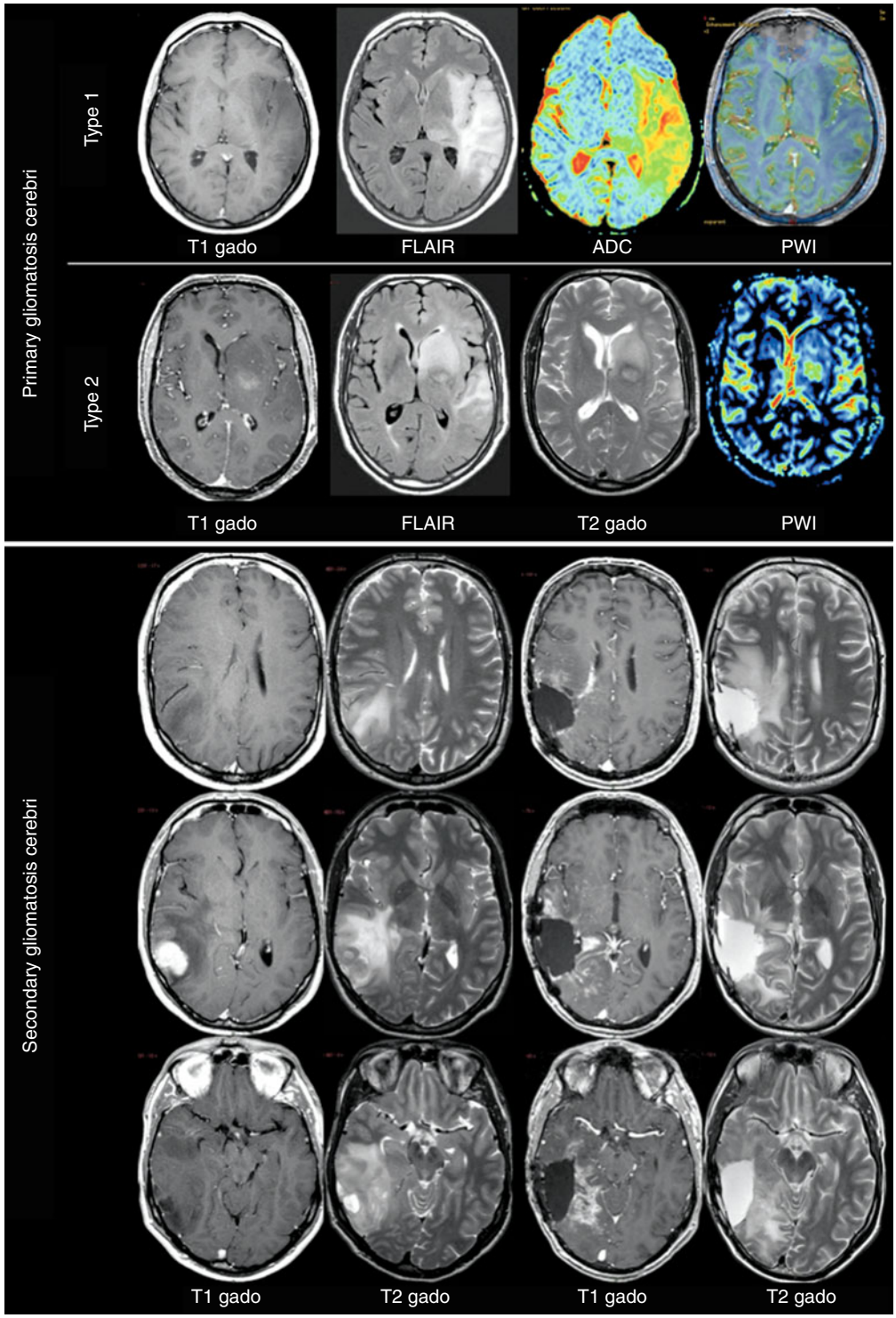

Fig. 4.1 Radiological aspects of GC. Primary type 1 GC: RMI: hypo-intense T1-weighted + gadolinium; hyperintense FLAIR; increase of apparent diffusion coefficient (ADC); low perfusion-weighted imaging (PWI). Primary type 2 GC: hypo-intense with a contrast-enhancing mass T1-weighted + gadolinium; hyperintense FLAIR; contrast-enhancing mass T2-weighted + gadolinium; high perfusion-weighted imaging of the tumor mass (PWI). Secondary GC: *two left panels*: diffuse anaplastic astrocytoma; *two right panels*: postsurgical evolution; gliomatosis cerebri aspect (Courtesy of Pr. T. Duprez, UCL, Belgium)

between gray and white matter. The limits of tumor infiltration cannot be appreciated at gross examination. In type 2, the tumor may appear as well defined.

Microscopic Examination (Fig. 4.2)

A wide variety of histological aspects are seen. Various patterns of tumor cell infiltration occur, varying from highly cellular regions to areas so lightly infiltrated that the diagnosis of tumor can barely be done using only H&E-stained sections. Tumor cells most frequently presented scanty cytoplasm that makes them appear as naked nuclei. On occasion, the cytoplasm may appear eosinophilic or as a clear perinuclear halo. The degree of nuclear atypia also varies, ranging from normal-looking nuclei to highly atypical ones. Frequently, the naked nuclei appear elongated or triangular. Mitosis can be seen, particularly in the most cellular areas, whereas necrosis and vascular proliferation are generally absent at the onset, but may appear during evolution, or be seen in case of GC type 2. Infiltration of gray matter is associated with formation of secondary structures of Scherer: tumor cells accumulated along blood vessels, around neurons, or in the subpial region.

Immunohistochemistry (IHC)

As in DLGG, IHC is useful to confirm the tumor nature of the process and also to identify its type of cellular differentiation. For this purpose, we are using a panel of antibodies direct against vimentin, GFAP, p53, R132H-*IDH1*, alpha-internexin, and a proliferative marker, such as KI67. Whenever required, we also perform FISH to search for 1p19q-codeletion and sequencing to look for minor mutation of *IDH1-2*.

In GC with astrocytic differentiation, immunostaining for vimentin and GFAP is observed, although the results are difficult to interpret in samples of low cellularity. In this situation, positivity for R132H-*IDH1*, p53 and/or KI67 may be a major help for the diagnostic.

In case of oligodendroglial differentiation, R132H-*IDH1* is generally positive, whereas negative results are obtained with vimentin and, in most cases, with GFAP. As for oligodendroglioma, p53 may discretely label a small fraction of tumor cells. Alpha-internexin can be positive, as it is a surrogate of the 1p19q-codeletion [74]. In case of negative results with alpha-internexin, a molecular-based technique as FISH is recommended to search for 1p19q-codeletion.

Grade

WHO classification defines GC as a grade III lesion [7]. However, in the litterature, GC is frequently graded based on the sample analyzed, and according to the WHO grading scheme of diffuse glioma, varying from II to IV. It is not infrequent that different regions within the same neoplasm are reported with different grades, thus reflecting the heterogeneity of GC. The ICD-O code is 938 1/3.

Genetics

Most of the genetic studies question the clonality, or not, of GC [75–78] and its relationship to diffuse glioma. To tackle the latter question, genetic anomalies associated with diffuse glioma (*TP53* mutation) and its progression (10q deletion, *PTEN* mutation and CDKN2A pathway alteration), as genetic characteristics of primary glioblastoma (*EGFR* amplification, 10q deletion, *PTEN* mutation) were looked for in GC [12, 35, 69, 77–80]. As results varied according to the series, a consensus about GC being clonal or not, or being part of diffuse glioma could not be reached. But, generally the current opinion considers GC being a clonal lesion with heterogeneous secondary genetic changes related to tumor progression, and being part of diffuse glioma.

A major improvement in understanding this apparent genetic inconsistency, and in understanding the relationship between GC and DLGG, came from the identification of *IDH1* mutations in GC [37, 69, 81–83]. These mutations were observed in both type1 and type 2 GC [37]. As in DLGG, part of the *IDH1*-mutated GC also harbors a 1p19q-codeletion [37, 69, 83], alpha-internexin

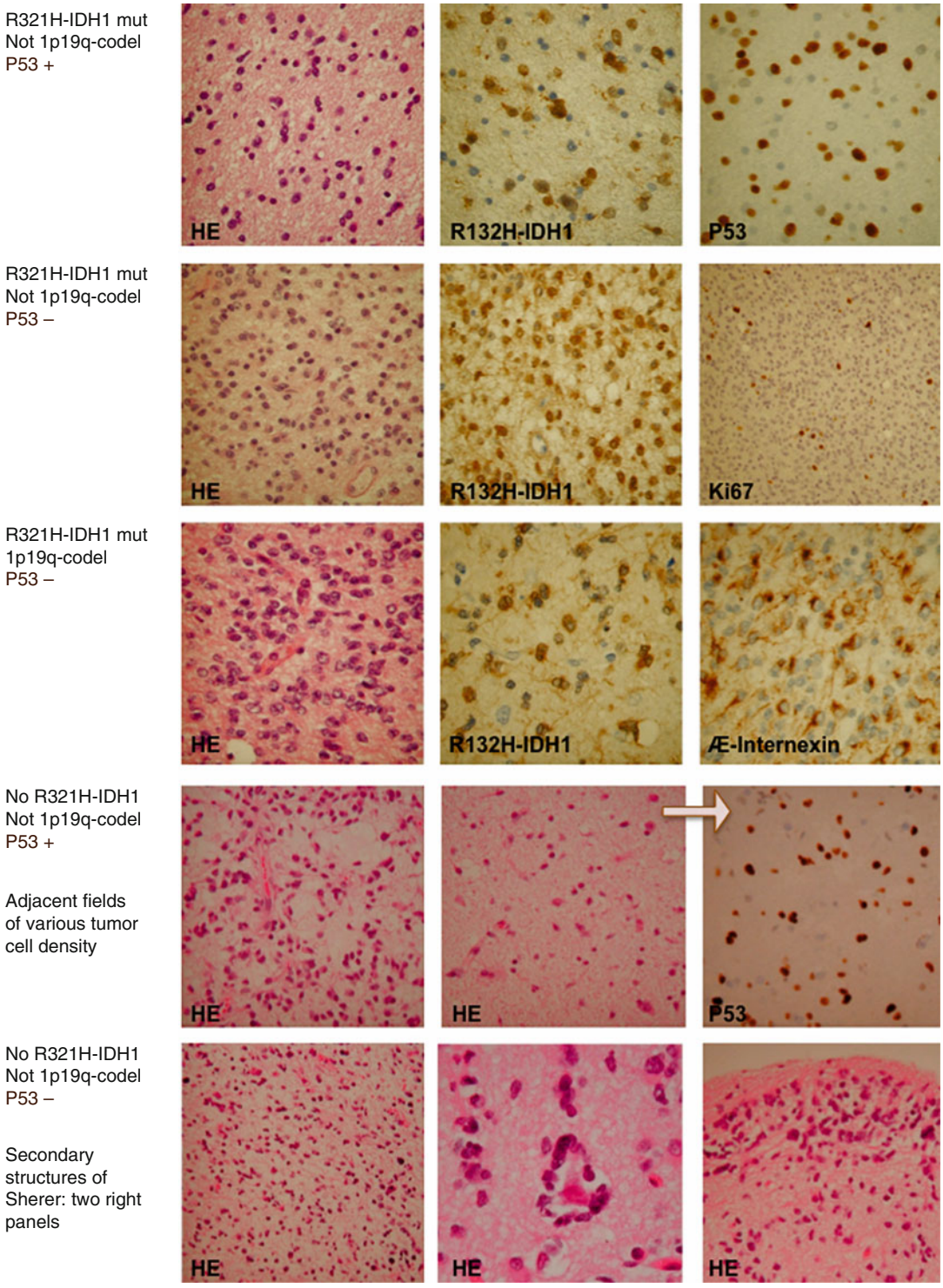

Fig. 4.2 Histological aspects of the five genetic patterns of GC

being a surrogate for this codeletion [37, 83]. In our series, as in DLGG, 1p19q-codeletion and nuclear accumulation of p53 are mutually exclusive [37]. *IDH1*-mutated but not-1p19q-codeleted GC, as not-*IDH1*-mutated GC could additionally demonstrate nuclear accumulation of p53 or not [37].

Finally, similarly to DLGG, by combining these various genetic anomalies (*IDH1* mutation +/–, 1p19q-codeletion +/–, p53 nuclear accumulation +/–), five different genetic patterns could be attributed to GC, as illustrated in Fig. 4.2.

Mutations of *IDH1-2* in DLGG, in acute myeloid leukemia (AML), and in enchondromas are associated with accumulation of the dextrogyre form of 2-hydroxyglutarate (2-D-HG) [84] and with hypermethylation of the tumoral genome [85, 86]. This has not yet been described for *IDH1*-mutated GC but is more likely to occur. As for DLGG, promoter methylation of *MGMT* has been identified in GC [69, 81]. In both tumor entities, the *MGMT* status does not correlate with the presence, or the absence, of an *IDH1-2* mutation [69].

Finding DLGG and GC frequently mutated for *IDH1-2* is an interesting observation. Indeed, as *IDH*-mutations are heterozygote, it implies that one of the alleles keeps its regular function. In a hypoxic situation, which is the one encountered by tumor cells of DLGG and GC, glucose is directed away from the tricarboxylic acid cycle (TCA). Therefore, citrate, the main energetic source of cancer cells, theoretically fails to be produced. However, in such hypoxic conditions, *IDH1* and *IDH2* are also able to reverse their normal function [87, 88]. Instead of transforming isocitrate to æ-ketoglutarate, they can use glutamine-derived-æ-ketoglutarate to produce isocitrate and from there, citrate. This gives cancer cells the opportunity to constitute their reservoir of energy.

At the same time, the mutated allele, thanks to its acquired new function, will lead to the accumulation of 2-D-HG [84] and from there, to HIF1æ and VEGF [89]. GC is not using this opportunity to form new vessels contrary to DLGG. Instead, it continues to invade. *HIF1æ*, a well-documented transcription factor that increases the expression of invasion-related genes, may be more beneficial to GC than to DLGG [90]. Also in GC, no alteration in *CDKN2A* has been demonstrated [79], and in glioma cell motility has been associated to a reduced proliferative activity [91].

A way to understand this behavior dissimilarity in between GC and DLGG is to propose that, beyond *IDH1-2* mutation and its subsequent 2-D-HG accumulation, there are additional anomalies that are needed for tumorigenesis. These anomalies could also favor either proliferation/angiogenesis or invasion. Biology illustrates this "beyond" view. 2-Hydroxyglutaric aciduria is an autosomal recessive disorder associated with 2-HG accumulation (OMIM #236792, #600721, #613657). Patients affected by this disorder are prone to develop brain tumors, namely, glioblastoma. But this only happens when the levogyre form of 2-HD (2-L-HG) is accumulated, and not the dextrogyre one (2-D-HG), which is the one detected in *IDH*-mutated tumors and considered as oncometabolite [84]. Also the pattern of *IDH1-2* mutation varies according to cancer type. R132H-*IDH1* is mostly observed in gliomas [84]. R132C-*IDH1* is the main mutation for enchondroma and fusiform hemangioendothelioma [86]. R140-*IDH2* is the principal mutation in acute myeloid leukemia [92]. In addition, the frequency of *IDH1* mutation is lower in GC (41 % out of a total of 186 GC tested in the literature) than in DLGG (70 %). The pattern of *IDH1* mutation is dissimilar too: in GC, all the reported *IDH1-2* mutations are R132H-*IDH1* at the exception of one, a R132S [81]; on the other hand, in DLGG, 90 % of the mutations happen to be R132H, while the 10 % remaining correspond to the other R132-mutations and to *IDH2* mutations [84].

All this suggests that beyond *IDH1-2* mutations, there is a "specific element" that favors a type of *IDH*-mutation. This element would also be needed to potentiate the tumorigenic effect of 2-D-HG accumulation. Finally, it could also account for tumor behavior differences.

Prognosis

Prognosis of GC is generally poor. On meta-analysis, median overall survival is 14.5 months, men having a longer survival than women (17 and 11.5 months, respectively [14]). Few patients were reported with long survival of 20 and 22 years [93, 94].

Previously suggested markers of prognosis include low-grade histology [14], patient age

[14, 95], Karnofsky Performance Scale higher than 80 [96, 97], and lack of contrast enhancement [96, 97]. Interestingly also, asymmetrical type of infiltration appears of better prognosis than diffuse bilateral symmetric pattern (Fig. 4.3b [69]).

Today, as for DLGG, *IDH1*-mutation and methylation of *MGMT* promoter region emerge as the prevailing prognostic markers of longer overall survival and progression-free survival [69, 83]. By extracting the data from three published series [37, 81, 83], we were able to reanalyze a total of 129 GC. We could confirm *IDH1* mutation to be associated with a statistically significant longer survival (log rank alpha = 0.00002, Fig. 4.3a).

Treatment

Because of the highly infiltrative character of GC, surgery is restricted to debulking procedures and/or to biopsies. On meta-analysis from the ANOCEF database, radiotherapy has been demonstrated to improve quality of life but not the overall survival [14]. Today, radiotherapy is favored as a salvage treatment, since irradiation

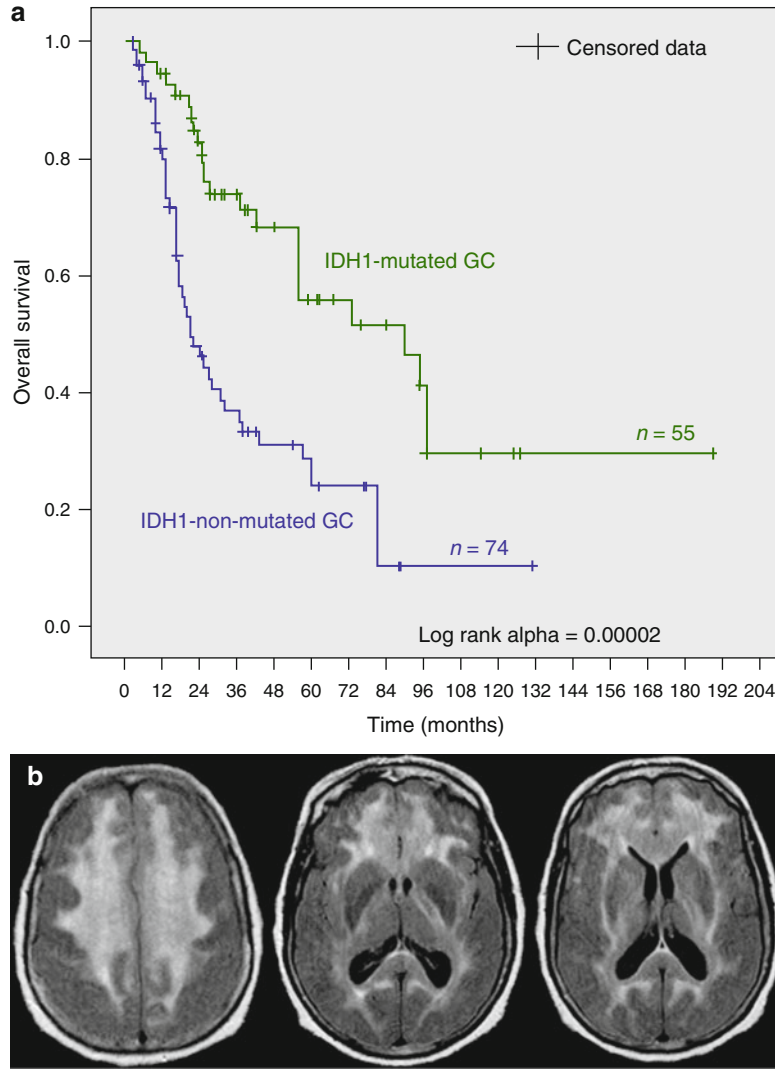

Fig. 4.3 Prognostic factors of GC. (**a**) Kaplan-Meier curve from data extracted from the literature: overall survival for GC with and without *IDH1* mutation (Courtesy of Dr. Narasimhaiah, UCL, Belgium). (**b**) Symmetrical infiltration of a GC, worse prognosis than asymmetrical one (Courtesy of Dr. Taipa, Hospital Santo Antonio, Portugal)

of a large target volume with 45 Gy is not exempt from neurotoxicity. In addition, up-front chemotherapies have demonstrated their value [14].

Among the various chemotherapy regimens attempted, the most frequently used were PCV (procarbazine, CCNU, vincristine) and, most recently, temozolomide. Retrospective analyses demonstrated these regimens to increase overall survival [11, 98]. Similar results were observed in the only published prospective multicentric trial in which procarbazine and lomustine were used [69]. The overall survival increases to a mean of 24 months against 11 months for patients treated with radiotherapy alone [69]. A retrospective study identified combined immunohistochemistry positive staining for the R132H-*IDH1* mutant protein and for alpha-internexin to be strongly associated with response to chemotherapy [83].

Conclusion

Whereas diffuse low-grade gliomas (DLGG) represent a relatively homogeneous group of neoplasms, the issue of whether GC belongs to the same entity requires the analysis of the similarities and differences which have been considered above, and that will be discussed in this section.

Traditionally, tumors are classified according to their putative cell of origin. DLGG and GC consist of glial cell presenting similar degrees of differentiation. Therefore, GC and DLGG should be grouped under the same entity, glioma. However, since Nevin introduced GC its position with respect to DLGG was debated mainly because of its propensity to be extremely widespread.

At the turn of the millennium, a group emphasized the value of genetic evaluation as an essential tool to classify tumors [99]. To date, a subset of DLGG and a subset of GC appear to share mutations in a gene family, *IDH1* and *IDH2,* that have been implicated in early step of the glioma tumorigenesis [100]. This would be a further reason why we should group GC and DLGG.

However, 2-D-HG, the product accumulated in *IDH*-mutated cells, does not appear per se to be tumorigenic in all biological condition, therefore questioning its quality of oncometabolite. The example of 2-hydroxyglutaric aciduria seems to clarify this point: this is a metabolic disease which is associated with the accumulation of either 2-D-HG or 2-L-HG. However, only when the levogyre form (2-L-HG) is accumulated, the patient is prone to develop cancer including glioblastoma. This compels us to suggest that there is a need for further abnormalities, in addition to *IDH*-mutations, to trigger the tumorigenic effect of 2-D-HG. Furthermore, observation of various patterns of *IDH1-2* mutations in cancers, including GC and DLGG as discussed in the genetic section, strengthens the hint of an additional mechanism, cancer-type related, that could influence the type of *IDH*-mutation, but also other features of the cancer such as invasion.

Adverse effect of treatment has brought upon us a human model of secondary GC. A number of the glioblastomas treated with an anti-VEGF molecule, bevacizumab, develop an extensively infiltrative behavior associated with clinical features reminiscent of GC [30–32]. This prompted us to go into deeper evaluation of this phenomenon, as tumor invasion constitutes the main clinical difference between GC and DLGG. A search in PubMed, for the words “glioma, invasion, and VEGF”, resulted in 78 records. Interestingly, they reveal HGF, its receptor c-Met, and VEGRF2 as candidate molecules associating VEGF and glioma invasion [101, 102]. STRING, a database dedicated to the analysis of known and predicted protein interactions, demonstrated a direct link between these four molecules, with combined scores varying between 0.961 and 0.999 (www.string-db.org).

VEGF production has been detected in GC [103], as in cell cultures from gliomas of different WHO grades [104]. It plays a role in glial cell proliferation [105]. Interestingly, when glial cells stop growing, they start to migrate [91, 106, 107]. In culture, the relation between VEGF and glial cell proliferation is dose dependent [105]. In tumors, this relation may

be more complex. For example, VEGF also upregulates CXCR4 [108], a chemokine involved in glioma invasion [109]. Therefore, in the reality of a glioma, the relationship between VEGF level and proliferation/migration is probably not binary, but influenced by other molecules that may also account for differences in behavior between GC and DLGG.

HGF and its receptor, c-Met, are frequently co-expressed in malignant gliomas. The status of this tyrosine kinase receptor and its ligand is not known in GC, a gap that would be of interest to fill. HGF is a multifunctional growth factor playing a role not only in glioma invasion but also in its growth and angiogenesis [110]. Its invading role seems to be linked to MMPs [110, 111]. But again, the relationship between c-Met activation and glioma invasion does not appear to be linear. To illustrate this point, *PTEN* loss, a frequent genetic alteration in glioblastoma, intensifies c-Met-induced malignancy in these tumors [112]. *PTEN* alterations are uncommon in GC [12, 35, 78], thus showing again that further genetic alterations may differ between GC and DLGG.

At this point, one may feel lost, not knowing anymore if GC resembles or not to DLGG. Indeed, GC and DLGG share various features but also differ by others. The same would have occurred, if we had completed the similar analysis in between astrocytoma and oligodendroglioma that are both DLGG. Astrocytoma and oligodendroglioma do not share their cell type, but a cell lineage, which is the same for GC: the glial cell lineage. Astrocytoma and oligodendroglioma partly share part of genetic anomalies, with *IDH1-2* mutations being the common denominator for some of them, as for a subset of GC. Nonetheless astrocytoma and oligodendroglioma differ for other genetic anomalies, as for example, 1p19q codeletion or p53 mutation, which by the way are observed in some GC.

In conclusion, GC presents genetic similarities with DLGG that are in keeping with glioma. Meanwhile, future identification of anomalies specifically associated to GC but not to astrocytoma and oligodendroglioma, could be anticipated, as already discussed. Astrocytoma and oligodendroglioma share an invasive quality, meanwhile much less extensive than in GC. But mostly, astrocytoma and oligodendroglioma share with GC clinicopathological evolution from a less malignant tumor at the time of the diagnosis to a more malignant one at the time of the patient death, which is the concept of low-grade tumors. Although many issues still deserve clarification, and evidence is lacking for others, we suggest that GC be included in the group of DLGG.

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Contribution of Molecular Biology to the Classification of Low-Grade Diffuse Glioma

5

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Abstract

Low-grade diffuse gliomas WHO grade II (diffuse astrocytoma, oligoastrocytoma, oligodendroglioma) are characterized by frequent *IDH1/2* mutations (>80 %) that occur at a very early stage. In addition, diffuse astrocytomas frequently (~60 %) carry *TP53* mutations, which constitute a prognostic marker for shorter survival. Oligodendrogliomas show frequent 1p/19q loss (~70 %), which is associated with longer survival. Molecular classification on the basis of *IDH1/2* mutations, *TP53* mutations, and 1p/19q loss showed a predictive power similar to histological classification with respect to patient survival. Only secondary glioblastomas that have progressed from low-grade or anaplastic astrocytomas, but not primary glioblastomas, share frequent *IDH1/2* mutations with oligodendroglial tumors, suggesting that primary and secondary glioblastomas may develop from different progenitor cell populations.

Keywords

Diffuse astrocytoma • Oligodendroglioma • Glioblastoma • *IDH1* mutation • *TP53* mutation • 1p/19q loss

Histological Classification of Low-Grade Diffuse Gliomas

The 2007 WHO classification recognizes three histological types of grade II low-grade diffuse glioma: diffuse astrocytoma, oligoastrocytoma, and oligodendroglioma. Diffuse astrocytoma is a well-differentiated and slow-growing tumor but shows a consistent tendency to diffusely infiltrate surrounding brain structures. Therefore, these tumors tend to recur after surgical resection, and this is often associated with progression to more malignant histological types, i.e., anaplastic astrocytoma (WHO grade III) and eventually secondary glioblastoma (WHO grade IV) [1].

Oligodendroglioma is a well-differentiated, slow-growing, diffusely infiltrating tumor of adults, typically located in the cerebral hemispheres and composed predominantly of cells morphologically resembling oligodendroglia [1].

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Progression from oligodendroglioma to anaplastic oligodendroglioma (WHO grade III) is not consistent [1].

Oligoastrocytoma is composed of a conspicuous mixture of two distinct neoplastic cell types morphologically resembling the neoplastic cells in oligodendroglioma and low-grade astrocytoma [1]. However, cases with discrete tumor areas exhibiting either oligodendroglial or astrocytic differentiation are rare. More commonly, oligoastrocytomas show an intimate mixture of oligodendroglial and astrocytic tumor cells. Oligoastrocytoma also includes cells with phenotypic characteristics that are intermediate to those of the two cell types, i.e., with oligodendroglial and astrocytic differentiation [1].

Histological criteria for the diagnosis of low-grade diffuse glioma, in particular oligoastrocytoma, may be subjective owing to the pronounced phenotypic heterogeneity of the astroglial and oligodendroglial cell lineages and the lack of reliable immunohistochemical markers to define oligodendroglioma cells [1]. Therefore, there is considerable interobserver variability in the histological diagnosis of low-grade diffuse glioma, particularly oligoastrocytoma [2–5].

Primary and Secondary Glioblastoma

Most glioblastomas (WHO grade IV) develop very rapidly after a short clinical history without evidence of a less malignant precursor lesion. These "primary" or "de novo" glioblastomas typically affect elderly patients [1, 6, 7]. Much less frequently, glioblastoma develops through progression from diffuse astrocytoma (WHO grade II) or anaplastic astrocytoma (WHO grade III). These "secondary" glioblastomas typically develop in younger patients [1, 6, 7]. In the past, the distinction between primary and secondary glioblastomas was made on the basis of clinical data: tumors were considered to be primary if the diagnosis of glioblastoma was made at the first biopsy, without clinical or histological evidence for the presence of less malignant precursor lesion, whereas the diagnosis of secondary glioblastoma required histological and/or clinical evidence of a preceding low-grade or anaplastic astrocytoma [6, 8].

Accumulating evidence suggests that primary and secondary glioblastomas are characterized by distinct genetic and epigenetic alterations as well as different expression profiles at RNA and protein levels [6]. Genetic alterations that are significantly more frequent in primary glioblastomas than secondary glioblastomas include loss of heterozygosity (LOH) 10p (47 % vs. 8 %), *EGFR* amplification (36 % vs. 8 %), and *PTEN* mutations (25 % vs. 4 %) [6, 9]. Alterations that are significantly more frequent in secondary than primary glioblastomas are *TP53* mutations (65 % vs. 28 %), LOH 19q (54 % vs. 6 %), and LOH 22q (82 % vs. 41 %) [6, 10, 11]. However, until the identification of *IDH1* mutations, none of these alterations could reliably distinguish between glioblastoma subtypes. Since primary and secondary glioblastomas are histologically largely indistinguishable [12], these subtypes remained conceptual and were not used for diagnosis or treatment decisions [6, 8, 12].

IDH1/2 Mutations Are Frequent and Early Genetic Alterations Shared by Astrocytic and Oligodendroglial Diffuse Gliomas

The presence of *IDH1* mutations (at codon 132) in glioblastomas was first reported in an analysis of 20,661 protein-coding genes [13]. *IDH2* mutations (at codon 172) were also found in gliomas lacking *IDH1* mutations, although much less frequently [14]. Subsequent studies in several laboratories demonstrated that *IDH1/2* mutations are not only very frequent (>80 %) in secondary glioblastomas and their precursor lesions (diffuse astrocytoma and anaplastic astrocytoma) but also similarly frequent (>80 %) in oligodendrogliomas (oligodendroglioma WHO grade II and anaplastic oligodendroglioma WHO grade III) and oligoastrocytomas (oligoastrocytoma WHO grade II and anaplastic oligoastrocytoma WHO grade III) [14–16]. In contrast, *IDH1/2* mutations are very rare (<5 %) or absent in primary glioblastomas and pilocytic astrocytomas, as

well as other neoplasms of the nervous system, including ependymomas, medulloblastomas, and meningiomas [14–17]. *IDH1/2* mutations are also largely absent or very rare in tumors at other organ sites, including the bladder, breast, stomach, colorectum, lung, liver, ovary, and prostate [14, 17]. The exceptions so far reported include chondrosarcoma (~55 %) [18], cholangiocarcinomas of intrahepatic origin (23 %) [19], acute myeloid leukemia (AML, up to 20 %) [20–25], angioimmunoblastic T-cell lymphoma (AITL, 20 %) [26], melanomas (~10 %) [27], and anaplastic thyroid cancer (approx. 10 %) [28] (Fig. 5.1).

In low-grade diffuse gliomas, in addition to frequent *IDH1/2* mutations, about 60 % of diffuse astrocytomas carry a *TP53* mutation, while oligodendrogliomas show frequent 1p/19q loss (~70 %) [1, 15, 29–31]. Most low-grade diffuse gliomas have either *IDH1/2* mutations plus *TP53* mutations or *IDH1/2* mutations plus 1p/19q loss (Fig. 5.2) [5, 15]. *IDH1/2* mutations are likely to occur before *TP53* mutations or 1p/19q loss. Of 40 patients with diffuse glioma who had both *IDH1* mutations and other genetic alterations (*TP53* mutation or 1p/19q loss) at the last biopsy, 33 (83 %) had both *IDH1* mutations and other genetic alterations from the first biopsy [15]. In four patients, the first biopsy had an *IDH1* mutation alone, while the second biopsy showed both *IDH1* and *TP53* mutations. In three patients, the first biopsy had an *IDH1* mutation alone, while the second biopsy showed both *IDH1* mutations and 1p/19q loss [15]. There was no case in which an *IDH1* mutation occurred after the acquisition of a *TP53* mutation or loss of 1p/19q [5, 15]. Furthermore, low-grade diffuse gliomas carrying only *IDH1/2* mutations are more frequent (17 %) than those carrying only a *TP53* mutation (2 %) or those showing only 1p/19q loss (3 %) [5].

Acquisition of 1p/19q loss in cells with *IDH1/2* mutations may be the driving force toward oligodendroglial differentiation in low-grade diffuse gliomas [5, 15, 30]. It has been shown that oligodendrogliomas with the typical histological signature of oligodendroglioma

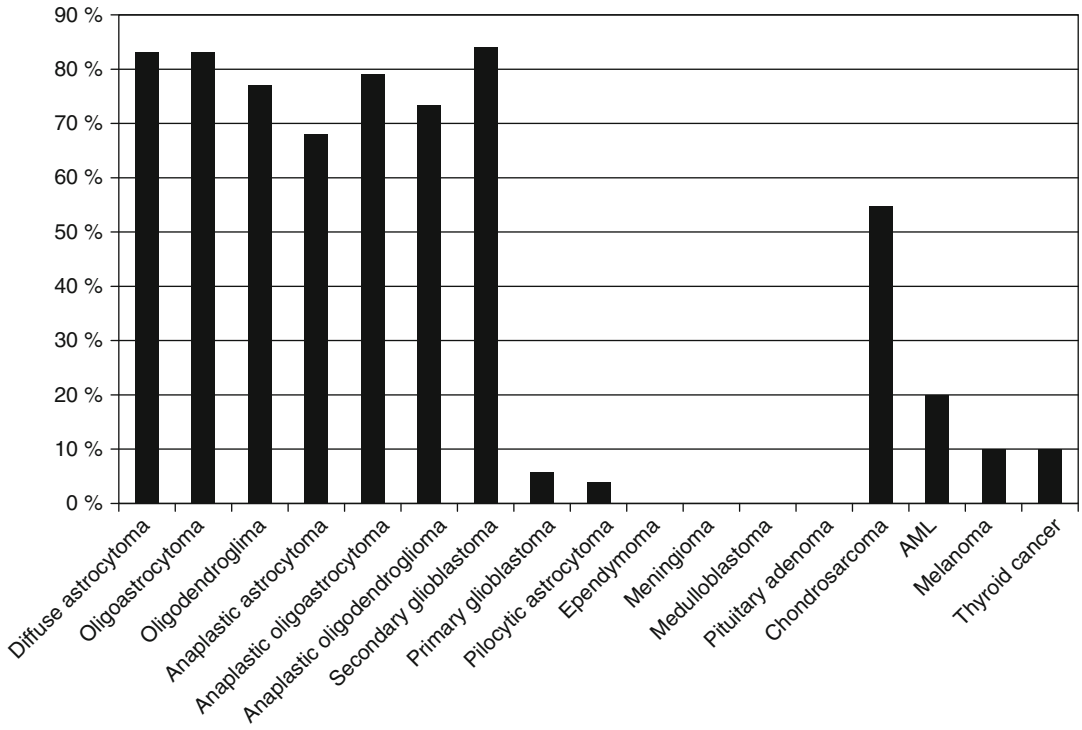

Fig. 5.1 Frequency of *IDH1/2* mutations in human neoplasms

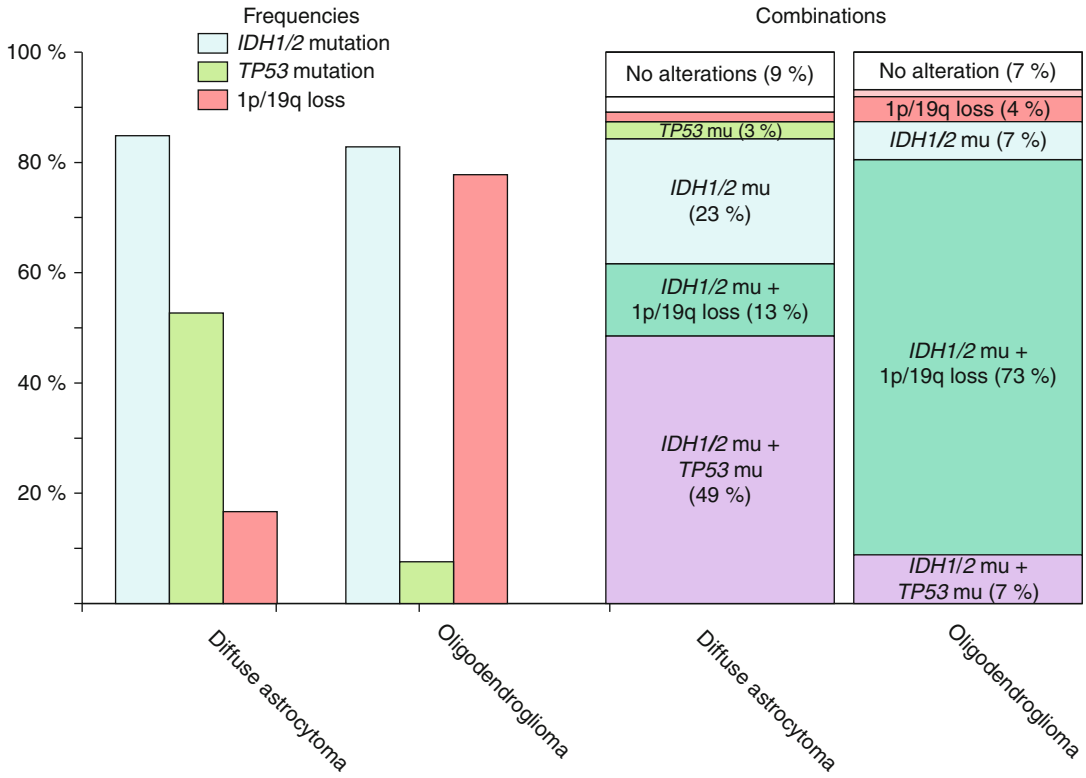

Fig. 5.2 Frequency and combinations of genetic alterations in diffuse astrocytomas and oligodendrogliomas

(e.g., honeycomb appearance of most neoplastic cells) showed loss at 1p/19q in the vast majority of cases (>90 %) [30]. The astrocytic phenotype/astrocytic differentiation may be associated with *IDH1/2* mutations, since the majority (66 %) of low-grade diffuse gliomas containing only an *IDH1/2* mutation were histologically diagnosed as diffuse astrocytoma; alternatively the astrocytic phenotype may develop in cells with *IDH1/2* mutations that subsequently acquire *TP53* mutations [5].

Molecular Bases of Common Genetic Alterations in Low-Grade Diffuse Gliomas

IDH1/2 Mutations

IDH1 and IDH2 are enzymes that catalyze the interconversion of isocitrate and α-ketoglutarate (α-KG), resulting in the production of NADPH in the citric acid (Krebs) cycle [21, 32–36].

Heterozygous *IDH1* mutations impair the enzyme's affinity for its substrate and dominantly inhibit wild-type IDH1 activity through the formation of catalytically inactive heterodimers [37]. *IDH1* mutations decreased the enzyme activities in oligodendroglioma cells [14], and downregulation of either IDH1 or IDH2 significantly reduced the proliferative capacity of cancer cells [38]. Forced expression of mutant IDH1 in cultured cells reduced formation of α-KG and increased the levels of hypoxia-inducible factor-1α (HIF-1α), a transcription factor, and its targets such as GLUT1, VEGF, and PGK1 [37], which are involved in angiogenesis, survival, and invasion in malignant glioma cells. However, immunohistochemistry revealed HIF-1α upregulation in tumor cells adjacent to areas of necrosis in gliomas irrespective of *IDH1* mutations [39].

IDH1/2 mutation results in a gain of enzymic function in the form of the NADPH-dependent reduction of α-KG to produce 2-hydroxyglutarate (2-HG) in gliomas [40] as well as in AMLs [38]. 2-HG production, but not the dominant-negative effect, is a shared function of the several different *IDH1/2* mutants analyzed (IDH1-R132H, IDH1-R132C, IDH1-R132G, IDH1-R132L, IDH1-R132S; IDH2-R172K, IDH2-172 G, IDH2-R172M) [38, 40, 41]. It has been shown that malignant gliomas carrying an *IDH1* mutation contain an increased (up to 100-fold) concentration of 2-HG [40]. It is of interest to note that children with excessive accumulation of 2-HG due to inborn errors of 2-HG metabolism have an elevated risk of brain tumor [42], while *IDH2* heterozygous germline mutations were detected in 15 unrelated patients with 2-HG aciduria [43].

IDH1/2 mutations are associated with a hypermethylation phenotype in gliomas and AMLs. Noushmehr et al. [44] reported a distinct subset of glioblastomas with concerted CpG island methylation at a large number of loci frequently carry *IDH1/2* mutations. Similarly, in a study of 131 brain tumors, hypermethylation of CpG loci was strongly associated with *IDH1/2* mutation [45]. Similar hypermethylation signatures were detected in AMLs carrying *IDH1/2* mutations [35]. In AMLs, *IDH1/2* mutations were mutually exclusive with mutations in 5-methlycytosine hydroxylase 2 (*TET2*, an α-KG-dependent enzyme), and *TET2* mutations were associated with epigenetic defects similar to those seen in *IDH1/2* mutants [35]. *TET2* promoter methylation, but not *TET2* mutation, was detected in a small fraction of gliomas lacking *IDH1/2* mutations [46]. Recent evidence suggests that 2-HG plays a critical role in abnormal methylation, since it is a competitive inhibitor of multiple α-KG-dependent dioxygenases, such as histone demethylases and the TET family of 5-methlycytosine hydroxylases [47].

In summary, *IDH1* and *IDH2* mutations lead to simultaneous downregulation of α-KG and upregulation of 2-HG [35, 40], resulting in HIF-1α upregulation, and a hypermethylation phenotype due to genome-wide histone and DNA-methylation alterations.

TP53 Mutations

TP53 plays an important role in many cellular processes, such as the cell cycle, response to DNA damage, apoptosis, and cell differentiation [48]. After DNA damage, TP53 is activated and induces transcription of genes such as $p21^{Waf1/Cip1}$ [49, 50]. MDM2 is induced by wild-type TP53 [51, 52] and binds to mutant and wild-type TP53, thereby inhibiting the ability of TP53 to activate transcription [53, 54]. $p14^{ARF}$ binds to MDM2 and inhibits MDM2-mediated TP53 degradation and transactivational silencing [50, 55]. $p14^{ARF}$ is negatively regulated by TP53 [50]. Thus, loss of normal TP53 function may result from alterations in TP53, MDM2, or $p14^{ARF}$. Promoter methylation of the *p14*^{ARF} gene was observed in 20–30 % of diffuse astrocytomas [56, 57] and oligodendrogliomas [58], whereas *p14*^{ARF} homozygous deletion and *MDM2* amplification are largely absent in low-grade diffuse gliomas.

The type and distribution of *TP53* mutations in diffuse astrocytomas and secondary glioblastomas are similar, being characterized by frequent G:C → A:T mutations at CpG sites, particularly at codons 248 and 273 [8, 29]. This contrasts with findings for primary glioblastomas, in which *TP53* mutations are more evenly distributed throughout the exons, and G:C → A:T mutations are less frequent than in secondary glioblastomas [7, 8]. These results suggest that the acquisition of *TP53* mutations in primary and secondary glioblastomas may occur through different molecular mechanisms [8].

1p/19q Loss

Oligodendrogliomas are characterized by frequent co-deletion of 1p and 19q; in most cases, the entire 1p/19q arms are involved [59–62]. Jenkins et al. [63] showed that this is due to unbalanced translocation between chromosomes 1 and 19 [t(1;19)(q10;p10)]. A balanced whole-arm translocation between chromosomes 1 and 19 forming two derivative chromosomes, one composed of 1q and 19p, the other of 1p

and 19q, and subsequent loss of der(1;19)(p10;q10) then results in the simultaneous 1p and 19q loss observed in oligodendroglioma with retention of the der(1;19)(q10;p10) seen in these cases [64]. Isolated deletions of 19q are also common in astrocytic and oligodendroglial tumors [62, 65, 66], but isolated deletions of 1p are rare in gliomas and are associated with a poorer prognosis [61, 67].

Molecular cytogenetic deletion mapping studies have suggested that the minimal regions of deletion and, by implication, the putative candidate genes reside within 1p36 and 19q13.3 [61, 62, 68]. Recent exomic sequencing in seven oligodendrogliomas showed somatic mutations in the *CIC* gene (homologue of the *Drosophila* gene capicua) at 19q13.2 in six cases and in the *FUBP1* gene that encodes far-upstream element (FUSE) binding protein on chromosome 1p in two tumors [69]. Similarly, another study of exome sequencing showed somatic mutations and insertions/deletions in the *CIC* gene in 13/16 (81 %) oligodendrogliomas with 1p/19q co-deletion [70]. This finding was validated by deep sequencing of 13 additional tumors, which revealed 7 others with *CIC* mutations, thus bringing the overall mutation rate in oligodendrogliomas in this study to 20/29 (69 %) [70]. Astrocytomas and oligoastrocytomas lacking 1p/19q loss revealed that *CIC* alterations were very rare (2 %) [70]. In contrast, Bralten et al. [71] reported the absence of common somatic alterations in genes on 1p and 19q in seven oligodendrogliomas analyzed.

Prognostic Value of Common Genetic Alterations in Low-Grade Diffuse Gliomas

IDH1/2 Mutations

It is well established that *IDH1* mutations are a significant prognostic marker of favorable outcome in patients with glioblastoma [14, 72]. This is, however, likely to be due to distinct biological behaviors of primary glioblastomas typically lacking *IDH1* mutations and secondary glioblastomas typically carrying *IDH1* mutations.

In low-grade diffuse gliomas, interpretation of survival data may be more complex, because *IDH1* mutations are frequently copresent with either *TP53* mutations or 1p/19q loss [5, 15]. In one study ($n=49$), there was a significant association between the presence of *IDH1* mutations and longer overall survival in patients with diffuse astrocytoma [73]. In another study (404 gliomas of grades II–IV; 100 being classified as WHO grade II), univariate and multivariate analyses showed that *IDH1* mutations were prognostic for a more favorable outcome [74]. Houillier et al. [75] showed that 1p/19q loss, but not *IDH* mutation, was associated with prolonged progression-free survival of low-grade diffuse glioma patients, although *IDH1* mutation and 1p/19q co-deletion were associated with prolonged overall survival. In our recent study, when low-grade diffuse gliomas (diffuse astrocytoma, oligoastrocytoma, oligodendroglioma; $n=360$) were combined in univariate or multivariate analyses, the presence of *IDH1/2* mutations was not prognostic for patient survival [5].

TP53 Mutations

The prognostic value of *TP53* mutations in low-grade gliomas has been controversial. In a study of diffuse astrocytomas and oligoastrocytomas (159 cases), *TP53* mutation was significantly associated with progression-free survival, but not with overall survival [76]. Ishii et al. [77] reported a tendency for shorter survival in patients (34 diffuse astrocytomas/oligoastrocytomas) with *TP53* mutations, but the results were not statistically significant. Watanabe et al. [57] found that *TP53* mutations were not significantly prognostic of survival of patients with diffuse astrocytomas ($n=46$). We have previously reported in a population-based study that *TP53* mutations are predictive of shorter survival in patients with low-grade diffuse gliomas ($n=122$) [29]. In our recent study ($n=360$), when low-grade diffuse

gliomas were combined, *TP53* mutations were prognostic for shorter survival [5].

1p/19q Loss

Concurrent deletion of chromosomes 1p and 19q, a typical genetic alteration in oligodendroglioma, is a well-established predictive marker in oligodendrogliomas [63, 78, 79], i.e., associated with increased chemosensitivity and a more favorable clinical outcome [61, 63, 80, 81]. In our recent study ($n=360$), when results for low-grade diffuse gliomas were combined, 1p/19q loss remained to be prognostic for longer survival [5].

Molecular Classification of Low-Grade Diffuse Gliomas

Since the vast majority (>90 %) of WHO grade II diffuse gliomas carry at least one of these alterations (*IDH1* mutation, *TP53* mutation, and/or 1p/19q loss) (Fig. 5.2) [5], it may be possible to develop a molecular classification that complements and eventually replaces histological typing. In our recent study ($n=360$), patients with low-grade diffuse glioma with *IDH1/2* mutations plus 1p/19q loss survived significantly longer than those with *IDH1/2* mutation plus *TP53* mutation [5]. Patients with diffuse astrocytoma showed a similar survival curve to that of patients with low-grade diffuse glioma with *IDH1/2* mutation plus *TP53* mutation; survival of patients with oligodendroglioma was similar to that of patients with low-grade diffuse glioma with *IDH1/2* mutation plus 1p/19q loss [5]. Thus, with respect to clinical outcome of patients with low-grade diffuse gliomas, the power of molecular classification on the basis of *IDH1/2* mutations, *TP53* mutations, and 1p/19q loss is similar to that of histological classification [5].

A molecular classification of low-grade diffuse gliomas would be valuable, since histological diagnosis of these tumors may be difficult in a substantial fraction of cases, with marked interobserver variability, particularly for oligoastrocytomas. Oligoastrocytomas carry either *IDH1* mutation plus *TP53* mutation (approx. 40 %) or *IDH1* mutation plus 1p/19q loss (approx. 45 %) [1, 29]. However, *TP53* mutations and 1p/19q loss are mutually exclusive [29, 30, 82, 83], indicating that, despite their histologic heterogeneity, oligoastrocytomas are genetically monoclonal and carry genetic alterations similar to either diffuse astrocytomas or oligodendrogliomas. This was also supported by our recent analyses ($n=360$), showing that the frequency and combination of genetic alterations in oligoastrocytomas are similar to those when all diffuse gliomas combined [5, 11]. Thus, oligoastrocytoma is not a distinct tumor entity, but one subset appears to be genetically related to diffuse astrocytomas, while another is genetically related to oligodendrogliomas.

A small fraction (7 %) of low-grade diffuse gliomas lack common alterations, i.e., are triple negative for *IDH1/2* mutations, *TP53* mutations, and 1p/19q loss [5]. This may suggest the presence of not yet identified additional genetic pathway(s) in the development of low-grade diffuse gliomas. Array CGH analysis in triple-negative low-grade diffuse gliomas showed loss at 9p21 ($p14^{ARF}$, $p15^{INK4b}$, $p16^{INK4a}$ loci) and 13q14–13q32 (containing the *RB1* locus) in several cases. Further analyses revealed that alterations in the RB1 pathway (homozygous deletion and promoter methylation of the $p15^{INK4b}$, $p16^{INK4a}$, and *RB1* genes) were significantly more frequent in triple-negative than in non-triple-negative cases, and they were significantly associated with unfavorable patient outcome [84]. These results suggest that a fraction of low-grade diffuse gliomas lacking common genetic alterations may develop through a distinct genetic pathway, which may include loss of cell-cycle control regulated by the RB1 pathway.

In summary, the molecular profile of low-grade diffuse gliomas based on *IDH1/2* mutations, *TP53* mutations, and 1p/19q loss provides a more objective classification and correlates well with clinical outcome. Despite their histological heterogeneity, oligoastrocytomas are

Table 5.1 Genetic alterations diagnostic for astrocytic and oligodendroglial gliomas

Tumor	WHO grade	Genetic alterations typically present	Genetic alterations typically absent
Pilocytic astrocytoma	I	*BRAF* fusion (>80 %)	*IDH1/2* mutation
Oligodendroglioma	II	*IDH1/2* mutation (>85 %), 1p/19q loss (>70 %)	10q loss
Anaplastic oligodendroglioma	III	*IDH1/2* mutation (>85 %), 1p/19q loss (>70 %)	
Diffuse astrocytoma	II	*IDH1/2* mutation (>85 %), *TP53* mutation (~65 %)	10q loss
Anaplastic astrocytoma	III	*IDH1/2* mutation (>85 %), *TP53* mutation (~65 %)	
Secondary glioblastoma	IV	*IDH1/2* mutation (>85 %), *TP53* mutation (~65 %), 10q loss (>60 %)	
Primary glioblastoma	IV	10q loss (>60 %), *EGFR* amplification (~40 %), *PTEN* mutation (~25 %)	*IDH1/2* mutation

genetically clonal neoplasms, one subset being genetically related to diffuse astrocytomas, while the other type is genetically related to oligodendroglial tumors, indicating that oligoastrocytoma is not a distinct entity. We recommend that the working group of the next WHO classification (5th edition) reevaluate this issue.

Genetic Alterations Useful for Diagnosis of Low-Grade Diffuse Gliomas

Much progress has been made in establishing the genetic profile of gliomas, in particular through next-generation sequencing [13, 85]. Some genetic alterations are helpful in confirming the histopathological diagnosis (Table 5.1). Screening for *IDH1/2* mutations and/or immunohistochemistry using antibodies to specific *IDH1* mutants [86] is useful for reliably distinguishing between primary and secondary glioblastomas, between diffuse gliomas and pilocytic astrocytomas or other CNS neoplasms, and between the infiltrating zone of low-grade diffuse gliomas and non-tumorous tissues [14–16, 87]. Since pilocytic astrocytomas are characterized by frequent *BRAF-KIAA1549* fusion (70 %) [88], combined molecular analysis of *BRAF* and *IDH1* reliably distinguishes pilocytic astrocytoma from diffuse astrocytoma [87] (Table 5.1).

Genetic Pathways in the Development of Astrocytic and Oligodendroglial Diffuse Gliomas

The identification of *IDH1/2* mutations was a breakthrough, since it significantly changed our understanding of genetic pathways in the development of gliomas. Primary and secondary glioblastomas are now reliably defined by the absence or presence of *IDH1/2* mutations, respectively [72]. Taking *IDH1* mutations as a genetic marker of secondary, but not primary, glioblastomas corresponds to the respective clinical diagnosis in 385/407 (95 %) glioblastomas at the population level [72]. *IDH1/2* mutations are very early and frequent genetic alterations common to diffuse astrocytic and oligodendroglial tumors [11, 14–16], suggesting that they may originate from the common precursor cells. The additional loss of 1p/19q in cells with *IDH1/2* mutations may lead to the acquisition of the oligodendroglial phenotype. Among glioblastomas, only secondary glioblastomas share a common cellular origin with oligodendrogliomas, whereas primary glioblastomas may derive from different precursor cells lacking *IDH1/2* mutations. Our current concept of genetic pathways to astrocytic and oligodendroglial diffuse gliomas is summarized in Fig. 5.3.

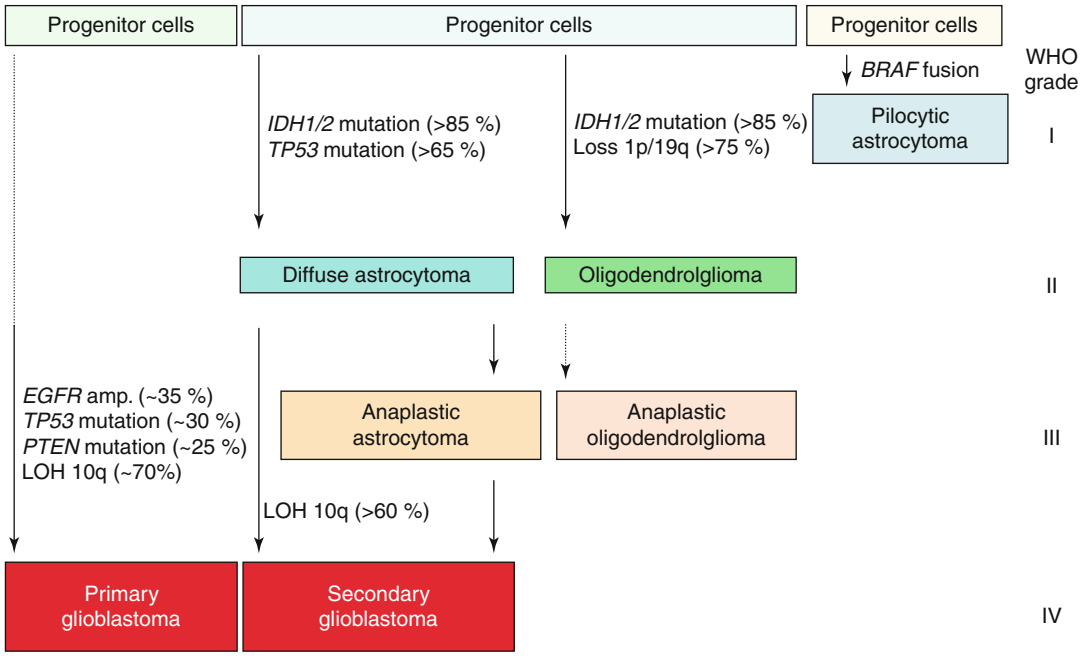

Fig. 5.3 Current concept of genetic pathways to astrocytic and oligodendroglial gliomas

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Part II

Basic Science and Experimental Research in DLGG

Cellular Origin of Grade II Gliomas

6

Pierre-Olivier Guichet and Jean-Philippe Hugnot

Abstract

Low-grade, diffuse gliomas comprise a heterogeneous group of tumors that display different phenotypes, genetic alterations, and clinical features. Important advances made over the past decade have significantly contributed to clarifying the cellular origin of these tumors. The identification of new markers for cells derived from the neural lineage combined with the detailed characterization of stem cell and progenitor cell populations that reside in the adult brain has fueled the development of original approaches to identify the cell type from which these tumors are derived. In this chapter, we will summarize our current knowledge in this dynamic field of research.

Keywords

Oligodendroglioma • Neural stem cells • Oligodendrocyte progenitors • Transgenic mice • Low-grade diffuse gliomas • Glia

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Introduction

In the human brain, glial cells appear to be as numerous as neurons [1]. This observation has been attributed to the evolution of the nervous system, which has favored an increasing ratio of glial cells to neurons [2]. The selection for a higher relative number of glial cells highlights the importance of the glial component of brain cells for accomplishing complex and rapid tasks culminating in the astonishing cognitive performance of the human brain. For instance, oligodendrocytes and Schwann cells are responsible for the myelin sheath that insulates axons and greatly increases the rate of conduction of neural signals. Far from being simple, supportive glue

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for neurons ("neverkitt," as historically named [3]), the diversity of the cellular functions accomplished by glial cells has increasingly been recognized over the previous two decades [4]. One example of how glial cells are becoming better recognized for playing a more dynamic role in the human brain involves synapses, which are now considered as a tripartite cellular association between two neurons and one astrocyte. The macroglial cells have been classically divided into astrocytes and oligodendrocytes, and these cells have been named after their cellular morphology. However, it has become apparent that this dichotomy is rather simplistic for describing the variety of these cells in the brain, especially in higher-order vertebrates. Therefore, we will start this chapter by briefly reviewing our current knowledge of the diversity of glial cells and their precursors in the adult central nervous system (CNS). Because glial cells are particularly important for proper brain function, they have undoubtedly been profoundly associated with the pathogenesis of a vast number of CNS diseases. For instance, a previous study has shown that in the familial form of amyotrophic lateral sclerosis (ALS), astrocytes expressing ALS-linked mutated superoxide dismutase 1 (SOD1) release factors that are selectively toxic to motor neurons [5]. Regarding brain cancers, mutations occurring in glial cells lead to the development of gliomas, which are the most frequent form of primitive cerebral tumors. Gliomas have been classically divided into oligodendrogliomas and astrocytomas based on the fact that the predominant population of tumor cells in these tumors shares morphological similarities with oligodendrocytes and astrocytes, respectively [6]. There are several grades of gliomas; however, this chapter will focus on low-grade diffuse gliomas that develop in adults (grade II gliomas according to the World Health Organization, G2G). We will describe the various methodologies used to provide clues on the cellular origin of these tumors and then summarize their phenotypic features. Finally, we will discuss data obtained from various animal studies analyzing low-grade diffuse gliomas.

Diversity of Glial Cells and Precursor Cells in the Adult Human Brain

Most of the current knowledge on adult glial cells has been derived from studies performed on rodent nervous tissues. However, primates and rodents have phylogenetically diverged at least 100 Ma ago. This long period of divergent evolution has allowed for the selection of unique developmental molecular and cellular mechanisms, which are used by primates to form both a large gyrencephalic cortex with an expanded prefrontal lobe and a large subcortical region of white matter. Comparisons of astrocytes in adult primates and rodents have also revealed major differences between primate and rodent species. In rodents, astrocytic cells are generally divided into fibrillary and protoplasmic astrocytes, which are mainly present in the white and gray matter, respectively. These astrocyte subsets differ from each other by their intermediate filament content. In contrast, in primates and particularly in humans, morphological differences and distinct locations have allowed for the description of six astrocytic cell types [7]. Cell populations found in layer 1 include interlaminar astrocytes with long fibers, and cell populations found in layer 5–6 include polarized astrocytes; both of these astrocyte subpopulations appear to be unique to humans and primates. In healthy rodents, astrocytes do not actively proliferate [8]; however, it remains unknown whether the different astrocyte subtypes of the primate cortex also do not actively proliferate under normal conditions. In contrast, in the adult human brain, cells involved in myelination appear to actively proliferate, and these types of cells can typically be found in various stages of development, suggesting that they are derived from a cell lineage. One example of this is the identification of oligodendrocyte progenitors (OPC), pre-oligodendrocytes, and mature oligodendrocytes in the human brain [9], which can be distinguished by the markers NG2 and O4 and the protein constituents of myelin, MAG, MOG, and MBP. Oligodendrocyte progenitors are the main

proliferating cell population in the adult brain [10, 11]. The role this proliferation might play remains obscure and one possible explanation is that it may be necessary for the adult myelination associated with learning and memory [12]. In addition to astrocytes and oligodendrocytes, synantocytes comprise another abundant glial cell population that has been more recently characterized [13]. Synantocytes have a complex morphology reminiscent of astrocytes, but they do not express GFAP. In fact, synantocytes share cellular markers with oligodendrocyte progenitors such as NG2, and they may possess the capacity to generate new oligodendrocytes in certain contexts. Synantocytes have been shown to form direct synaptic junctions with axons, respond to neurotransmitters, and interact with astrocytes. Synantocytes are considered an important but incompletely characterized cellular subset of the neuroglial network.

Over the past two decades, evidence has accumulated supporting the existence of a population of neural stem cells (NSCs) in the adult human brain [14]. NSCs are multipotent cells located in specific regions of the brain called niches and can generate both glial and neuronal cells in vitro and in vivo. The NSC niche acts as a nest and a barrier to nourish, protect, and regulate the fate of the NSCs. These niches are highly organized structures that provide cellular and molecular cues for the strict control of stem cell properties (e.g., self-renewal, differentiation, and quiescence). Typically, NSCs preferentially express genes involved in canonical developmental pathways such as Notch, SHH, Wnt, and BMP [15]. These pathways accurately regulate the proliferation/quiescence, differentiation/self-renewal, and migratory/stationary characteristics of stem cells. In addition, the physical architecture of the niche facilitates interactions between stem cells and specific cells such as vascular cells. Two niches have been well characterized in the human brain: the subventricular zone (SVZ) and the hippocampus. These two regions generate neurons in adults; however, their activity declines with age [16, 17]. The SVZ can also generate oligodendrocyte progenitors, which can be recruited in pathological situations such as multiple sclerosis [18]. Within the niche, NSCs are thought to be dormant, slowly proliferating cells that express a splice variant of GFAP termed GFAPdelta [19]. Whereas in rodents, NSCs express the CD133 marker, human NSCs are CD133-negative [20]. In rodents, the SVZ predominantly produces neuroblasts that migrate to the olfactory bulb; however, the SVZ in macaques appears to also provide new neurons for the associative cortex [21]. Although the SVZ-related adult cortical neurogenesis needs to be confirmed by additional studies, preferably in other primates, this finding opens new avenues to better understand human brain function and plasticity. In contrast with rodents, the SVZ and the hippocampus appear to be two of several regions that harbor multipotent cells in primates. Several studies have reported the in vitro isolation of multipotent precursor cells directly from the human cortex [22, 23]. A2B5+ multipotent progenitor cells have also been identified in human subcortical white matter [24]. These cells can generate nonadherent spheres in culture called neurospheres that are capable of generating neurons and glia both in vitro and after xenografting into rodent brain.

Collectively, these data explicitly illustrate that similarly to other organs, the human adult brain contains a significant population of dispersed and localized neural precursor cells whose functions remain largely unknown. As stated by Floyd Bloom, a former editor of Science magazine, “the gain in brain lies mainly in the stain.” Thus, it is likely that new markers and imaging technology will soon unravel a largely underestimated cellular complexity in the human brain. In rodents and most likely in primates, the proliferation of these cell populations appears to be closely associated with both physical and psychological activity. Therefore, one could speculate that brain cells that undergo mitosis are more prone to the accumulation of mutations, which could lead to tumors.

Grade II Gliomas

Grade II gliomas (G2Gs) affect approximately 4,000 people annually in the USA with an average patient age of 35. Several types of G2Gs exist, but the main subtypes are oligodendrogliomas, astrocytomas, and oligoastrocytomas, which can be distinguished histologically. These tumors tend to progress to high-grade gliomas within 5–10 years, at which point the patient rapidly progresses to death. However, G2Gs are clinically heterogeneous tumors and have unpredictable biological behavior such that approximately 10–20 % of patients have a significantly increased rate of tumor growth and progress more rapidly to anaplasia. Genetically, the majority of these tumors harbor a mutation in the isocitrate dehydrogenase 1 gene (IDH1), which is commonly associated with additional chromosomal aberrations such as 1p19q deletions in oligodendrogliomas or p53 mutations in astrocytomas. Frequent alterations of the CUC1 and FUBP1 genes have also recently been reported in oligodendrogliomas [25]. G2Gs are also heterogeneous with respect to their location within the brain, and those located within the insular lobe (approximately one-third of G2Gs) less frequently harbor 1p19q deletions [26]. Treatments for G2Gs typically attempt to prevent malignant progression as long as possible while preserving the patient's quality of life. One important feature frequently observed in G2Gs is a compensatory reorganization of the brain. For instance, patients with G2Gs growing in the Broca's area (an important area for language) will not display language deficits because language control becomes transferred to another area of the brain that is either contiguous to Broca's area or more remotely located [27]. This characteristic brain plasticity is often used during awake surgery to optimize the limit of the tumor resection. Another important feature of G2Gs is their ability to infiltrate the entire brain (approximately 4 mm per year) along white matter fibers and the associated vessels [28]. The ability to disseminate is responsible for the tumor relapse observed in patients within a few years after the initial resection. There is currently no curative treatment for G2Gs, and the number of new molecules specifically targeting these tumors is low.

Approaches to Determine the Cellular Origin of G2Gs

The cellular origin of G2Gs remains unknown and thus requires clarification. However, identifying the cell type from which G2Gs derive is a complex problem replete with pitfalls, and several different approaches have been used to attempt to find the answer. An obvious method is to compare the characteristics of tumoral cells with those of nonneoplastic cells found in normal tissue under the assumption that the tumoral cells should most closely resemble the cells from which they are derived. Several cellular features are typically analyzed: the cellular morphology, the presence of various markers (most frequently proteins and carbohydrates), and the expression of specific genes. Over the past decade, this type of comparative analysis has been considerably enhanced by major advances in high-throughput technologies. These have made it possible to obtain the complete tumor molecular profiles for carbohydrates and protein/transcript expression, the so-called glycome, proteome, and transcriptome, respectively. These comparative analyses provide very valuable information; however, comparison is not reason and these approaches have some inherent limitations for defining the cell of origin. First, mutations can substantially modify the phenotype of the tumor founder cells. For instance, mutagenic events can lead to the reexpression of immature features and markers in tumoral cells, which could generate doubt concerning their origin. Second, molecular and histological data are generally obtained retrospectively several years after the original mutagenic events occurred in the tumor founder cells. During this long period of time, the tumor characteristics are likely to evolve, particularly with respect to the accumulation of new mutations. Third, one must consider the possibility that the tumor founder cells that have migrated outside of their normal environment or that are situated in a pathological context such as inflammation can

adopt new phenotypes with little similarity to the original phenotype. Studies based on the phenotype of tumor cells placed in culture face even more obstacles with respect to gaining insight into the origin of G2Gs. In in vitro studies, the cells are generally cultured with a high concentration of growth factors in the presence of a high percentage of oxygen, which are likely to induce phenotypic modifications. Fourth, it is conceivable that a mutation occurring in a rare founder cell is propagated by its progeny in the context of a very different phenotype, which will then dominate the tumoral cellular population. The migration of the daughter cells away from the founder cell population tends to further complicate the process of retrospectively determining the founder cell population. Finally, tumors are typically composed of several different cell populations. The cause of tumor cell heterogeneity is believed by many to be derived from a cancer stem cell pool, which, similar to normal stem cells, maintains the capacity to generate the different cell types in a given tissue and thus produce the variety of cells found in tumors. Another theory postulates that the cellular diversity of tumors could arise from stochastic differentiation-dedifferentiation events that occur in the cells. Regardless of the cause of tumor cell diversity, this heterogeneity renders comparative analyses difficult to interpret, especially when studying highly heterogeneous tumors such as glioblastomas. An even more complicated picture in terms of defining the cellular origin of tumors was recently depicted by Fomchenko et al. [29]. Whereas gliomas are thought to be derived from the clonal expansion of a single mutated cell, which then accumulates additional mutations in its progeny, Fomchenko et al. used an experimental murine model to show that cells that were not originally at the origin of the glioma could be recruited to the glioma microenvironment during tumor progression. Once they were within the tumor microenvironment, these cells acquired features typically observed in tumor cells including tumorigenicity when transplanted into other mice.

A completely different approach to define the founding cell population of gliomas is based on transgenic mouse studies. The transgenic mouse approach typically involves the overexpression of oncoproteins such as K-Ras or growth factors such as PDGF in a particular cell type and is generally achieved by using a cell-specific promoter (for instance, GFAP for astrocytes) to target the expression of a given oncogene to a selected population of cells. Elegant studies such as these allow the exploration of the competency of a given cell type to generate specific subtypes of brain tumors. Although transgenic mouse studies have provided very meaningful data, there are several limitations to this approach. The specificity of the promoter used in these studies is crucial. Previous studies have demonstrated that promoters once thought to be cell-specific are in fact also active in other cell types. For instance, GFAP is expressed in astrocytes as well as in NSCs in the subventricular zone [30]. Another limitation of this approach is, as discussed above, the limited cell diversity in the mouse brain compared to primates. One must also consider that genes specifically expressed in a cell population in mice can be expressed in a completely different cell type in the human brain. For instance, Dcx is specifically expressed by young neurons in the adult mouse brain, whereas Dcx is expressed in young neurons and subpopulations of astrocytes in the human brain [31, 32]. In 2011, a sophisticated and elegant transgenic mouse study was designed to define the cellular origin of gliomas using the method mosaic analysis with double markers (MADM). MADM allows the precise identification of the cellular subtype showing an aberrant growth pattern during the premalignant phase of tumor progression [33].

Phenotype of Oligodendroglioma Tumors

At face value, the term oligodendroglioma may imply that these tumors are composed of tumoral cells derived from the transformation of oligodendrocytes. However, there is a lack of current data to support such a view. At the ultrastructural level, a fraction of oligodendroglioma cells can display concentric arrays of membranes

(the so-called membrane laminations, whorls, or scrolls). This could be interpreted as a remnant of the myelinating process still active in these cells [34–38]. Oligodendroglioma cells also display a large number of mitochondria, polygonal crystalline structures in the cytoplasm, and cytoplasmic microtubules [39]. At the histological level, low-grade oligodendroglioma cells characteristically have a "fried egg" morphology with a round to oval central nuclei and a clear and large cytoplasm. The cells are arranged in compact cell nests resembling honeycombs and are separated by arborizing tumor vessels, which can form a "chicken wire" pattern. Calcifications are often found in these tumors.

Despite abundant literature on the expression of various oligodendrocyte markers in tumors, the phenotype of oligodendroglioma cells is still poorly defined with respect to the oligodendrocyte lineage. Notably, these studies have been based primarily on immunohistochemistry, which may vary substantially between laboratories as a consequence of protocol variation that may differ in the type of fixatives and the quality of the antibodies used. Protocol variation may explain some of the conflicting results obtained by different laboratories. In this section, we will summarize our current knowledge on the phenotypic characteristics of low-grade oligodendroglioma cells with respect to the expression of the main markers used to delineate the different steps of the oligodendrocytic lineage. Briefly, the oligodendrocyte lineage begins with oligodendrocyte progenitor cells characterized by the expression of the markers NG2, A2B5, and PDGFRα [9]. Oligoden-drocyte progenitor cells tend to downregulate these markers as they enter the pre-oligodendrocyte stage, which is characterized by the strong expression of the antigens O4 and Nogo-A. Finally, oligodendrocytes mature and express myelin proteins such as MBP, MOG, MAG, and the galactocerebroside (GalC). Oligodendrogliomas do not appear to express MAG protein, which indicates that the tumoral cells are most likely incapable of conducting the myelination process [40, 41]. The expression of MBP is only observed by a fraction of oligodendroglioma, with diverging results between studies, and by only a fraction of tumoral cells [41–47]. Other markers of relatively mature oligodendrocytes (GalC/O1, PLP, and CNPase) show variable expression between tumors suggesting that variability may exist in the degree of differentiation between oligodendrogliomas [45–48]. Nogo-A is strongly expressed in the majority of oligodendrogliomas [49–51], which is particularly interesting because it is expressed by pre-oligodendrocytes, and its expression might reflect a substantial presence of pre-oligodendrocytes in oligodendrogliomas. The majority of oligodendrogliomas also express several membranous markers (such as A2B5, PDGFRa, NG2) and transcription factors (Ascl1, Olig2, and Nkx2.2) for OPCs [48, 52–59]. They also express Sox10, a transcription factor which is thought to be expressed in more mature oligodendrocytic cells [60, 61]. Taken together, while it seems apparent that oligodendrogliomas contain immature cells of the oligodendrocyte lineage, it remains unclear whether there is a single self-renewing cell type which is halted in the differentiation process or alternatively whether a pseudo-oligodendrocytic lineage exists with the ability to produce tumoral cells at different stages of differentiation. The latter scenario is supported by recent results from Persson et al. [56], which have shown that distinct NG2+ and NG2– cells exist in human oligodendrogliomas. Given this observation, it would be interesting to determine whether the ratio of oligodendrocyte progenitors to more differentiated oligodendrocytic cells differs between tumors. It is conceivable that several oligodendroglioma subtypes could exist based on the prominence of oligodendrocyte progenitors, pre-oligodendrocytes, or oligodendrocytes, which should be called oligoprogenitogliomas, pre-oligodendrogliomas, and oligodendrogliomas, respectively.

In addition to the expected oligodendrocyte lineage markers, several studies have revealed the unexpected expression of markers that are commonly considered specific for the neuronal lineage [62]. These markers include Dcx [63], synaptophysin, chromogranin [64, 65], neurofilament [65, 66], beta III tubulin [67], alpha internexin [68], and synapsin [64]. Large-scale profilings of oligodendrogliomas have also indicated that tumors

with 1p19q loss of heterozygosity (LOH) overexpress genes related to neurogenesis [69, 70] and that these tumors are related to the proneural subtype of high-grade gliomas, which also tend to express immature neuronal markers. Oligodendroglioma cells have also been shown to express functional receptors for neurotransmitters such as GABA and glutamate [71, 72]. However, it is worth nothing that the NeuN marker (now identified as the Fox-3 protein [73]), which is thought to be specific for mature neurons, is not expressed in oligodendrogliomas [74–76]. The detection of proteins characteristic of neurons could be related to studies presenting evidence for electrical activity including action potentials in oligodendroglioma cells [77, 78]. The identification of neuritic structures, neurosecretory granules, and synapses in oligodendroglioma cells by electron microscopy [64, 77] has confirmed that at least a fraction of these cells display characteristics that are typical of neuronal cells. However, it must be emphasized that markers and properties once thought to be specific for neurons have been described in other cell types. Furthermore, normal oligodendrocyte progenitors can make synapses with neurons [79] and express receptors for neurotransmitters [80–82]. Thus, it remains unclear whether neuronal markers found in oligodendroglioma cells are in fact normal traits for oligodendrocyte progenitor cells, which in fact can generate neurons and oligodendrocytes during development [83]. Alternatively, genetic alterations such as 1p19q LOH could derepress a neuronal gene network that is normally silent in oligodendrocytic cells and could lead to the expression of neuronal markers.

In addition to neuronal markers, the presence of oligodendroglioma cells expressing the astrocytic marker GFAP has been reported by several labs [42, 84–88]. Cells that stain both for MBP and GFAP have been observed by Tanaka et al. [43], and these cells have been more frequently observed in high-grade oligodendroglial tumors [85]. These cells are called gliofibrillary oligodendrocytes, and their presence may define a subgroup of oligodendrogliomas referred to as gliofibrillary oligodendrogliomas [88]. Exhibiting features common to both oligodendrocytes and astrocytes, these cells could be regarded as an intermediate morphological form of these two cell types [84, 86, 89]. Similar to the previous discussion for neuronal markers, several hypotheses could account for the presence of these cells. First, it appears that during normal development, some myelin-forming glia stain positively for GFAP [87, 90] and that the human GFAP promoter is active in oligodendrocyte progenitor cells [91]. These observations collectively suggest that gliofibrillary oligodendrocytes may represent a subtype of tumoral cells that express at least one trait of a progenitor cell possibly through a dedifferentiation process. A second plausible explanation is that gliofibrillary oligodendrogliomas are composed of oligodendrocyte progenitors in the process of differentiating toward the astrocytic lineage. In fact, it has been observed that oligodendroglioma cells cultured in serum-supplemented medium lose their oligodendrocytic features and acquire astrocytic markers [92]. Based on this evidence, oligodendroglioma cells might be bipotential cells similar to O2A cells, which can differentiate into GFAP+ cells or oligodendrocytes depending on the culture conditions [93]. This hypothesis speculates that the differentiation of oligodendroglioma cells to astrocytic cells results from the presence of astrocytic differentiating factors, which could be present in a subset of tumors. Finally, considering that the type of mutations found in oligodendrogliomas clearly influences their phenotype [94], one might also assume that gliofibrillary oligodendrogliomas result from particular mutations leading to the aberrant activation of pathways leading to the astrocytic differentiation of oligodendrocytic cells.

Collectively, these data indicate the existence of phenotypically different classes of low-grade oligodendrogliomas. The variety in oligodendroglioma tumors could result from the differences in the type of mutations accumulated, which clearly influences the phenotype and prognosis of low-grade tumors. In addition, the identity of the cells from which the tumor might originate (oligodendrocytes, pre-oligodendrocytes, or oligodendrocyte progenitors) may also have a strong impact on the phenotype of the tumor.

The possibility must also be considered that G2Gs that develop in different brain regions may be phenotypically distinct based on interactions with their microenvironment. At the genetic level, G2Gs located in the insula appear to harbor 1p19q deletions less frequently than G2Gs arising in other regions of the brain [26, 95]. The cellular organization of the insular region is distinct from that of other cortical areas [96, 97]; therefore, it is possible that insular G2Gs might develop distinct features.

Phenotype of Low-Grade Diffuse Astrocytoma and Oligodendrocytoma Tumors

Astrocytoma and oligoastrocytoma tumors are two types of G2G tumors that are commonly distinguished from oligodendrogliomas. Astrocytomas and oligodendrogliomas are distinguished based on their morphologic similarities to mature astrocytes and oligodendroglia, respectively [61]. However, interobserver variability imparts a subjective influence on the diagnosis of these subtypes of G2Gs [98]. It is important to note that some authors consider that at least a fraction of diffuse astrocytoma and oligoastrocytoma cells are in fact isolated infiltrative oligodendroglioma cells entrapped in GFAP+ processes within the white matter [99]. By not identifying these cells as infiltrative oligodendroglioma cells, these tumor cells become misinterpreted as astrocytoma cells. This case of mistaken identity could explain why a large number of markers for oligodendrogliomas such as Olig2, A2B5, and O4 [58, 98, 100–102] have also been reported in astrocytomas. However, oligodendrocytoma and astrocytoma tumors display markers and genetic alterations that distinguish them from oligodendroglioma. The markers Trk A, B, and C; G-CSF; Ezrin; VEGF; glutamine synthetase; and strong nuclear staining for p53, FABP7, and Id4 are more specific for astrocytomas and oligoastrocytomas [98, 103–107], whereas the markers Nogo-A, rPTPbeta/zeta, OLIG2, ASCL1, and NKX2-2 are more commonly expressed in oligodendrogliomas [57, 108, 109]. High-throughput techniques have also demonstrated that oligodendrogliomas and astrocytomas differ in their gene expression profiles [110]. In addition, oligodendrogliomas are associated with longer overall patient survival than diffuse astrocytomas [111]. At the genetic level, the deletion of 1p19q is a genetic feature of oligodendrogliomas, whereas duplications of parts of chromosome 7 and mutations of the gene TP53 are more typical of astrocytomas [98, 112]. Based on these phenotypic analyses, it is impossible to draw conclusions with regard to whether the founder cell type for astrocytomas differs from that of oligodendrogliomas. The simplest hypothesis suggests that astrocytomas and oligodendrogliomas are derived from two distinct cell lineages. However, a more sophisticated hypothesis predicts that specific mutations could lead to the astrocytic differentiation of oligodendrocyte lineage cells, which would further develop as an astrocytoma. An elegant study performed by Dai et al. supports this view [94] by showing that Akt hyperactivity in oligodendroglioma cells induced by PDGF stimulation results in cells with an astrocytic differentiation pattern both in vitro and in vivo. These data imply that the activation of different pathways can have a major influence on tumor histology. Finally, it is also conceivable that a specific tumor microenvironment (inflammation) or location (white or gray matter) may influence the dominant feature (astrocytoma or oligodendroglioma) observed in tumors arising from the same cell type. For example, oligodendrogliomas with 1p19q LOH were most often associated with white matter tracks, whereas astrocytomas frequently touch the ventricles [56]. Importantly, in the majority of oligoastrocytomas, the oligodendroglial and astrocytic components have similar genotypes [112]. This observation favors the theory of a common cellular origin for oligodendrogliomas and astrocytomas. If this were indeed the case, then the predominance of either tumor cell type would be influenced by genetic and/or environmental conditions.

Blockage of Differentiation in Low-Grade Oligodendroglioma

The presence of OPC-like cells in G2Gs suggests that these cells should differentiate into oligodendrocytes; however, this is not the case. In vitro and in vivo studies in mice have firmly established that the overactivation of the PDGFR or EGFR pathways by overexpressing their respective ligands or receptors halted the differentiation process responsible for the progression to more mature oligodendrocytes [113–118]. This differentiation block is accompanied by cell proliferation and extensive migration in the brain. Similarly, experimental gliomagenesis generated by in utero exposure to the carcinogen *N*-ethyl-*N*-nitrosourea (ENU) led to the formation of NG2+ low-grade oligodendrogliomas. In vitro, these cells could differentiate into O4+ late-stage OPCs but could not generate MBP+ mature oligodendrocytes [54]. Studies of human oligodendrogliomas in vitro also suggest that these cells are also compromised in their differentiation capacity [56, 119]. In human G2Gs, the presence of EGFR and FGFR and their ligands has been reported [120, 121] suggesting the possible existence of autocrine loops that could antagonize the differentiation process. Interestingly, defective asymmetric division of oligodendroglioma cells has been observed, which could account for the inhibition in the differentiation process. Asymmetric division is an evolutionarily conserved cellular mechanism that is broadly used to generate cell diversity, notably during development. In this process, the unequal distribution of molecules during division generates two morphologically different cells that have different fates. Normal NG2+ OPCs in mice and in humans can divide asymmetrically to generate both NG2+ and NG2− cells, which have drastically different fates [113]. Importantly, the inheritance of NG2 by one cell is associated with EGFR co-segregation, increased self-renewal, and reduced differentiation, whereas the cell lacking NG2 differentiates into an O4+ cell. Compared to normal OPCs, in a murine oligodendroglioma model and in oligodendroglioma human cell cultures, the rate of asymmetric division of NG2+ cells decreases, which could lead to an accumulation of undifferentiated cells and premalignant lesions. Moreover, Sugiarto et al. observed that the proteins implicated in asymmetric division are downregulated in human oligodendrogliomas supporting the deregulation of asymmetric division in gliomas [113].

Finally, one major step in our understanding of how common G2G mutations affect differentiation was achieved in 2012 [122]. Using an adipocyte differentiation model, it was demonstrated that IDH1 mutation can prevent the histone demethylation that is required for lineage-specific progenitor cells to differentiate into terminally differentiated cells. It is likely that similar mechanisms act in OPC to prevent their differentiation into mature oligodendrocytes.

Insights on the Cellular Origin of G2Gs from Animal Work and Cell Culture

Phenotypic analyses provide only partial insight regarding the cellular origin of G2Gs. Additional insight can be obtained by experimental gliomagenesis in animals using transgenic mouse experiments or more conventional chemical-induced gliomas. Using transgenic mice, at least two cell types have been shown to be competent in the generation of oligodendrogliomas. Lindberg et al. have shown that PDGF-B overexpression in CNPase+ cells, which comprise OPCs and mature oligodendrocytes, could induce gliomas resembling human low-grade oligodendrogliomas with a high incidence [123]. Using the promoter S100b, which mainly targets mature astrocytes and OPCs, and an activated allele of epidermal growth factor receptor (v-erb), Persson et al. have reported that NG2+ OPCs can generate oligodendrogliomas in mice [56]. More recently, using MADM, which initiates p53/NF1 sporadic mutations, Liu et al. have shown that aberrant growth occurs only in OPCs prior to full malignancy [33]. Following transformation, these tumor cells display prominent OPC characteristics. Liu et al. went on to show that

introducing p53/NF1 mutations in OPCs led to gliomagenesis. With respect to human tumors, FACS analysis has shown that oligodendrogliomas contain a minority of NG2+ cells (5–30 %); however, these cells showed significantly higher tumorigenicity compared to those of the NG2- fraction [56]. This finding indicates that G2Gs might contain different tumoral cell populations and that only a subset of these tumoral cells can sustain tumor growth and propagation. This situation is somewhat reminiscent of the isolation of cancer stem cells in high-grade gliomas [124]. Collectively, these studies underline the implication of OPC cells in the generation of oligodendrogliomas. In the human brain, previous studies have estimated that mitotic OPCs expressing the Olig2+ marker represent a pool of ten million cells [11], and these proliferating cells likely represent ideal targets for mutagenic events occurring during replication.

OPCs appear not to be the only cell type capable of generating oligodendrogliomas. Three independent studies have reported that targeting GFAP+ cells in vivo with the polyoma virus middle T antigen, EGFRvIII, or PDGF can generate gliomas with mixed astrocytoma and oligodendroglioma morphological features [114, 125, 126]. Dai et al. further reported that the overexpression of PDGF in GFAP+ cells in vitro converted these cells into PDGFRa+ PLP+ Id4+ (however O4– NG2–) cells with an elongated bipolar morphology reminiscent of glial precursor cells. This observation is reminiscent of earlier work by Tenenbaum et al. [92], who showed that cells derived from human oligodendrogliomas and cultured in serum-supplemented medium lose their oligodendrocytic antigenic markers and acquire astrocytic markers. However, after transplantation of these cells into rodent brains, the cell reexpressed oligodendrocytic markers. This phenotypic switch between OPC and GFAP+ astrocytic cells may account for the ability of these two cell types to generate oligodendrogliomas in mouse models.

While several studies point to OPCs as a plausible cell type for the generation of grade II gliomas, the possible involvement of NSCs, which are located in specialized niches such as the SVZ, in gliomagenesis has also been explored. NSCs typically express CD133 and form multipotent neurospheres capable of being passaged. Rebetz et al. have shown that in three astrocytomas and one oligoastrocytoma, there were very few CD133+ cells and that CD133+ cells coexpress CD31, which is a marker for endothelial cells [102]. In contrast, Thon et al. reported the presence of CD133+ cells that did not express the endothelial progenitor cell marker CD34 in seven out of ten grade II gliomas (mainly diffuse astrocytomas) [127]. These cells were reported to grow as passageable, multipotent neurospheres. However, the CD133+ and CD34- cells were not tested for tumorigenicity, for instance, by transplanting them in immunocompromised animals. In mice, glioma tumors can recruit non-tumoral NSCs from the SVZ. Thus, it is possible that CD133+ cells observed in G2Gs represent non-tumoral NSCs attracted by the tumoral environment. Regarding oligodendrogliomas, Persson et al. and Galli et al. were not able to isolate neurosphere-forming cells from 2 to 5 grade II oligodendrogliomas, respectively [56, 128]. Similarly, Persson et al. have found that human oligodendroglioma cells grow as adherent cultures, are not tripotent, and differentiate mainly into oligodendrocyte-like cells [56]. Collectively, these data do not support a NSC origin for G2Gs. The case against NSCs as the founding cell type for G2Gs is also supported by the location of these tumors which infrequently develop near the ventricles where SVZ NSCs are known to reside [56].

Conclusion

Research in neural development and in degenerative diseases such as multiple sclerosis produces an ever-increasing amount of molecular data on the generation and differentiation of neural cells and especially astrocytes and oligodendrocytes. The discovery of new genes and mechanisms involved in the generation of neural cellular diversity is rapidly feeding the field of neurooncology. In parallel, a detailed exploration of the diversity of glial cells in the human brain is needed to identify new markers

that define specific cellular subtypes and cellular steps during the differentiation process. This research will undoubtedly provide a clearer picture on the molecular and cellular origin of G2Gs.

G2Gs inexorably degenerate into high-grade gliomas. Thus, it is crucial to define new therapeutic strategies to target these tumoral cells before they develop into a more aggressive form where the accumulation of additional mutations could increase their resistance to treatments. A better understanding of the cellular origin of G2Gs and the diversity of tumoral cells present in these tumors combined with a detailed description of the molecular alterations that inhibit the differentiation process in these tumors will certainly generate a fruitful and rational background for the development of innovative treatments for gliomas.

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Deciphering the Molecular and Cellular Basis for Dissemination of Diffuse Low-Grade Gliomas

7

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Abstract

Although initially silent, diffuse low-grade gliomas (DLGGs) always progress into a more aggressive pathology, eventually causing death of the patient. Their diffusive nature makes them difficult to fully remove by the surgical approach. Understanding the molecular pathways ruling DLGG dissemination would open up new lines of treatments aiming at limiting their spread throughout the brain. However, the rare occurrence of these tumors, the difficulties in growing them in culture, and the quasi-absence of DLGG-derived cell lines have definitely impeded the progress of knowledge on this topic. This explains the very few data available today on DLGG invasion and calls for more efforts from the scientific community to tackle this complex challenge. Here after reporting the main studies which have approached the problematic of DLGG dissemination, we propose some analogies with oligodendrocyte precursor migration and suggest some promising directions to take. We then raise central issues making DLGG dissemination difficult to study with our present state of knowledge and technical possibilities. Deciphering the migratory strategies adopted by DLGG to invade the brain would be a major advance for the development of therapies aiming at maintaining DLGG in a confined and resectable nutshell.

Keywords

Oligodendroglioma • Oligodendrocyte progenitors • Migration • Diffuse low-grade glioma • Molecular basis

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Introduction

DLGGs are sometimes erroneously considered as good prognosis tumors as they will ineluctably degenerate into high-grade gliomas. As already noted by Virchow in the nineteenth century [1], gliomas do not exhibit an obvious border that separates tumoral from healthy brain tissue, making surgical resection difficult and often incomplete. Residual tumoral cells will continue to grow and invade the brain while acquiring further mutations. Development of therapies that would reduce the dissemination of these cells and confine them to a limited area would be greatly beneficial, making the surgical approach fully efficient. Therefore, it is important to understand at the anatomical, cellular, and molecular levels how glioma cells disseminate into the brain so as to build an integrated model which could be used to elaborate new therapeutic approaches. Very good reviews on the molecular mechanisms underlying high-grade glioma invasion already exist (see, for instance, [2–14]), so the present chapter does not intend to cover this topic again. In contrast very little is specifically known on how DLGG cells spread into the brain parenchyma. It is not yet established if DLGG invasion is simply a slower but identical version to high-grade glioma dissemination. However, given that glioblastomas (GBMs) and DLGGs differ substantially in their genetic alterations, it is likely that low- and high-grade glioma migration relies both on common and different modalities. The rare occurrence of DLGGs, the difficulties in growing them in culture, and the quasi-absence of DLGG-derived cell lines have definitely impeded the progress of knowledge on this topic. Although animal models for DLGGs have been developed [15], these models do not include the classical mutations found in these tumors in man (notably IDH1 mutations and 1p19q codeletions). Therefore, the relevance of these models for studying DLGG dissemination remains to be established. In this chapter, we will first focus on the available models used to study DLGG dissemination and on the few molecular data obtained so far. In the second part, we will consider data obtained on the migration of normal oligodendrocyte precursor cells as a possible source for guiding our understanding of glioma cell dissemination. Finally, we will point out important issues to address in order to move forward on this topic.

In Vitro Models for Studying DLGG Invasion

As described in another chapter of this book (see Chap. 3), DLGGs comprise mainly three distinct populations of cells that can be discriminated at the anatomopathological level and that present different genetic alterations and molecular signatures. Increasing observations and publications suggest that these distinct types of DLGGs (namely, oligodendroglioma (OG), astrocytoma, and oligoastrocytoma) engage in different routes of migration, suggesting different molecular pathways involved. We will first review the publications reporting a different mode of migration for OGs versus astrocytomas and then discuss three studies identifying proteins specifically involved in OG diffusion.

Different Modes of Migration Within DLGG Populations

Glioma cells appear to invade the normal tissue in three ways: (1) migration along the nerve fibers which possibly leads to the perineuronal satellitosis (i.e., clustering of neoplastic cells around neurons) frequently observed in OGs, (2) migration along the vessels, and (3) cellular accumulation and spread through subpial sites. Considering the diversity and the complexity of these migration modalities, multiple assays (see, for instance, [16–19]) have been used to model high-grade glioma invasion in vitro. In contrast, very few studies aimed at exploring DLGG cell migration in vitro [20–23]. An original model

developed by Colin et al. used freshly obtained tumoral explants (500 μm^3) which they placed on poly-D-lysine-coated coverslips to analyze glioma cell migration. This study revealed that OG (grade II and III) explants tended to generate few migrating cells. A more sophisticated approach was used by Palfi et al. [20] who implanted DII-labeled glioma fragments into 400-μm-thick slices of 7 days-old mice brains. In this organotypic assay, invasive glioma cells showed patterns of dissemination and phenotypes similar to those observed in vivo. This assay allowed a quantitative analysis of the invasive potential of gliomas of different types and grades. They observed that there was no overt difference between grade II and grade III glioma invasion in this assay. However, there was a clear difference between OGs and astrocytomas. OGs tended to be less invasive than astrocytomas and grew as circumscribed tumoral masses. In contrast, astrocytomas were more infiltrative.

Further investigations on the correlation between molecular alterations and the in vitro growth patterns established that tumors with 1p19q loss were less invasive in this assay, which is very consistent with the prominent association of this genomic alteration with OGs. OGs with 1p19q codeletion show a proneural phenotype characterized by a high level of expression of genes which are classically expressed during central nervous system development and in neuronal cells such as alpha-internexin [24]. In addition, electrical activity including action potentials has been described in OG cells [25]. Thus, it is possible that somehow this neuronal-like phenotype may endow the cells with a less invasive capacity compared to astrocytomas.

Different genetic alterations typically associated with astrocytomas and OGs may also account for the difference in the invasion pattern observed by Palfi et al. Indeed, it has been found that mutations in p53 and ATRX (alpha-thalassemia/mental retardation syndrome X-linked) are mainly found in astrocytomas [26], whereas genomewide sequencing has recently revealed the frequent alteration of Fubp1 and Cic genes in OGs [27]. How these alterations impact on cellular migration is totally unknown, but this will certainly be a major subject of investigation in the next decade.

Interestingly, Persson et al. [28] reported that 1p/19q-deleted OGs preferentially arose in white matter regions, while low-grade astrocytoma tumors were more frequently associated with the lateral ventricles. Furthermore, the pattern of infiltration seems to differ in astrocytomas and OGs with the latter tending to infiltrate the brain tissue by perineuronal satellitosis. Collectively, these data suggest important differences in the mode of invasion between low-grade astrocytoma and OG cells as a result of differences in genetic alterations, cellular phenotypes, or/and brain locations.

Molecular Components Driving DLGG Migration

A paper presenting evidence of a protein involved in OG migration in vivo was published in 2002 [29]. Indeed, in this study, the authors showed that OG cells in vivo expressed the standard form of CD44 (CD44s), in particular in sites of dissemination, and that CD44s expression was correlated with grade in OG. CD44 role in cancer dissemination has been widely described [30], but no definite demonstration of its involvement in DLGG migration has been offered so far.

This demonstration that CD44s are expressed in OG leads to several presumptions on the role of hyaluronan (HA) on OG migration, as CD44 is a HA receptor. Indeed, Radotra and collaborators [31, 32] used a Matrigel system to study glioma cell migration and found that adding HA to the Matrigel increased migration in a dose-dependent manner. They further investigated the mechanisms by which glioma cells migrate in HA-enriched Matrigel and found that CD44 was involved in this process, as blocking CD44 with a specific antibody led to a significant reduction

in glioma cell migration. This study was done in vitro on an anaplastic astrocytoma-derived cell line rather than OG cells. Nevertheless, given the preferential migration of OG cells along HA-rich white matter fibers and the previously shown demonstration that CD44 is expressed in OGs [29], it seems likely that CD44s and HA play a role on OG migration in vivo. As HA inhibits oligodendrocyte maturation [33], it can be hypothesized that HA participates in the dissemination of OG cells by maintaining them in an undifferentiated precursor-like state which is prone to migration.

In 2006, McDonald et al. used OG tumor sections from 177 patients to identify the genetic consequences of the 1p36 deletion observed in 60–80 % of OGs [34]. They identified SHREW1 (also known as AJAP1) as the unique gene present in the deleted region and found that OG cells from 30 patients showed significantly reduced SHREW1 expression as compared to normal tissue. SHREW1 is a membrane protein involved in adherent junctions. The authors demonstrated that restoration of SHREW1 leads to reduction of cell migration and they speculated that SHREW1 could be considered as a tumor suppressor involved in OG progression. Interestingly, when overexpressing SHREW1 in an established GBM cell line (U251), no effect on cell migration was observed. Indeed in GBM cells, further advanced in the cancerous process, SHREW1 overexpression is not sufficient to trigger migration arrest. This latter information is a great reminder that studies based on GBM cell line experimentations are not suitable to investigate DLGG biology.

Finally, based on the observation that OGs with 1p19q are less aggressive than their non-deleted counterparts, Rostomily et al. [35] undertook the comparative proteomics of OGs with or without 1p19q deletion. They found that about 10 % of the differentially expressed genes were involved in invasion/migration. The list of genes presented in their Appendix II could be used to decipher the differential migratory properties of OG with or without 1p19q deletion and open up new lines of investigations on the invasive properties of OG.

Insights from Oligodendrocyte Progenitor Migration

Given the suspicion that OG tumors are derived from the tumorigenesis of oligodendrocyte precursor cells (OPCs) (see Chap. 6), it is likely that OGs share some common molecular properties with OPCs. So as to propose new potential targets worthy of investigation as potential OG migration drivers, we will list and review the main pathways known to direct OPC migration and propose how these data could be used and applied to research on OG invasion.

Several categories of molecules are involved in OPC migration: (a) long-distance signaling proteins play a chemoattractive or chemorepelling effect on OPCs, thus controlling their direction; (b) proteins of the extracellular matrix provide a beneficial or repulsive substrate to migrating cells; and (c) structural proteins expressed by the migrating OPC provide a favorable cytoskeleton configuration. A comprehensive review on OPC migration provides substantial information on the signals involved in this process [36]. Of these signals, we present here the ones that are likely to be shared by OGs.

The first obvious signal involved in OPC migration and likely to play a role on DLGG survival is PDGF. In OPCs, PDGF-induced migration is mediated by Cdk5, involving the phosphorylation of the nonreceptor tyrosine kinase Fyn [37]. Cdk5 phosphorylates the WAVE2 protein which forms a multiprotein signal transduction complex binding to receptor kinases and actin, with an effect on cell shape and motility [38]. Overexpression of a WAVE2 construct where the site of phosphorylation has been mutated leads to reduction of PDGF-mediated migration of OPC. WAVE2 has been shown to be involved in cellular migration [39] and plays a pivotal role in melanoma cell migration [40] and other cancer metastasis [41]. Given the role of WAVE2 on OPC migration [37] and on several types of cancers, WAVE2 is likely to be involved in DLGG invasion. Comparative proteomics between OGs with or without 1p19q deletion identified WAVE3 as a protein significantly more expressed in tumor cells with

deletion than in cells without deletion [35]. Proteins of the WAVE (or WASF) family could thus supply informative clues on the migratory properties of DLGG.

The best characterized chemotactic signals driving OPC migration during development are semaphorins (3F and 3A) and netrin-1 [42]. Indeed, semaphorin 3A was shown to have a repulsive effect on OPCs in explants, whereas semaphorin 3F and netrin-1 attract OPCs. One study investigated the expression of seven class-3 semaphorins, SEMA4D, VEGF, and the NRP1 and NRP2 receptors in 38 adult glial tumors and showed that Sema3A expression was similarly expressed in low-grade and high-grade gliomas [43]. Sem3A has been shown to promote dispersal of GBM cells [44] and it would be interesting to investigate its role on DLGG migration. Interestingly, Nasarre et al. showed that Sem3A can have either a chemorepellent or a chemoattractant effect on GBM cell lines depending on the present partners [45], thus making it a prime target for glioma migration investigations.

Finally, one important point to emphasize is the specific pattern of migration adopted by OPCs depending on their localization in the brain. Indeed, using heterotypic quail/chick xenografts, Olivier et al. [46] demonstrated that OPCs transplanted from the rostral to caudal brain domain and vice versa changed their migratory properties. Their neat approach identified several routes of migration followed by OPCs depending on their original location and emphasized the key role of environmental cues upon OPC migration. Based on these data, it is easily predictable that gliomas arising from OPC tumorigenesis might comply with the same rules. This hypothesis is consistent with a recent study on GBM [47] showing that SVZ-derived or cortex-derived GBM cells (GBM6 and GBM9, respectively) present different invasive properties. It is thus necessary, when studying DLGG dissemination, to report precisely their site of appearance, so as to be able in the future to predict the more likely pattern of diffusion of a tumor based on its initial localization in the brain.

Issues to Be Addressed

There are several issues that would deserve further explorations in order to clarify the means by which DLGG cells disseminate into the brain:

1. Identifying infiltrated tumoral cells in the normal tissue: Low-grade tumoral cells exhibit only a mild nuclear atypia so they are difficult to distinguishing from normal cells in infiltrated tissues. So until recently it was very difficult to delimit the actual dissemination of tumoral cells which probably extend far beyond MRI-defined abnormalities. Indeed, using serial stereotactic biopsies and MIB1/Olig2 staining, Pallud et al. [48] found that tumoral cells can be observed at sites up to at least 15 mm beyond brain imaging aberrations. A major advance in the field is the development of an antibody recognizing the mutated form of the IDH1 enzyme (R132H) which is commonly found in DLGGs [49]. This antibody allows the precise detection of isolated tumoral cells by immunohistochemistry. Using this tool, Sahm et al. analyzed the infiltration pattern of three anaplastic OGs and considering the widespread dispersion of tumoral cells concluded that gliomas should be addressed not as a focal but as a systemic disease [50]. Similar analysis performed on DLGGs would reveal the actual extends of the tumor.
2. Distinguishing migratory versus nonmigratory glioma cells: The tumoral tissue is composed of cells in different states (for instance, proliferative *vs.* quiescent cells) and it is likely that in DLGG, only a fraction of the cells are actually invading the normal tissue. It will be very useful to uncover reliable markers to identify and purify these cells in order to analyze precisely the molecular components and active pathways involved in this process. Whereas such markers are starting to be uncovered for high-grade gliomas, thanks to differential microdissection of the tumor core versus invasive margin [51], such tools are not yet available for DLGGs. In addition, it appears that DLGGs exhibit two main patterns of spatial organizations: solid tumor plus a diffuse halo of infiltrating cells or dispersed

cloud of cells with no solid bulk [52, 53]. The molecular and cellular mechanisms behind this heterogeneity of patterns are not documented and warrant further exploration.

3. Differential migration in the white and gray matters: Brain white and gray matters differ in many aspects (cellular composition, vascular density, organization, biochemical composition) so it is expected that glioma cells invade these two components in a different way. In particular, the white matter contains myelin which constitutes the insulating sheath for neurons but which is also a nonpermissive substrate for neurite growth, attachment, and spreading of a lot of cell types. Interestingly, Amberger et al. [54] found that low-grade astrocytomas appeared to be strongly sensitive to inhibitors present in myelin, whereas OGs were able to spread and migrate on this substrate. Using the C6 glioma line which showed no spreading inhibition on myelin, this group went on to show that these cells expressed the MMP14 metalloprotease (MT1-MMP). This protease degraded the Nogo protein, a major inhibitor present in myelin [55], and allowed glioma cells to spread on this substrate [56]. It is anticipated that such a mechanism might operate for low-grade OG cell dissemination, but this remains to be formerly demonstrated. In the normal brain, oligodendrocytes are closely associated with nerve fibers, whereas astrocytes interact closely with vessels to form the blood–brain barrier. Therefore, spreading of OGs along nerve fibers and of astrocytoma cells along vessels could represent a pathological counterpart of the normal situation.

Future Prospects

Controlling DLGG cell dissemination in the brain would represent a major advance for treating these diseases. In the nonpathological brain, neural precursor cells are produced and migrate in at least two locations (hippocampus and the subventricular zone). Therefore, any treatment aiming at reducing low-grade glioma cell invasion may potentially interfere with these processes and may have serious side effects. It is thus central to identify molecular targets and/or signaling pathways specific to tumoral cells so as to spare normal cells. As described before, useful insights could be obtained by exploring both the migration of normal and pathological brain cells to find a potential glioma Achilles' heel to target. Not only migrating cells represent deadly infiltrated snipers sneaking into the brain, but these cells may also be more resistant to treatment; as in high-grade gliomas, they may be and less prone to apoptosis apoptotic [57, 58]. Hence, deciphering the molecular and cellular basis for dissemination of diffuse low-grade gliomas represents a major goal to find innovating cures.

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The Molecular Biology of Diffuse Low-Grade Gliomas

8

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Abstract

The World Health Organization (WHO) grading scheme for glial neoplasms assigns grade II to three infiltrating (non-circumscribed) gliomas: diffuse astrocytomas, oligodendrogliomas, and oligoastrocytomas. Although commonly referred to collectively as among the "low-grade gliomas", these three tumors represent molecularly and clinically unique entities. Each is the subject of active basic research aimed at developing a more complete understanding of its molecular biology, and the pace of such research continues to accelerate. Additionally, because prognostication and management of these tumors has historically proven challenging, translational research regarding grade II infiltrating gliomas continues in the hopes of identifying novel molecular features that can better inform diagnostic, prognostic, and therapeutic strategies. Unfortunately, the basic and translational literature regarding the molecular biology of WHO grade II infiltrating gliomas remains nebulous. Our goal for this chapter is to present a comprehensive discussion of current knowledge regarding the molecular characteristics of these three WHO grade II tumors on the chromosomal, genomic, and epigenomic levels. Additionally, we discuss the emerging evidence suggesting molecular differences between adult and pediatric low-grade, infiltrating gliomas. Finally, we present an overview of current strategies for using molecular data to classify low-grade, infiltrating gliomas into clinically relevant categories based on tumor biology.

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Introduction

The term "diffuse low-grade glioma" is commonly used to refer to one of three glial neoplasms assigned to World Health Organization (WHO) grade II: diffuse astrocytoma, oligodendroglioma, or oligoastrocytoma [1]. The WHO system is a purely histologic system that is the most common strategy for classifying gliomas, and "low grade" is often used to describe those gliomas with a microscopic appearance that is "histologically benign". Use of the terms "low-grade glioma" and "histologically benign", however, are falling out of favor, the former because it aggregates a number of dissimilar disease processes with unique molecular, phenotypic, and clinical characteristics and the latter because the absence of aggressive histologic features does not necessarily correlate with a "benign" clinical course in glioma patients. Nonetheless, these entities differ both clinically and molecularly from WHO grade III and IV gliomas, and so they are often discussed together.

Molecular investigation of WHO grade II astrocytomas, oligodendrogliomas, and oligoastrocytomas is an area of active research and occupies a unique position in the world of translational oncology. Because prognostication and management of these tumors has historically proven challenging, the translational research paradigm has been embraced by investigators working on these tumors in the hopes of identifying novel molecular features that can better inform diagnostic, prognostic, and therapeutic strategies. Arguably the most notable translational achievement in neuro-oncology has come from research on WHO grade II gliomas, where chromosomal characteristics are now being routinely used to inform discussions of prognosis and strategies for adjuvant therapy.

Despite these translational successes, the literature regarding the molecular biology of diffuse, low-grade gliomas remains nebulous. The goal of this chapter is to present a comprehensive discussion of the current knowledge regarding the molecular characteristics of these tumors on the chromosomal, genomic, and epigenomic levels. We have endeavored to clarify the many points of potential confusion and apparent contradiction that exist among this body of work, and we have attempted to organize this data into a logical and organized framework through which it can be more readily understood. We first discuss the specific chromosomal, genomic, and epigenomic features of WHO grade II astrocytomas and oligodendrogliomas. Next, we briefly address the oligoastrocytomas, or "mixed gliomas," whose molecular biology generally represents a combination of that of the astrocytoma and oligodendroglioma. Finally, we make additional comments regarding the pediatric diffuse gliomas and provide an overview of the current literature discussing potential molecular strategies for classifying the diffuse, low-grade gliomas.

Background

Diffuse Astrocytoma

The synonymous terms "diffuse astrocytoma" and "low-grade, diffuse astrocytoma" (AII) refer to tumors of astrocytic origin with relatively low proliferative activity and without obvious anaplastic features on histologic examination [2]. The category comprises three histologic variants,

including *fibrillary astrocytoma*, *protoplasmic astrocytoma*, and *gemistocytic astrocytoma* (sometimes described as "variants") [1, 3]. Overall these tumors represent approximately 1.6 % of all gliomas and 2.1 % of astrocytomas and account for 2,700–4,600 new brain tumor diagnoses per year in the USA [2]. They occur with peak incidence in the young adult population (ages 20–34), where they represent approximately 10.2 % of primary CNS tumors, 30.0 % of all gliomas, and 25.2 % of all malignant brain tumors [4]. In this age group their survival rates at 1, 5, and 10 years are 91.6, 58.5, and 40.7 %, respectively [4]. However, these tumors are observed across all age groups and are associated with relatively longer survival times in the pediatric population and with relatively shorter survival times in older adults [4].

In the adult population, most AIIs will ultimately progress to anaplastic astrocytomas and then to "secondary" glioblastomas [1, 5, 6]. This tendency suggests that AIIs represent an early stage in the evolution of secondary glioblastoma, and many of the molecular characteristics described in AIIs are likely to be early steps along the path to full-scale malignant transformation of the astrocyte. For this reason it is difficult to describe a set of genomic and epigenomic features that are unique to this grade of glioma, and descriptions of the molecular biology of AIIs should be viewed through this lens.

Many molecular investigations include a small number of AIIs as one part of larger experimental samples containing various grades of glioma. These studies tend to identify genomic and epigenomic changes that occur with relatively low frequency in AIIs and become more prevalent as gliomas progress to higher grades. Reporting the relative frequency of such changes in AIIs adds little to a focused discussion of AII-specific molecular biology, and interested readers should refer to any of a number of texts on high-grade gliomas that place these findings in the context of the molecular pathogenesis and evolution of glioblastoma [7, 8]. Instead, in this section we summarize those molecular features that appear common to a large proportion of AIIs. These molecular features may logically be assumed to represent at least some of the functionally significant, early subcellular changes involved in the process of malignant astrocytic transformation, and understanding these features may be the most clinically relevant approach to interpreting the molecular biology of AIIs.

Oligodendroglioma

The synonymous terms "oligodendroglioma" and "low-grade oligodendroglioma" (OII) refer to tumors of oligodendroglial histology with low proliferative activity and without obvious anaplastic features on microscopic examination [2]. There are no specific histologic variants of OII [1]. Among all grades of glioma (excluding glioblastoma), oligodendroglioma histology is outnumbered by astrocytic histology by a factor of 3 [1, 8]. They occur with peak incidence in the third to fifth decades [1, 8], and the 1-, 5-, and 10-year survival rates for OIIs in adults are 94.2, 79.5, and 63.6 %, respectively [4]. OIIs are less common in pediatric patients [1], but when they do occur in this age group, they are associated with better survival rates than those for OIIs in adults [4].

OIIs have recently become the subject of considerable attention in translational neuro-oncology research because they represent the first primary brain tumor that can be routinely and consistently stratified by molecular features into two clinically distinct subgroups. OIIs with "deletions" of chromosome 1p ± 19q are associated with a relatively longer survival and may exhibit improved response to adjuvant therapy, whereas those in which chromosome 1p ± 19q is intact behave more aggressively [1]. This finding supports the long-standing concerns of many neuro-oncologists that histologic subtypes of glioma may not adequately capture all clinically relevant variability among these tumors [9, 10] and serves as important proof of principle for ongoing investigations for molecular subclassification of gliomas.

Chromosomal Abnormalities

Diffuse Astrocytoma

The most common chromosomal abnormalities in AII are trisomies or polysomies of chromosome 7 [1, 3], with gains of 7 or 7q observed in approximately 50 % of these tumors [11, 12]. Gains in 8q have also been reported to occur with some consistency in AIIs [13], and gains of 5p, 9, and 19p have also been inconsistently observed [3, 8, 14]. Chromosomal losses in AIIs have been reported most commonly involving chromosome 17p [8, 13, 15] and less frequently on chromosomes 6q [16], 10p, 13q, 19q, and 22q and the sex chromosomes [3, 8, 14].

Oligodendroglioma

The most common chromosomal abnormality in OIIs, occurring in approximately 50 % of these tumors (although some report 80+ %) [2, 17–24], is a combined "loss" of the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q) [1, 8, 17]. These tumors demonstrate *loss* of one entire copy of these chromosomal arms due to an unbalanced t(1;19)(q10;p10) translocation [25, 26], and this finding is commonly (although technically inaccurately) described as "1p/19q codeletion". Conversely, partial deletions of these loci [1] or isolated loss of 1p [8] are rare. Of the two chromosomal losses, 1p has the greater specificity, as 19q losses have been observed in other histologic types and grades of glioma [27]. Notwithstanding, 1p/19q codeletion is not completely specific to OIIs, as it has also been occasionally reported in astrocytomas, oligoastrocytomas [8], and glioblastomas [28]. Combined losses of 1p/19q appear to be mutually exclusive of several other molecular abnormalities commonly associated with gliomas, including loss of heterozygosity (LOH) on 17p and *TP53* mutation [29–32]. This suggests that the molecular pathway leading to the 1p/19q codeleted OII may be distinct from those involved in other forms of glioma pathogenesis [14].

The exact molecular mechanisms associated with the development of the unique t(1;19)(q10;p10) translocation in OIIs are not yet fully understood. Recent evidence suggests that the centromeric regions of chromosomes 1 and 19 show a high degree of sequence homology [33]. This has been hypothesized to result in centromeric co-localization of chromosomes 1 and 19, which might promote centromeric instability and thus favor the translocation [26, 33]. Additional investigations regarding the specifics of this process and the clinical and molecular significance of this finding are ongoing.

Additional chromosomal abnormalities have also been reported in OIIs, although less frequently than 1p/19q codeletions. These include deletions involving chromosomes 4, 6, 11p, 14, and 22q [18, 20] and occasional losses of chromosomes 9 and 10 [1]. Array-based comparative genomic hybridization has also suggested submegabase deletions associated with OIIs, including 300–550 kb regions on 11q13 and 13q12 [34]. The validity and consistency of these focal deletions remains to be determined.

Genomic Abnormalities

Diffuse Astrocytoma

TP53

The *TP53* gene localizes to chromosome 17p13.1 and its protein product (p53) is involved in several cellular processes, including cell cycle regulation, response of cells to DNA damage, cell differentiation, and cell death [35]. Activated p53 induces transcription of $p21^{Waf1/Cip1}$, which regulates cell cycle progression at G_1 via its activity on cyclin-CDK complexes [15, 16]. The activity of p53 is modulated by MDM4 (MDMX) as well as by MDM2, the latter of which is modulated [36] by $p14^{ARF}$.

Sixty (60 %) to 80 % of AIIs have allelic loss on 17p that includes the *TP53* locus [8, 14, 15], and most AIIs with the retained locus exhibit *TP53* mutations [8, 37–39]. This makes complete absence of wild-type p53 the most common

genomic abnormality in AIIs [8, 14]. The incidence of *TP53* mutations is higher in secondary than in primary glioblastoma [40, 41] but does not increase appreciably between AIIs and glioblastoma [42–45], lending genome-level support to the hypothesis that AIIs represent an early stage in the evolution of secondary glioblastoma [1, 2, 36]. This hypothesis is further supported by the findings that common *TP53* mutations both in AIIs [46] and in secondary glioblastomas [41] occur at codons 248 or 273 (while the *TP53* mutations observed in primary glioblastomas are more broadly distributed) and that G:C A:T mutation in CpG islands are more frequent in secondary than in primary glioblastoma. The latter observation suggests that different mechanisms may lead to the acquisitions of the *TP53* mutations seen in these two glioblastoma subtypes [8, 41].

Isocitrate Dehydrogenase

The enzyme isocitrate dehydrogenase (IDH) catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate in the citric acid cycle and uses $NADP^+$ as a proton acceptor [47]. A total of five IDH isozymes have been described, although IDH1 and IDH2 are currently believed to be the most relevant to glioma biology. The IDH1 enzyme localizes to the cytosol and peroxisome [47], while the IDH2 enzyme assumes the more classic, mitochondrial localization [48]. A genome-wide analysis of glioblastoma identified *IDH1* (2q33) [48, 49] gene mutations in 12 % of these tumors [50], prompting additional investigations into the potential role of *IDH* mutation in glioma biology. Subsequent studies demonstrated that *IDH* mutations are most common in WHO grade II and III gliomas as well as in secondary (but not primary) glioblastomas [51, 52]. Approximately 80 % of AIIs have been shown to harbor *IDH1* gene mutations, and *IDH2* (15q26.1) gene mutations are often present in the residual fraction [51]. This finding makes *IDH* gene mutations the most common and consistent genetic abnormality in AIIs reported to date. Notably, there does not appear to be a statistical association between *IDH* mutations and *TP53* mutations in AIIs [53], although these data remain inconsistent [36, 54].

The specific IDH1 mutation observed in low-grade gliomas is almost always (>90 %) [55] a point mutation at position 132, where wild-type arginine is replaced by histidine in the mutant form (R132H) [53]. Other rare mutations at this position include substitutions of arginine with cysteine (R132C), serine (R132S), leucine (R132L), glycine (R132G), or valine (R132V) [51, 53]. These mutations are all heterozygous, and no truncation or frame shift mutants have yet been described [56]. Position 132 belongs to an evolutionarily conserved region representing the binding site of the isocitrate substrate [53], and the R132 mutations result in reduced enzymatic activity toward isocitrate [51, 52, 57]. Recent kinetic studies have demonstrated that R132 mutations alter the relative affinity of the IDH1 active site, favoring α-ketoglutarate over isocitrate and resulting in increased production of α-hydroxyglutarate in cells harboring the mutation [58]. Structural investigations have suggested a mechanistic explanation for this observation related to its effects on subunit dimerization [59], and a "dominant inhibition" model whereby concurrent underproduction of α-ketoglutarate and overproduction of α-hydroxyglutarate may favor oncogenesis has been proposed [56]. Supplementary hypotheses include contributions to oncogenesis through induction of the HIF-α pathway [57], while others suggest that IDH mutations may not be oncogenic but may instead represent protective mechanisms that interfere with the metabolism of tumor cells [60].

IDH2 is the only human protein homologue of IDH1 that uses $NDAP^+$ as a proton acceptor [51], and its arginine at position 172 (R172) is exactly analogous to R132 in IDH1. Five point mutations have been identified in IDH2, resulting in replacements of R172 with glycine (R172G), methionine (R172M), lysine (R172K), serine (R172S), and tyrosine (R172Y) [51, 61, 62]. Kinetic and structural studies of IDH2 have not been as extensive as those for IDH1, but the strong similarities between these isozymes and the involved mutations suggest comparable underlying biology.

PDGFR

The platelet-derived growth factor receptor (PDGFR) is a tyrosine kinase receptor that interacts with the RAS pathway (and thus the PI3K/PTEN/AKT/mTOR pathway) [36] via the SOS-Grb2 intermediary [63, 64]. As downstream pathways also modulated by the epidermal growth factor receptor (EGFR), PDGFR-associated pathways have been of considerable interest in glioma research [36]. This has the potential to lead to some degree of confusion regarding the relative importance of these pathways in AII versus glioblastoma, and it is therefore important to clarify the current molecular evidence regarding PDGFR pathways in AIIs.

A number of preclinical and translational studies have reported putative roles for various components of the PDGF/PDGFR proteins in the biology of glioblastoma [36, 65, 66]. However, despite being perpetuated throughout the glioma genomics literature [1, 36] as being overexpressed in up to 60 % of AIIs [1, 14], firm evidence for PDGFR overexpression in AII is sparse. Two small studies from the early 1990s [67, 68], each including only five AIIs in their analyses, reported that PDGFR-α appeared overexpressed in gliomas of all grades, including AIIs. Attempts to validate this finding have been inconsistent [69, 70], and ascribing an important, functional role to PDGFR-α in AII on the basis of current evidence appears premature. This distinction is even more important given numerous reports suggesting a role for the overlapping EGFR/RAS/PI3K/PTEN/AKT/mTOR pathway in the biology of primary but not secondary glioblastoma [36] and the possible mutual exclusivity between p53 mutations and EGFR overexpression [43]. Moreover, EGFR overexpression is currently considered to be one factor that distinguishes primary from secondary glioblastoma, as it is observed in approximately 40 % of the former but is rare in the latter [36, 41, 43, 71, 72]. Given these data, it appears that the tyrosine kinase receptor pathways may be of much greater significance to primary glioblastoma biology than to the biology that defines the AII-secondary glioblastoma spectrum.

Other Genomic Abnormalities

A comprehensive meta-analysis [73] of studies specifically reporting on gene expression in low-grade gliomas performed through 2006 identified only 11 studies [69, 74–83] describing specific patterns of gene expression in grade I and/or grade II gliomas. The investigators summarized these results and then verified the most commonly reported gene expression patterns using RT-PCR [73]. With regard to gene expression in AIIs, the authors reported data from six studies [69, 74, 75, 77, 80, 83] comparing expression in AIIs versus normal controls. They found consistent evidence for underexpression of the *TYRO3* gene and for overexpression of the genes, *CD9*, *TIMP3*, *CSPG2*, *EGFR*, *PDGRFA*, and *NTF3*, as well as a single report of overexpression of *KCNN3* [73]. Comparison between AIIs and glioblastoma revealed no instances of specific gene overexpression in AIIs relative to glioblastoma but found consistent evidence for relative underexpression of *NCAM1*, *FN*, *EGFR*, *VEGF*, *IGFBP2*, *IGFBP3*, and *IGFBP5* as well as an isolated report of underexpression of *MMP16* [73].

In light of the previous comments regarding PDGFRA and EGFR, additional clarification regarding some of these genomic findings [73] is necessary. Review of the source publications in which *PDGFA* and *EGFR* expression differences were noted [69, 80, 83] demonstrates relatively small sample numbers, and two of the three [69, 83] studies were reported by the same research group. One of these studies [69] reported >2-fold overexpression in *PDGFRA* to be present in only two of ten AIIs analyzed. Accordingly, we caution against drawing firm conclusions from these data regarding the actual role of *EGFR* and *PDGFRA* in AIIs, as considerable evidence (described above) suggests that these genomic features are more consistently associated with higher-grade gliomas.

Additional reports involving AII genomics include those that characterize expression and propose potential roles for human herpesvirus-6 variants [84], the *LGI1* [85] and *BR-3* [86] gene products, and the *SoxD* and *SoxE* gene families [87] in AII biology and in malignant progression

of gliomas. Additional research is necessary before definitive conclusions can be made regarding the putative roles and overall significance of these candidate molecules.

Oligodendroglioma

1p/19q Candidate Genes

Despite consistent and convincing evidence for 1p/19q deletions in OII, the specific gene(s) whose loss is associated with the unique clinical phenotype of codeleted OIIs (see below) remains unclear. Proposed candidate genes on 1p include *Notch2* (1p13-p11) [88], *DIRAS3* (1p31) [89], *CDKN2C* (1p32) [90], *RAD54* (1p32) [91], *CITED4* (1p34.2) [92], *CAMTA1* (1p36) [93], *DFFB* (1p36) [94], *TP73* (1p36.3) [95], and *SHREW1* (1p36.32) [96]. Because 19q is completely lost in the OII translocation, mapping studies for identification of candidate gene regions on this chromosome have focused on brain tumors of other histologic types with partial deletions of 19q [27, 97–100]. These studies have suggested a potential role for several genes on the 19q3 region [27, 98–100], but additional investigations have not demonstrated consistent mutations of these genes [101]. Epigenomic studies (see below) suggest potential roles for *ZNF342* (19q13) [102], *p190RhoGAP* (19q13.3) [103], *EMP3* (19q13.3) [104], and *PEG3* (19q13.4) [105, 106], but definitive evidence for any of these candidate genes has yet to be demonstrated [1, 14, 107].

Isocitrate Dehydrogenase

As in AIIs, IDH1 (and/or IDH2) mutations are common in OIIs [36, 51, 53, 61, 62] and have been observed in >80 % of these tumors [51]. Many of the studies regarding the specific mutations and their functional significance have been conducted on mixed populations of AIIs and OIIs, and thus, the IDH1 R132 and the IDH2 R172 mutations are believed to be the relevant abnormalities in both tumor types. Although the high rate of IDH mutations in both AIIs and OIIs initially suggested that these mutations were independent of other molecular features that differentiated these tumor types [36, 51, 53, 61, 62], more recent evidence suggests that there may be a high degree of correlation between IDH mutations and chromosome 1p/19q codeletions [62]. Many of these investigations are conducted with populations containing a mixture of OIIs and AIIs [53, 62] and do not stratify independently by 1p/19q status and WHO grade, limiting the ability to study the relationship in detail. One investigation where stratification was performed, however, demonstrated 1p/19q codeletions in 85 % of tumors with IDH mutations, while no tumors with wild-type IDH were found to be 1p/19q codeleted [51]. The pathophysiologic significance of this finding remains to be determined.

Other Abnormalities

EGFR amplification has been reported in approximately 50 % of OIIs, although this represents older data from small studies of relatively few tumors [108]. PDGFA and PDGFB as well as their receptors (PDGFR-α and PDGFR-β) appear to be overexpressed in a large percentage of OIIs [109], making this finding more common among these tumors than in AIIs. More recently, overexpression of *rPTPβ/γ* has been reported to distinguish OIIs from AIIs [110].

Epigenomic Abnormalities

Diffuse Astrocytoma

Epigenomic investigations represent a relatively recent area of research in the molecular biology of AIIs. The most robust epigenomic data involves the *ARF* gene [111, 112], which localizes to the *CDKN2A* (*INK4/ARF*) locus on chromosome 9p21 [111, 113]. Its gene product, $p14^{ARF}$, binds to MDM2 and stabilizes both MDM2 and p53 [111, 113–115]. Accordingly, methylation of the $p14^{ARF}$ promoter results in decreased production of the p14 gene product and relative destabilization of MDM2 and p53. In a single study, *ARF* ($p14^{ARF}$) promoter hypermethylation has been documented in 26 % of AIIs, which was frequently observed in AIIs without primary p53

mutations [112]. All AIIs in this study harboring *ARF* ($p14^{ARF}$) promoter methylation ultimately progressed to secondary glioblastomas. Similarly, promoter hypermethylation of the DNA-repair gene *O^6-methylguanine-DNA methyltransferase* (*MGMT*) has also been observed in 63 % of AIIs [112]. Interestingly, limited data suggests that *MGMT* hypermethylation is associated with p53 mutation but is mutually exclusive to *ARF* ($p14^{ARF}$) gene hypermethylation [14, 112]. Additional reports suggest epigenomic silencing of the *PCDH-γ-A11* (5q31) [116], *PTEN* (10q23.31) [117], and *EMP3* (19q13) [118] genes in AII, and further investigations are likely to reveal additional instances of epigenomic abnormalities in these tumors [119, 120].

Oligodendroglioma

OIIs demonstrate lower levels of MGMT expression than AIIs [2, 121]. Some evidence suggests that up to 60–80 % of OIIs may exhibit hypermethylation of the *MGMT* promoter [122–124] (more common than in AIIs) and that this hypermethylation correlates with chromosome 1p/19q loss [122, 125], while others have not observed these effects [126, 127]. Additional genes that have been found to be hypermethylated in some OIIs include *CDKN2A* (9p21), *CDKN2B* (9p21), *ARF* (9p21), *RB1* (13q14), *TP73* (1p36.3), *DAPK1* (9q34.1), *ESR1* (6q25.1), *TIMP3* (22q12.3), *THBS* (15q15), and *GSTP1* (11q13) [20, 124].

Clinical Correlations

Diffuse Astrocytoma

Few molecular markers have demonstrated prognostic significance in AIIs. The evidence is most comprehensive for the putative relationship between p53 status and clinical outcomes, but even here the results remain unclear. Early investigations demonstrated no apparent relationship between p53 expression levels and overall survival [128]. The literature presents conflicting evidence regarding a potential relationship between abnormalities in p53 and malignant progression, with data arguing both for [129] and against [44] a potential association. Several studies agree, however, that p53 mutation does appear to be associated with an increased likelihood of tumor recurrence [44, 46, 129]. One possible explanation for these nebulous findings may be that the relationship between p53 status and clinical outcomes varies between subtypes of AII. For instance, some investigators have suggested that much of the overall prognostic impact of p53 status may be related to its disproportionate association with the gemistocytic AII subtype [46]. Another possible explanation may be that specific p53 mutations are associated with unique prognostic profiles. This is exemplified by the apparent correlation between codon 175 *TP53* mutation and an increased risk of progression and malignant transformation [46].

Other genomic and epigenomic changes may also have prognostic implications. *IDH1* and *IDH2* gene mutations have been suggested as markers of more favorable survival phenotypes [61, 62, 130], although many of the studies in which this has been demonstrated do not necessarily separate AIIs from oligodendrogliomas. It therefore remains possible that disproportionate overrepresentation of oligodendroglioma in the experimental samples of these studies influenced the results, and the ultimate generalizability of these potential prognostic biomarkers specifically to AIIs remains to be determined. EGFR [70, 72] (although uncommon in AIIs) and PDGFR [70] overexpression may be associated with shorter survival times in patients with AIIs. Additionally, *MGMT* promoter methylation has been associated with response to chemotherapy and thus to improved survival in AII patients [131].

Oligodendroglioma

Perhaps the most widely reported molecular finding with a clinical correlation is the relationship between the combined loss of chromosomes 1p/19q and improvements in survival [24, 132–135] and response to chemo- [136–139] and radiotherapy

[140]. Data regarding the prognostic significance of *TP53* mutation status and/or LOH 17p13 specifically in OIIs is limited, but some evidence suggests that these may be independent, unfavorable predictors of overall and progression-free survival [141, 142]. Gains on chromosome 8q may also be associated with poor outcomes in OIIs, but this data is derived from a relatively small study on a population of oligodendrogliomas of mixed WHO grades [143]. While other correlations between molecular markers and survival or response-to-therapy phenotypes have been reported [14, 20], these have almost always been studied primarily in OIIIs, making their generalizability specifically to OIIs unclear.

Oligoastrocytoma

Oligoastrocytomas (OAII), also called "mixed gliomas" [1], represent a unique WHO class of grade II glioma that is characterized by tumors exhibiting a mixture of astrocytic and oligodendroglial histologic morphology. Molecular evidence suggests that this histologic class may comprise an unbalanced mixture of two primary tumor genotypes, AII and OII [1, 20, 29]. This is supported by the observation that 30–50 % of OAIIs exhibit chromosome 1p/19q codeletions [17, 19, 23, 29] (OII-like), while approximately 30 % carry *TP53* gene mutations [17, 19, 29, 31] (AII-like). Moreover, OAIIs with 1p/19q codeletions have been observed to exhibit more prominent oligodendroglioma-like features on microscopic examination, whereas those with *TP53* mutations are more histologically similar to astrocytoma [29].

One study has proposed that chromosomal data may be useful for subdividing OAIIs into four subclasses [144]. This approach may be reasonable if OAII is a genotypically distinct tumor type but may introduce unnecessary complexity if it is nothing more than a mixture of AII and OII genotypes. This proposed scheme has not been further validated, but it underscores the translational relevance of determining the true genotypic nature of OAII. Without such data only broad correlations of genotype with phenotype are possible for this WHO class, such as recent investigations suggesting that 1p/19q codeletions may be a generally favorable prognostic factor in OAIIs [145].

While addressing this issue is important, it remains difficult to draw from current data firm conclusions regarding the degree to which OAII biology is novel versus the extent to which the biological observations in OAII can be explained simply as a mixture of AII and OII genotypes. One directly related but seldom-discussed factor that should be considered when interpreting molecular analyses of OAIIs is the method of extraction of molecular material from the tissue samples. Experimental protocols that homogenize tissue blocks are likely to extract biological samples for analysis that are heterogeneous mixtures of the molecular constituents of both the oligodendroglia-like and astrocytoma-like tumor regions, while those that use microdissection of specific regions may be more likely to isolate molecular material that is biased toward one of the two constituent cell types. Studies employing the latter methodology are presently lacking, but such investigations are necessary if comprehensive, comparative molecular analyses of the fundamental similarities and differences between tumors classified as OAII, AII, and OII, as well as careful investigations of the clonal origins of OAIIs, are to be performed.

Pediatric Grade II Infiltrative Gliomas

Clinical evidence shows that WHO grade II infiltrative astrocytomas in pediatric patients have a lower rate of malignant transformation than those in adults (10 % vs. 90 %) [146]. These findings suggest that, despite identical WHO classification, pediatric grade II infiltrative gliomas may represent a unique disease process that could be expected to harbor a novel genotype. Current evidence regarding this hypothesis is nebulous, and it is difficult to draw definitive conclusions regarding the molecular comparability of adult and pediatric grade II infiltrative gliomas. While a complete discussion of the molecular differences between adult and pediatric glioma genomics is outside the scope of this

chapter, a brief overview of the current status of this data is beneficial in order to draw attention to this persistent ambiguity.

Most investigations of specific molecular differences between pediatric and adult low-grade gliomas have thus far been conducted at the chromosomal level. While 50 % or more of adult infiltrating gliomas may have some form of chromosomal abnormality [11, 12, 17–23], rates for comparable abnormalities in pediatric patients have been reported to be relatively lower [147–154]. Notwithstanding, chromosomal abnormalities in these pediatric tumors are not rare [154]. For example, rates of chromosome 1p and 19q loss in pediatric populations may be similar to [155] or greater than [156] those in adults, although they do not appear to be associated with the same prognostic significance in children [155].

Definitive conclusions regarding the actual rate of chromosomal abnormalities in pediatric diffuse infiltrating grade II gliomas, as well as the clinical significance of these findings, are difficult to determine definitively based upon current data. Most relevant studies combine (often disproportionately) grade II gliomas with gliomas of other grades for aggregate analyses of "low-grade gliomas". Aggregation with either pilocytic astrocytomas, in which chromosomal abnormalities are known to be uncommon, or with anaplastic (grade III) gliomas, in which prognosis may differ, may significantly bias results [147–156]. When the primary data are presented such that infiltrating glioma karyotypes can be examined independently [147, 148, 153, 154], the rates of chromosomal abnormalities generally appear higher in the grade II subgroup than is reported for the aggregate data set. This suggests that disproportionate inclusion of pilocytic astrocytomas may artifactually dilute the commonly reported rates of chromosomal abnormalities in pediatric infiltrative low-grade gliomas and that these may, in fact, approach those of the adult population. Similarly, conclusions regarding the prognostic implications of 1p/19q status in grade II gliomas may not be generalizable from the population of predominantly grade III patients in which it was studied [155]. Data interpretation is further complicated by the relatively low absolute number of infiltrating gliomas included in many of these studies.

Genomic profiling studies comparing adult and pediatric gliomas suggest that, in general, transcriptome-level differences may exist between these entities [157], but data on differential rates of expression of specific genes is currently limited. Some evidence suggests that EGFR overexpression may be relatively more common in pediatric tumors [158]. Conversely, OLIG2 expression may be relatively less common [159]. The clinical significance of these findings remains to be determined.

Molecular Classification of Low-Grade Gliomas

This chapter highlights a number of molecular characteristics of low-grade glioma subtypes that may have prognostic and therapeutic relevance. However, because the current WHO system relies solely on histologic features for classification [1], there is currently no formal mechanism by which molecular data can be used to improve the accuracy of glioma classification. Additionally, ambiguous WHO criteria can make classification of some low-grade gliomas challenging and can introduce subjectivity that may limit the reproducibility of glioma classification [160]. Accordingly, several investigators have suggested that molecular strategies for glioma classification be considered, and numerous efforts have been made toward developing these strategies for low-grade gliomas.

While a comprehensive review of the topic of molecular classification of low-grade gliomas is outside the scope of this chapter, an overview of the proposed general approaches to such classification is appropriate. Several proof-of-principle studies have demonstrated the ability to use molecular data to stratify low-grade gliomas into classes that overlap with the WHO scheme [9, 161]. From here, a number of specific strategies have been applied to the task of molecular classification of these tumors. Approaches based on the expression of single genes or gene products have been successful at resolving some of the difficulties associated with purely histologic differentiating between AII, OII, and OAII [110], and strategies employing various combinations of genomic and chromosomal data have

demonstrated similar success in this task [31, 162]. Classification techniques based solely on genomic data for a small subset of genes have also been successfully applied to the task of molecular stratification of various categories of low-grade gliomas [163], as have schemes that use more comprehensive sets of gene expression profiles [9, 82, 164]. Recently, epigenomic profiles involving patterns of CpG island methylation have also been used to define subsets of grade II gliomas with apparent differences in survival phenotype [165]. The actual methods for classification using molecular data vary from simple algorithms based on one or a few markers [31, 110, 163] to more complex mathematical models based on aggregate molecular data sets [9, 161, 162].

Issues regarding the practicality of implementation and utilization of molecular classification schemes for low-grade gliomas, the accuracy of putative molecular class discriminators, and the optimal approach for maximizing research, diagnostic, and clinical utility of molecular classification strategies are yet to be fully resolved [160]. Nevertheless, there is considerable optimism in the translational neuro-oncology community that molecular data will ultimately prove to be a useful adjunct for classification of low-grade gliomas.

Conclusion

Molecular and translational research in WHO grade II diffuse gliomas remains an area of active research through which several, practical discoveries have already been made. Future investigations in this arena will include attempts to clarify the relative importance of potentially clinically relevant molecular markers, including p53, chromosomes 1p and 19q, and IDH1 and IDH2; endeavors to expand upon preclinical discoveries of novel potential markers; and efforts to incorporate molecular markers into tumor classification strategies. The translational neuro-oncology community remains optimistic that significant progress to further understand the pathophysiology, clinical behavior, and optimal management of "diffuse low-grade gliomas" will continue to be made in the coming years.

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Proteomic Studies in Low-Grade Gliomas: What Have They Informed About Pathophysiology?

9

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Abstract

The study of normal, aberrant, and dysregulated proteins (proteomics) is now becoming an established technique in biological research. Proteomics has been widely applied in biological investigations of systemic cancers and also in high-grade gliomas (HGGs). However, relatively little work has been done on questions evaluating biological aspects of low-grade gliomas (LGGs). In this chapter, the proteomic literature on LGGs is critically and systematically reviewed. Protein lists from individual studies are summarized, and differences between "control" brain tissue and LGG, LGG and HGGs, LGGs with and without 1p/19q deletions, and the impact of IDH1 deletions are evaluated. Web-based bioinformatics tools, IPA and DAVID, are also used to assess protein-protein interactions between proteins differentially expressed in LGGs. Two highly significant and important functional protein networks are identified. One in silico network reveals underlying differences between LGG and control brain, and the other reveals underlying differences between HGGs and LGGs. The roles of cell proliferation, apoptosis, and aberrant subcellular processes are highlighted. In addition, the nascent literature on 1p/19q, and IDH1 deletions is reviewed. The findings from these studies show that systematic analysis of proteomic data in LGGs is much more informative than data

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derived from single studies. The lack of consistent proteomic differences identified between the various studies also highlights problems in proteomic methodologies and investigative study design. The results from this review provide novel insights into LGG biology and give some direction for focus of future studies.

Keywords
Astrocytoma • Oligodendroglioma • Proteomics • Protein-protein interactions • Functional protein networks • Oncogenesis

Abbreviations

GBM	Glioblastoma multiforme
HGG	High-grade glioma
LGG	Low-grade glioma

Introduction

Proteomics is the umbrella term for the study of protein content of tissues. It is the natural sequitur to the application of genomics to clinical medicine. As scientists seek the genetic mutations that underlie human diseases, proteomics clarifies and discovers the proteins that are up- or downregulated (since normal, dysregulated, and/or mutated genes are not always transcribed due to epigenetic factors) and detects posttranslational modifications that may result in a protein functioning aberrantly. This dissociation between genetic abnormalities and protein expression has been well demonstrated in gliomas [1]. Proteomics also allows for detection of downstream effects of genetic mutations; a mutated protein itself may have no direct effect, but the affected pathway it is part of might be modified, altering biological processes elsewhere. In this way proteomics provides a fundamental link between known genetic defaults and pharmacology. As yet we have not managed to create simple methods of correcting mutations, but if we understand aberrant protein-protein interactions, it may be possible to produce drugs that interfere and modify dysfunctional metabolic and proliferative pathways [2].

There are a number of different proteomic research methodologies now available. As computerization and technologies have advanced, the number of possible proteins that can be detected simultaneously has increased exponentially (see Fig. 9.1). Initially, it was only feasible to examine a single protein in isolation with the traditional method of western blotting using protein-specific antibodies (e.g., Chakravarti et al. [3]). This was useful for confirming specific hypotheses (often based on genomic information) but very limited in terms of understanding global proteome modifications. Two-dimensional gel electrophoresis (2DGE) combined with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) was then developed and allowed for an open detection of hundreds of proteins simultaneously with no preconceptual bias. A disadvantage of this "classical" high-throughput approach, however, is that it is an extremely labor-intensive process, involving the running of 2D gels, gel staining, protein spot matching, spot picking, protein digestion, and finally protein identification by mass spectrometry. More recent and sophisticated technology, liquid chromatography-mass spectrometry (LC/MS), has enabled much more rapid, less labor-intensive high-throughput studies and has enabled detection of even greater numbers and smaller quantities of proteins. In general, the mass spectrometry approach allows researchers to embark on a study with no preconceptions (as previously described) and therefore has the promise of exciting new discoveries; however, the enormous volume of data that is generated can often lead to shallow or underpowered analyses. LC/MS studies typically detect and quantify the levels of thousands of proteins, so require complex statistical analyses to rigorously confirm novel findings [4–6].

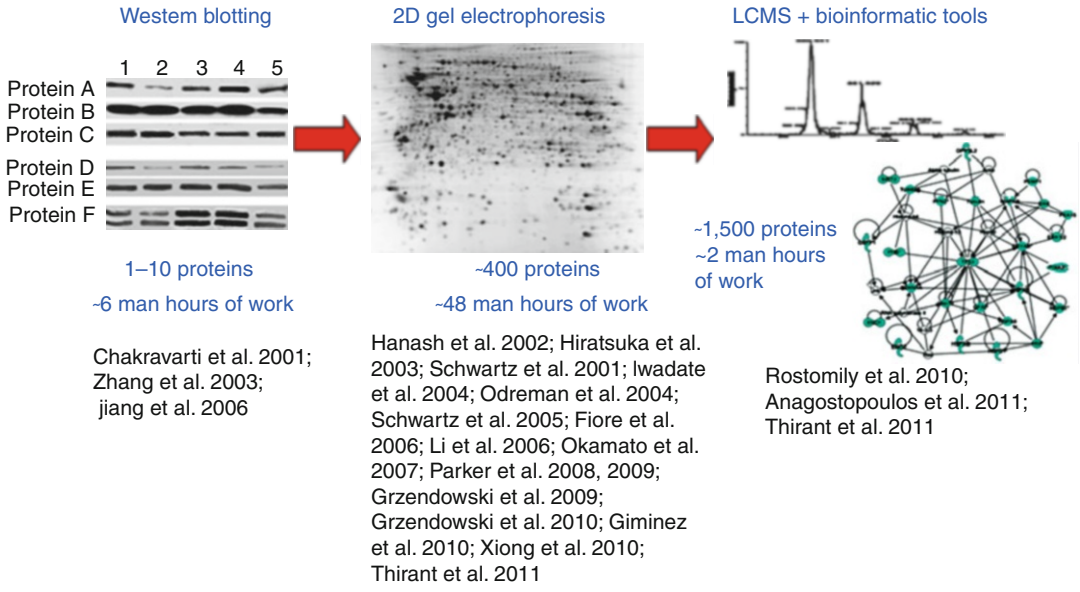

Fig. 9.1 Advancing proteomic technologies. This schematic summarizes how protein detection and identification technologies have advanced over the past two decades: from western blotting (detecting single proteins by targeting with protein-specific antibodies) to 2D gel electrophoresis (involving non-prejudicial, simultaneous detection of ~400 proteins) and to liquid chromatography-mass spectrometry (LC/MS) (comprising non-prejudicial, simultaneous detection of ~1,500 proteins) combined with downstream functional pathway/network analysis (Represented by an IPA network; www.ingenuity.com). In summary, the number of possible proteins detected simultaneously has increased exponentially, and there has been a trend towards less labor-intensive technologies. Proteomic studies of LGG, reviewed in this chapter (see Table 9.1), are listed by the technology used

This review focuses on the proteomics of low-grade gliomas (LGGs, WHO I–II) in comparison to the proteome of "healthy" control tissue and high-grade gliomas (HGGs, WHO III–IV). There have been several recent reviews of glioma proteomics [7–11], but these have not specifically addressed the topic of LGGs and indeed are most frequently focused on HGGs and insights offered by proteomics [10]. There are several key themes to this review. Firstly, we have reviewed papers that examine protein levels in LGGs relative to controls to investigate the initial changes required for glioma cells to become tumorigenic. Secondly, we have reviewed studies that have focused on the comparative proteomics of LGGs relative to HGGs to try and understand why the former progress into more aggressive WHO grade III and IV tumors. In addition, and perhaps more importantly, many of the studies evaluated listed specific proteins of interest without considering their importance in terms of protein-protein interactions and the functional networks key to specific proteins. We have therefore collated all the differentially expressed proteins described so far in LGG proteomic papers (relative to controls or HGGs) and have collectively analyzed these using two bioinformatics platforms (IPA and DAVID analysis). These powerful in silico Web-based tools and computer programs help facilitate biological insight into disease pathophysiology by identifying protein-protein interactions between proteins found altered in proteomic studies and introducing other key hub proteins that may also interact with these altered proteins [12, 13]. Fourthly, clinical and genetic studies have noted the association between deletion of the 1p and 19q chromosomal arms in oligodendrogliomas of both WHO II and III grades and better prognosis, however, as yet the downstream proteomic and metabolic reasons are not known [14]. We have therefore, where possible specifically evaluated proteomic studies comparing gliomas with and without these mutations. In addition, one proteomic study that investigates IDH1 genetic mutations (a mutation also correlated with better survival) is reviewed. The significance of the

findings from the review and their clinical implications are discussed.

Methods

Systematic Review of Human Low-Grade Glioma (LGG) Proteomic Literature

A comprehensive literature search of "PubMed," "Web of Science," and "Embase" was first performed using the general terms of "glioma" and "proteomics." Then to try and capture all appropriate LGG proteomic studies, the same three search engines were probed with the following more specific terms: "astrocytoma," "pilocytic astrocytoma," "subependymal giant cell," "oligodendroglioma," "oligoastrocytoma," "protein quantification." and "LC/MS." The final literature search for this review was carried out in 27 December 2011.

Once a list of potential papers was generated, they were sorted manually for inclusion against set criteria. Only peer-reviewed studies that used in vivo samples taken from human gliomas mid-surgery (i.e., no glioma cell line studies or studies of postmortem samples) were included in this review. The studies however could use a range of proteomic methodologies (e.g., western blotting, 2DGE/MALDI-TOF, or LC/MS), and control samples could be taken from a range of tissues including peritumoral brain, epileptic brains, and fetal astrocytes (see Table 9.1). Brief evaluation of the data determined that it was necessary to be able to differentiate comparative proteomic results in LGG from HGG and control samples.

Bioinformatics Analysis of Proteins Differentially Expressed in LGG

Differentially expressed proteins listed in the LGG proteomic literature (relative to controls or HGGs) (omitting proteins listed in the paper by Anagnostopoulos et al. [15], for reasons described below) were collectively analyzed using two bioinformatics platforms to assess putative biological pathways that incorporate these proteins and consequently gain further insight into LGG pathophysiology. First, proteins were uploaded to Ingenuity Pathway Analysis software (IPA; Ingenuity Systems, Mountain View, CA, USA, http://www.ingenuity.com). IPA contains a huge knowledge database of biological and chemical information extracted from the literature and can generate non-prejudicial protein-protein interaction networks from uploaded protein lists. Each network is assigned a score and ranked according to the inclusion of as many of the proteins inputted as possible. Second, proteins were uploaded to the Database for Annotation, Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov) [16]. DAVID also contains a knowledge base of biological information and has been used as a complementary method to IPA to discover functionally related protein groups enriched in the uploaded protein list, providing an overview of major biological functions associated with the list.

Results

A Summary of Extant LGG Proteomic Studies in the Literature

A total of 23 articles were identified from the literature [3, 14, 15, 17–36]. The papers all focus on the analysis of whole cell lysate samples except for the study by Rostomily et al. [34], which analyzes three distinct biochemical fractions (cytoplasmic-, nuclear-, and mitochondrial-enriched fractions). Several papers also employ microdissection techniques on selected tumors to try and circumvent the histological heterogeneity of the tumors [26, 30, 32, 36]. Unfortunately, the full manuscript for one article could not be accessed so has been omitted from further review [35]. From the remaining 22 articles, 17 papers have applied proteomics to look at protein changes in LGGs compared to either control or HGG samples. In addition, 3 papers evaluated the protein consequences of 1p and 19q chromosomal deletions and 1 paper evaluated the protein consequences of IDH1 mutation, and are discussed separately (see Table 9.1). Of the former 17 papers, 14 group-style analyses described less

Table 9.1 Overview of the human biopsy LGG proteomic literature

A. *Proteomic studies of LGG compared to control brain or HGG*

Date	Author/reference	*n*	Pathology WHO grade	Method	Control	Comparison	No. altered protein
2001	Chakravarti et al. [3]	96	56 GBM 13 anaplastic astrocytoma 1 anaplastic oligodendroglioma 12 LGG astrocytoma 12 other LGG	Western blot	Fetal astrocyte	LGG vs. GBM and LGG vs. control	1
2002	Hanash et al. [17]	23	11 grade I and II 12 GBM	2DGE	N/A	LGG vs. GBM	4
2003	Zhang et al. [18]	16	7 GBM 5 LGG 4 nonmalignant	Western blot	Cell line	LGG vs. control and LGG vs. HGG	4
2003	Hiratsuka et al. [19]	3	Grade II	2DGE and MALDI-TOF MS	Non-tumor brain	LGG vs. control	15
2004	Iwadate et al. [20]	10	10 grade II	2DGE and MALDI-TOF MS	Peritumoral or epilepsy	LGG vs. control	6
2004	Schwartz et al. [22]	20	5 nonmalignant 3 grade II 4 grade III 5 grade 4 3 others	2DGE and MALDI-TOF	Epilepsy	Grade II vs. III vs. IV	0
2005	Odreman et al. [21]	20	10 grade II astrocytes 10 GBM	2DGE and MS, plus western blot confirmation	N/A	LGG vs. HGG	15
2005	Schwartz et al. [23]	164	19 nonneoplastic 29 grade II 22 grade III 57 grade IV	2DGE and MALDI-TOF MS	N/A	LGG vs. HGG	0
2006	Fiore et al. [24]	5	2 grade I and II 3 grade IV	2DGE with MALDI-TOF MS	N/A	LGG vs. HGG	0
2006	Jiang et al. [25]	82	45 LGG (grades I, II, and III) 37 GBM	Western blot and protein lysate arrays	N/A	LGG vs. HGG	0
2006	Li et al. [26]	10	4 grade II 3 grade III 3 grade IV	2DGE with nano LC MS/MS	Epilepsy	LGG vs. control and LGG vs. HGG	9
2007	Khalil [27]	Excluded due to disassociation of a senior researcher					
2008	Park et al. [29]	1	Case study of grade II oligodendro-glioma progressing to grade III anaplastic oligodendroglioma	2DGE with MALDI-TOF MS	N/A	LGG vs. HGG case study	23

(continued)

Table 9.1 (continued)

Date	Author/reference	*n*	Pathology WHO grade	Method	Control	Comparison	No. altered protein
2009	Park et al. [30]	9	3 grade II 3 grade III 3 GBM[a]	2DGE and MALDI-TOF MS	N/A	LGG vs. control and LGG vs. HGG	2
2009	Grzendowski et al. [31]	?	Grade II oligodendroglioma Grade III oligodendoglioma	2DGE, MS, and western blotting	N/A	LGG vs. HGG	1
2010	Gimenez et al. [32]	15	5 grade II astrocytoma 5 grade III astrocytoma 5 grade IV astrocytoma	2DGE and MALDI-TOF MS	Epilepsy	LGG vs. HGG	1
2010	Xiong et al. [33]	36	20 grade I astrocytoma 16 grade II astrocytoma	2DGE and MALDI-TOF MS, plus western blotting	Peritumoral	LGG vs. control	25
2011	Anagnostopoulos et al. [15]	9	3 control 6 pilocytic astrocytoma – grade 1	2DGE with MALDI-TOF MS, plus western blotting	Nonmalignant brain	LGG vs. control	180
2011	Zhuang et al. [35]	Omitted because full manuscript details could not be accessed by the authors of this review					

B. *Proteomic studies of LGG that examine the effect of 1p/19q deletions*

Date	Author/reference	*n*	Pathology	Method	Comparison
2007	Okamoto et al. [28]	9	9 grade II and III oligodendro-gliomas 4 with 1p deletion 5 without 1p deletion	2DGE and LC/MS, plus western blotting	1p deletion vs. 10 undeleted
2010	Rostomily et al. [34]	10	10 grade II oligodendroglioma 5 with 1p/19q co-deletion 5 without 1p/19q co-deletion	ICAT and LC/MS	1p/19q co-deletion vs. 1p/19q undeleted
2010	Grzendowski et al. [14]	9	9 grade II oligodendro-gliomas 4 with 1p/19q co-deletion 5 without 1p/19q co-deletion	2DGE and MALDI-TOF MS, plus western blotting	1p/19q co-deletion vs. 1p/19q undeleted

Table 9.1 (continued)

C. *Proteomic study of LGG that examines the effect of IDH1 mutation*					
Date	Author/reference	*n*	Pathology	Method	Comparison
2011	Thirant et al. [36]	14	4 malignant glio-neuronal tumors	2D-DIGE and MALDI-TOF MS	IDH1-m vs. control
			IDH1 wild-type 10 grade II and III oligodendro-gliomas		IDH1-m vs. IDH-wt
			Containing mutant IDH1		

This table summarizes details of the clinical samples and methodology used in each of the publications identified from the literature that uses proteomics to study low-grade gliomas, in accordance with our search criteria (samples size *n*; tumor pathology utilizing the World Health Organization (WHO) system, method of tissue analysis, origin of "control tissue," and proteomic study design/comparison)

Abbreviations: *2DGE* two-dimensional gel electrophoresis, *2D-DIGE* two-dimensional-difference in gel electrophoresis, *GBM* glioblastoma, *LGG* low-grade glioma, *HGG* high-grade glioma, *MALDI-TOF* matrix-assisted laser desorption ionization-time of flight, *nano LC* nanoliquid chromatography, *ICAT* isotope-coded affinity tags

[a]Grade II, III, and IV samples described in this study are taken from different zones of GBM based on density, necrosis, and other histological features

than 25 proteins altered in LGG, so we have collated each of these protein lists and analyzed/discussed these collectively. One article was discarded due to dissociation of an eminent senior scientist from the publication [27], and another article entitled "Proteomics studies of childhood pilocytic astrocytoma" [15] has been kept separate for analysis and discussion due to the unique nature of the study. The paper by Anagnostopoulos et al. [15] describes a large 2DGE-LC/MS study that lists 180 differentially expressed proteins in childhood pilocytic astrocytoma (CPA), a small subset of gliomas – it was decided that the size of this sample in this minority subtype of LGG would skew meta-analysis and was therefore kept distinct. Lastly, one paper that describes a proteomic case study comparing an oligodendroglioma with an anaplastic oligodendroglioma in the same patient 15 months apart has been kept separate from meta-analysis but will be discussed. The proteins listed in the 14 group-comparison papers have been divided into "LGG vs. control" and "LGG vs. HGG" to help understand (a) proteomic disassociations between normal and LGG tumor that may be associated with tumorigenesis and (b) proteomic differences between LGG and HGG that may clarify the pathophysiology of progression of a glioma to one of a higher grade.

Protein Changes Between Normal Brain Tissue, LGG, and HGG

The results of all the proteins noted to be differentially expressed in the papers looking at LGGs compared to controls or HGG samples (i.e., protein changes between different glioma grades) are listed in Table 9.2 (excluding the paper by Park et al. [29] that describes a case study and the paper by Anagnostopoulos et al. [15] as the sheer volume of results in this paper which focuses on childhood pilocytic astrocytoma, a rare type of LGG, was considered to skew the protein list).

Comparative Proteomics of LGG and Control Tissue

Studies that have investigated the proteome of LGGs compared to "normal" control brain have identified a total of 45 differentially expressed proteins in LGGs: 31 upregulated proteins and 14

Table 9.2 List of proteins highlighted in extant LGG proteomic studies that are comparative to controls or HGGs

LGG vs. control	LGG vs. HGG
Upregulated in LGG:	Upregulated in HGG:
78 kDa glucose-regulated protein (HSPA5)	Alpha crystalline B chain (CRYAB)
Albumin (ALB)	Alpha-internexin (INA)
Alpha-enolase/enolase 1 (ENO1)	Beta-actin (ACTB)
Alpha crystallin B chain (CRYAB)	A-kinase anchor protein 9 (AKAP9)
Apolipoprotein A1 (APOA1)	Annexin A5 (ANXA5)
ATP synthase subunit d, mitochondrial (ATP5A1)	Antioxidant protein 2 (PRDX6)
ATP synthase H+transport, F1 α-subunit b (ATP5H)	Apolipoprotein AI (APOA1)
Catechol-O-methyltransferase (COMT)	Centromere protein F (CENPF)
Chain B_h Mag superoxide dismutase Q143n (mtSOD)	Creatine kinase B-type (CKB)
Chain d, crystal structure if human profilin Ii (PFN2)	Cyclin-dependent kinase 4 (CDK4)
Cyclin-dependent kinase inhibitor 1 (CDKN1A)	Cystatin B (CSTB)
Copine 1 isoform b (CPNE1)	Epidermal growth factor receptor protein (EGFR)
Dihydropyrimidinase-related protein 3 (DPYSL3)	Fibrinogen β-chain (FGB)
Fatty acid-binding protein, brain (FABP7)	G1-/S-specific cyclin-E1 (CCNE1)
Glutamate dehydrogenase 1 (GLUD1)	Glutathione-S-transferase P (GSTP1)
Glutathione-S-transferase P (GSTP1)	Heat shock protein beta 1 (HSPB1)
Heat shock 60 kDa protein 1 (HSPD1)	Laminin α-5 chain (LAMA5)
Heat shock 70 kDa (HSPA9)	Major vault protein (MVP)
Heat shock 70 kDa protein 8 isoform 2 (HSPA8)	Moesin (MSN)
Hemopexin (HPX)	Natural killer cell-enhancing factor A (PRDX1)
Heteronuclear ribonucleoprotein A2/B1 (HNRNPA2B1)	Nucleoside diphosphate kinase A (NME1)
Heat shock protein beta 1 (HSPB1)	Neurogenic locus notch homolog protein 3 (NOTCH3)
Heat shock protein 27 (HSP27)	RET proto-oncogene tyrosine kinase receptor (RET)
FHA domain-interacting nucleolar phosphoprotein (MK167)	Ryanodine receptor 3 (RYR3)
L3-phosphoserine phosphatase (PSPH)	Stathmin (STMN1)
Mitogen-activated protein kinase 1 (MAPK1)	Telomerase reverse transcriptase (TERT)
Neurocalcin delta (NCALD)	Terminal uridylyltransferase 4 (ZCCHC11)
Protein disulfide isomerase A3 (PDIA3)	Transthyretin (TTR)
Prohibitin (PHB)	
Protein disulfide isomerase E60 precursor (PDIA3)	
Transthyretin (TTR)	
Tubulin beta-2a (TUBB2A)	
Downregulated in LGG:	Downregulated in HGG:
Astrocytic phosphoprotein PEA-15 (PEA15)	cAMP-dependent protein kinase (PRKACA)
Cellular tumor antigen p53 (P53)	Dihydropteridine reductase (QDPR)
Creatine kinase U-type, mitochondrial (CKMT)	Glial fibrillary acidic protein, astrocyte (GFAP)
Dihydropyrimidinase-like 2 protein (DPYSL2)	p14ARF (CDKN2A)
Fructose-bisphosphate aldolase A (ALDOA)	p16 (CDKN2A)
Gamma enolase (neuronal) (ENO2)	Phosphatidylethanolamine-binding protein 1 (PEBP1)
Glial fibrillary acidic protein (GFAP)	Protein disulfide isomerase A3 (PDIA3)
Guanine nucleotide-binding protein (GNB1)	T-complex 1 e-subunit (CCT5)

Table 9.2 (continued)

LGG vs. control	LGG vs. HGG
NAD-dependent deacetylase sirtuin-2 (SIRT2)	Ubiquitin carboxyl-terminal hydrolase L1 (UCHL1)
OXCT protein (OXCT)	
Phosphatidylethanolamine-binding protein 1 (PEBP1)	
Peptidylprolyl isomerase A (PPIA)	
Profilin 2 (PFN2)	
Ubiquitin carboxyl-terminal hydrolase L1 (UCHL1)	

This table lists all the proteins that have been reported in the literature to be altered in expression level in LGGs compared to control samples (45 in total) and HGG samples (36 in total). Please note (a) the certainty of protein identifications varies across studies (some studies report the probability of a definitive protein match, and others do not) and (b) validation of identified proteins using alternative methodology (e.g., immunocytochemistry) only occurs in some studies and not others, but validation is essential to confirm true differences in expression. The proteins presented in this table are identified by their name with the gene name included in brackets

downregulated proteins (listed in Table 9.2). Seven proteins (apolipoprotein A1, alpha crystallin B chain, heat shock protein 27, protein disulfide A3, prohibitin, transthyretin, and profilin 2) were identified in more than one study (2/8 LGG vs. control studies). Six of these proteins showed consistency in the direction of expression change between studies, but 1 protein, profilin 2, was found upregulated in one study [33] and downregulated in another [19]. Interestingly, only 1 protein out of the total 45 differentially expressed proteins in LGG (vs. control) has been reported by Petrak and colleagues as a protein commonly identified in proteomic studies regardless of experiment, tissue, or species (viz., heat shock protein 27) [5].

Collating results from multiple papers is a useful method for allowing differences between methodologies and study power (two factors which affect the extent and/or subpart of the proteome captured) and gaining a global overview of known proteomic changes described in LGG. Entry of all the proteins into analytical tools such as IPA and DAVID also highlights biologically enriched functions and commonalities within the data and helps to make sense of the disparate lists of proteins that have been generated in the literature.

Our IPA of the collated list of proteins differentially expressed in LGG (compared to controls) generated three protein-protein interaction networks (with scores 52, 29, and 16, respectively; please refer to Ingenuity Systems software for an explanation of network scores). The top network generated is very high scoring (score 52; containing 21 proteins from the 45 proteins entered) and is interestingly assigned (by IPA) the functions "posttranslational modification, protein folding, and cell death," all processes known to contribute to oncogenesis. This finding is complemented by our independent analysis performed using DAVID where the top two pathways highlighted as enriched are "apoptosis" and "unfolded protein response," and all the proteins found in the DAVID "apoptosis" pathway are present in the top-scoring IPA network. Of note, TP53, a well-known tumor suppressor gene, is central to this IPA network (see Fig. 9.2). TP53 is downregulated in LGG compared to healthy brain tissue [37].

The DAVID analysis produced a total of 37 "functionally enriched" protein clusters, but the majority of these were ignored due to very low scores, which imply little statistical power. The top three protein clusters had scores >3 (a statistical score that warrants these clusters for discussion) and were assigned the following functions: (1) regulation of apoptosis (score 3.78; contained 13 proteins from the list), (2) unfolded protein response (score 3.6; contained 6 proteins from the list), and (3) unfolded protein response (score 3.33; data overlapped with cluster 2). These findings implicate increased glial cell growth juxtaposed to an imbalance between the rate of cell division and the rate of cell apoptosis. The results above suggest that the latter is the driving force accompanied by an inappropriate response to unfolded proteins.

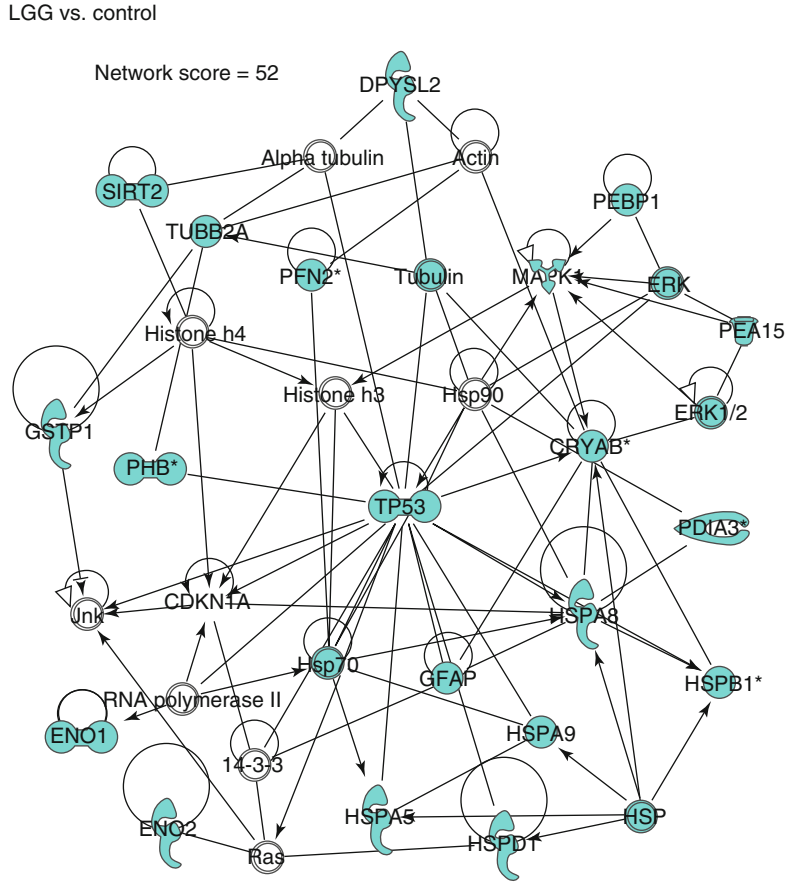

Fig. 9.2 LGG vs. control. Visual representation of the top IPA network generated from the list of collated proteins differentially expressed in LGGs relative to normal brain tissue (controls); the network is high scoring (score = 52) and coherent, containing 21 dataset proteins (in *blue*). Each node (shape) represents a protein and its association with other proteins is represented by a *line* (edge). Nodes have different shapes to represent different molecule types (please refer to Ingenuity Systems for complete node information). *Solid lines* represent direct interactions between proteins. Direct interactions are defined as those where two proteins make physical contact with each other with no intermediate step. This network contains a multitude of protein-protein interactions with oncogene TP53 at the center. The functions assigned (by IPA) to this network are "posttranslational modification, protein folding, and cell death"

Data from Anagnostopoulos et al. [15] was kept separate from the meta-analysis above. Anagnostopoulos et al. [15] looked specifically at childhood pilocytic astrocytomas (CPAs) compared to peritumoral tissue. The study applied 2DGE-MALDI-TOF MS technology to study CPA and identified a total of 180 differentially expressed proteins: 46 proteins identified in CPA but not control tissue and 134 proteins downregulated in CPA. These results stem from an enormous study of 18,000 proteins (from >24,000 protein spots) excised from 2D gels, encompassing proteins associated with cell motility (19 %), protein transport (15 %), cell metabolism (15 %), cell proliferation (12 %), and others (39 %) (analyzed using KEGG Gene Ontology annotation tool). Anagnostopoulos et al. [15] performed IPA on their dataset (and represent one of only a few proteomic studies of glioma that have applied bioinformatics downstream of the main proteomic analysis). The primary IPA network the authors identified centered on TP53, corresponding with the primary network we generated from our collective list of proteins identified in LGG vs. control studies (excluding Anagnostopoulos et al.'s [15] dataset). The ERK1 and ERK2 pathways were also identified as being aberrant; however, without further analysis, these pathways are too ubiquitous to present a realistic point of intervention [15]. This chapter provides a wealth of proteomic data and merits further analysis.

Comparative Proteomics of LGG and HGG

Studies that have investigated the proteome of LGGs compared to HGGs have identified a total of 36 differentially expressed proteins: 28 proteins upregulated in HGG and 9 proteins downregulated in HGG relative to LGGs (these are listed in

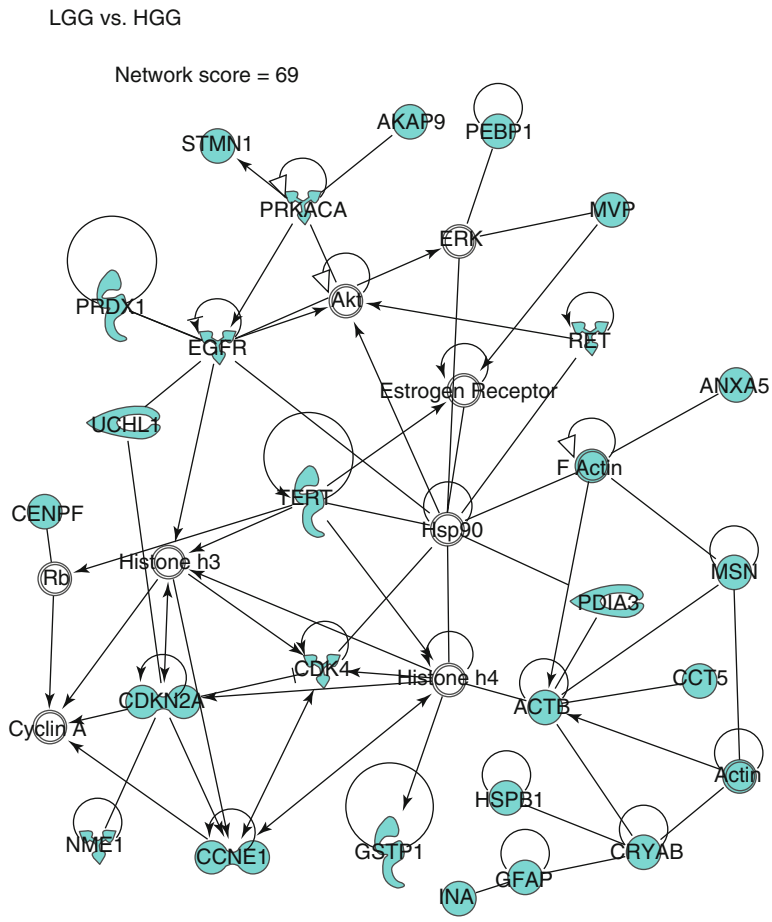

Fig. 9.3 LGG vs. HGG. Visual representation of the top IPA network generated from the list of collated proteins differentially expressed in LGGs relative to HGGs; the network is high scoring (score = 69) and coherent, containing 25 dataset proteins (in *blue*). As in Fig. 9.2, each node (shape) represents a protein and its association with other proteins is represented by a *line* (edge). Nodes have different shapes to represent different molecule types (please refer to Ingenuity Systems for complete node information). *Solid lines* represent direct interactions between proteins. Direct interactions are defined as those where two proteins make physical contact with each other with no intermediate step. This network has no clear hub but contains a multitude of protein-protein interactions between altered proteins described in the LGG vs. HGG literature. The functions assigned (by IPA) to this network are "cellular growth and proliferation, cancer, and cell cycle"

Table 9.2). There were no proteins identified in multiple studies from different research groups. However, 8 of the identified proteins were also identified in the previously described comparative studies of control brain vs. LGGs (glutathione-S-transferase P, glial fibrillary acidic protein, phosphatidylethanolamine-binding protein 1, ubiquitin carboxyl-terminal hydrolase isozyme L1, apolipoprotein A1, heat shock protein 27, protein disulfide isomerase A3, and alpha crystallin B chain). All of these eight proteins showed the same direction of response with increasing malignancy of glioma, except for protein disulfide isomerase A3 (PDIA3) which was upregulated in Iwadate et al. [20] (comparing LGG and control brain) and downregulated in Odreman et al. [21] (comparing LGGs and HGGs), suggesting a peak of PDIA3 expression in LGGs.

All 36 differentially expressed proteins were once again analyzed using IPA and DAVID software. IPA generated multiple networks but the top-scoring network appeared particularly significant due to its very high score of 69 (cf. score = 13 for network 2) and its incorporation of almost 70 % (25/36 proteins) of the protein list interrogated (see Fig. 9.3). IPA assigned the

network the functions "cellular growth and proliferation, cancer, and cell cycle." This is particularly interesting in contrast with our previous IPA analysis (of proteins altered in LGG vs. control) which highlighted cell apoptosis. It may be that the progression from healthy brain tissue to HGG passes through two distinct stages. Initially, there is a loss of apoptotic control to produce a LGG, and secondly there is an additional increase in cell proliferation leading to the HGG. Visual representation of the LGG vs. HGG protein interaction network appears more complex than the LGG vs. control network, with no obvious center or primary hub. It would be difficult to identify which areas to target therapeutically.

The DAVID analysis produced a total of 28 "functionally enriched" protein clusters, and once again the majority of these were ignored due to very low scores. The top six protein clusters however were assigned the following functions/localizations: (1) non-membrane-bounded organelle (score 3.77; contained 17 proteins from the list), (2) regulation of apoptosis (score 3.4; contained 11 proteins from the list), (3) organelle lumen (score 2.92; contained 13 proteins from the list), (4) regulation of intracellular transport (score 2.59; contained 4 proteins from the list), (5) response to oxidative stress (score 2.42; contained 5 proteins from the list), and (6) membrane-bounded vesicle (score 2.04; contained 7 proteins from the list).

These findings again suggest that the progression from LGG to HGG is a distinct one rather than a general, nonspecific or haphazard dysregulation of the balance between proliferation and cell death. The progression appears to involve not only loss of cell cycle control but also the degeneration of organelles and cellular transport, for example, we see the failure of multiple key subcellular systems. This may explain the characteristic nuclear and cytoplasmic atypia seen in HGGs but not LGGs. The increased cell proliferation accompanied by loss of apoptosis will stimulate angiogenesis as the tissue struggles to retrieve the oxygen needed for respiration and will eventually lead to a necrotic core where insufficient O_2 has led to ischemic cell death. Another use of proteomics is to look at changes to protein modification; for instance, α-tubulin is nitrated at Tyr224 in GBM tissue but not in grade 1 or noncancerous tissue. How this modification may change α-tubulin's role in neuronal cell growth or its interactions with other tubulin cells is unknown [24].

A Proteomic Case Study of Malignant Transformation from LGG to HGG

A dataset that has not been included in the meta-analysis above (because it stems from a single patient) but could be pertinent to understanding the malignant transformation from LGG to HGG comes from Park et al. [29]. This paper used 2DGE to investigate the protein levels between a low-grade oligodendroglioma from an individual (where there was near-total resection) and a histologically confirmed anaplastic oligodendroglioma that recurred in the same patient 15 months post-surgery. In total, 23 protein spots were found differentially altered in expression, 14 proteins were overexpressed in the low-grade oligodendroglioma, and 9 proteins were overexpressed in the malignant anaplastic oligodendroglioma. The criteria for determining a protein as differentially expressed are vague in this study (no statistics or methodological description of gel replication is described), so the results should be considered with caution. However, two differentially expressed proteins (peroxiredoxin 6 and RhoGDI alpha) were validated by western blotting on a second individual who showed progression from a low-grade oligodendroglioma to an anaplastic oligodendroglioma in 14 months. Interestingly, both these proteins have been highlighted in proteomic studies of HGGs [21, 38]. Both proteins are hypothesized to be molecular candidates that are predictive of malignant transformation, but further research is required to understand their relationship and precise roles in effecting downstream hypoxic, antioxidant, and Ras pathways to induce malignant transformation [29].

Comparative Proteomics of LGGs with and Without 1p/19q Deletions

Clinical and genetic studies have identified an interesting subset of LGGs focused on oligodendrogliomas [37, 39]. Patients with low-grade oligodendrogliomas who have lost the 1p and 19q chromosomal arms on one set of chromosomes

(i.e., 1p/19q loss of heterozygosity (LOH)) have improved clinical outcome [40]. Smith et al. [41] demonstrated that 1p/19q LOH tumors had a nearly 100 % 5-year survival compared to 60–70 % in those without LOH. The LOH appears to sensitize the tumor to treatment rather than improve untreated prognosis, although the data is not yet conclusive [42]. Despite the analysis of genes located on these arms, genomics has been unable to discover the reason for this improved outcome. Consequently, it represents an example of potentially rewarding proteomic application. A 2007 paper by Okamoto et al. [28] looked selectively at 1p LOH using 2DGE and silver staining for protein visualization. The study highlighted 19 proteins significantly altered in expression in patients with 1p LOH ($p<0.05$): 7 proteins downregulated and 11 proteins upregulated (see Table 9.3). One protein highlighted by the study was glyoxalase 1 (decreased in the LOH cohort) which has previously been associated with chemoresistance. Anticarcinogenic proteins were also noted at higher levels in the LOH group including nucleoside diphosphate kinase 1 and gelsolin. The authors suggest that the differences in protein expression detected may indicate that 1p LOH tumors have an earlier cellular developmental stage than other oligodendrogliomas [28].

A more recent paper published by Grzendowski et al. [14] also focused on WHO grade II oligoastrocytomas, analyzing 5 oligoastrocytomas with 1p/19q deletion and 4 oligoastrocytomas without. Using 2DGE, 22 proteins were found differentially expressed in cells with 1p/19q deletion; 19 proteins were downregulated and 3 proteins were upregulated (see Table 9.3). Interestingly, only two proteins, gelsolin and aldose reductase, were found in common with the study described above [28], but different isoforms of both proteins were detected. The detection of different isoforms of these proteins is probably due to different tumor types (oligodendroglioma and oligoastrocytoma) being analyzed in these two studies. Only two of the proteins identified by Grzendowski et al. [14] were mapped back to 1p or 19q. This may either be because the proteins coded for on 1p and 19q are too small to be detected by 2DGE or because the major effect of 1p/19q deletion is seen elsewhere. All three isoforms of glial fibrillary acidic protein (GFAP) were detected as downregulated in 1p/19q deletions; this is cited as possible evidence of these tumors being of a different genetic lineage rather than a progression from tumors without 1p/19q deletion. Grzendowski et al. [14] also investigated whether epigenetics could explain the changes observed in protein expression. Vimentin and villin were both expressed at lower levels in the 1p/19q deletion tumor cohort, and their promoter regions were hyper-methylated, suggesting the presence of genes controlling methylation on either 1p or 19q.

The most recent paper looking at 1p/19q deletion used advanced LC/MS proteomic technology together with isotope-coded affinity tagging (ICAT) [34]. In this paper, WHO grade II oligodendrogliomas were studied. As expected with more sophisticated technology, the number of proteins identified was much higher than in the previous two 2DGE studies (a total of 442 proteins were identified). Biochemical enriched fractions (cytosol-, mitochondria-, and nuclei-enriched fractions) were also targeted, allowing for greater and more specific identification of proteins compared to the whole cell lysate samples used in Okamoto et al. [28] and Grzendowski et al. [14]. A total of 101 proteins were found differentially expressed in at least one of the three fractions between oligodendrogliomas with and without 1p/19q deletion; 60 proteins were downregulated and 41 proteins were upregulated. The differentially expressed proteins were broadly classified according to their Gene Ontology and were all found members of Gene Ontology groups related to malignancy (invasion/migration, cell survival/death, proliferation, DNA repair, metabolism, and immune response). The authors then chose just a few proteins for validation by western blotting due to the large nature of the dataset generated. The study confirmed BCAN and transferrin (TF) as two novel markers of co-deletion that could be used as prognostic tools and suggested that metabolic reprogramming and differential invasiveness were consequences of 1p/19q co-deletion, but, due to the overwhelming quantity of data, was unable to take the analysis much further [34]. Differentially expressed proteins that were also identified in

Table 9.3 Differentially expressed proteins in LGGs with and without 1p/19q deletions

Protein name	Gene name	Uniprot accn no.	Rostomily et al. – data overlap
Downregulated in 1p LOH (Okamoto et al. [28])			
Glyoxalase I	GLO1	Q04760	–
Brain glycogen phosphorylase	PYGB	P11216	Down cytosol
Purine nucleoside phosphorylase	PNP	P00491	–
Phosphoglycerate kinase 2	PGK2	P07205	–
Haptoglobin-related protein	HPR	P00739	–
Cathepsin D	CTSD	P07339	–
Rho GDP dissociation inhibitor (GDI) alpha	ARHGDIA	P52565	–
Downregulated in 1p/19q deletion (Grzendowski et al. [14])			
Aldose reductase	AKR1B10	O60218	–
Alpha crystallin B chain	CRYAB	P02511	–
Annexin A1	ANXA1	P04083	–
Annexin A5	ANXA5	P09525	–
Chloride intracellular channel protein 1	CLIC1	O00295	–
DnaJ homolog subfamily B member 1	DNAJB1	P25685	–
Enolase-α	ENO1	P06733	–
Gelsolin precursor	GSN	P06396	–
Glial fibrillary acidic protein, splice isoform 1	GFAP	P14136	Up nuclear
Glial fibrillary acidic protein, splice isoform 2	GFAP	P14136	–
Glial fibrillary acidic protein, splice isoform 3	GFAP	P14136	–
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	P04406	Up nuclear
Heterogeneous nuclear ribonucleoproteins A2/B1	HNRPA2B1	P22626	–
Nucleoside diphosphate-linked moiety X motif 16	NUDT16	Q96DE0	–
Peroxiredoxin 1	PRDX1	Q06830	–
Peroxiredoxin 6	PRDX6	P30041	–
Sorbitol dehydrogenase	SORD	Q00796	–
Villin 2/ezrin	VIL2	P15311	–
Vimentin	VIM	P08670	–
Upregulated in 1p LOH (Okamoto et al. [28])			
Nucleoside diphosphate kinase 1 isoform a	NME1	P15531	–
Chaperonin-containing TCP1, subunit 2	CCT2	P78371	Up nuclear
Aldo-keto reductase family 1, member B1	AKR1B1	P15121	–
F-actin-capping protein beta subunit	CAPZB	P47756	–
Microtubule-associated protein, RP/EB family, member 1	MAPRE1	Q15691	–
Gelsolin***	GSN	P06396	–
Eukaryotic translation initiation factor 5A	EIF5A	P63241	–
T-complex protein 1	TCP1	P17987	–
Ubiquitin carboxyl-terminal esterase L3	UCHL3	P15374	–
14-3-3 protein zeta/delta	YWHAZ	P63104	–
Cofilin 1	CFL1	P23528	–
Glutathione-S-transferase M2	GSTM2	P28161	–

Table 9.3 (continued)

Protein name	Gene name	Uniprot accn no.	Rostomily et al. – data overlap
Upregulated in 1p/19q deletion (Grzendowski et al. [14])			
Peroxiredoxin 5, mitochondrial precursor	PRDX5	P30044	–
Platelet-activating factor acetylhydrolase IB-α subunit	PAFAH1B1	P43034	–
Syntaxin-binding protein 1	STXBP1	P61764	–

This table lists all the proteins that have been reported by Okamoto et al. [28] and Grzendowski et al. [14] to be altered in expression level in LGGs with and without 1p LOH and 1p/19q deletion, respectively. The proteins presented are identified by their protein name, gene name, and Uniprot accession number (a unique protein identifier). Rostomily et al. [34] identified 101 proteins altered in LGG with and without 1p/19q deletion and have not been listed due to the large number of identifications, but common protein alterations are indicated in the final column of this table and indicate the direction of change and subcellular fraction in which the protein alteration was detected

Okamoto et al. [28] and Grzendowski et al. [14] are highlighted in Table 9.3.

In summary, these papers identify markers of 1p/19q co-deletion. Further study needs to be done to see if interference in the pathways noted to be downregulated by 1p/19q co-deletion could improve sensitivity to chemotherapy and outcomes. This will require careful selection of proteins such as fascin 1 or BCAN and the development of pharmacological agents that can interfere with their protein-protein interactions.

Proteomics of LGG and IDH1 Mutations

In the last few years, another genetic mutation, namely, isocitrate dehydrogenase-1 (IDH1), has been identified to occur in a subset of 60–80 % of LGGs but rarely in high-grade glioblastomas [43–47]. Since the discovery of this mutation, patients with mutated IDH1 have been shown to have better survival rates, and IDH1 status is now deemed a reliable prognostic marker [44, 45, 48, 49]. Proteomics, as described above with respect to 1p/19q deletions, has the potential to reveal the molecular pathophysiological mechanisms that underlie IDH1 mutation and improved survival. One paper has recently sought to decipher protein alterations associated with IDH mutation [36]. The study compares oligodendrogliomas that contain mutated IDH1 (IDH-m) to wild-type IDH tumors (IDH-wt) such as glioblastoma with oligodendroglial components or oligoastrocytomas with core areas histologically indistinguishable from typical oligodendroglioma. IDH-m and IDH-wt tumors showed distinct proteomic patterns (in contrast to very similar global proteomes seen between four individual IDH1-m oligodendrogliomas contrasted to paired minimally infiltrated parenchymal control tissue). Analysis using 2D-DIGE found a total of 89 protein spots differentially expressed between IDH-m and IDH-wt tumors (>1.4-fold, $p<0.04$) with 40 proteins identified. Complementary principal component analysis also revealed unique patterns of clustered proteins between the two tumor types. Interestingly, IPA connected 38 out of the 40 identified proteins deregulated in IDH-m oligodendrogliomas in a network centered on oncogene MYC. Two notable groups of dysregulated proteins identified were (1) ERM proteins which cross-link actin, the cytoskeleton, and the plasma membrane and regulate cellular migration and invasiveness – ERM proteins were downregulated, indicating that IDH-m tumors may have better prognosis due to limited migratory properties – and (2) three proteins involved in aerobic glycolysis (fructose-bisphosphate aldolase C, phosphoglycerate mutase-1, and transaldolase) which were upregulated and hypothesized to reflect a favored shift towards aerobic glycolysis (the Warburg effect).

Discussion

Proteomic research of LGGs is a growing field but still much smaller than that of HGGs. This is

likely to be because the prognosis for LGG is often very good especially in young patients with WHO grade II tumors who have major resective surgery [50] and in addition the disease is less common [51, 52]. As a result, it does not exhibit the same imperative from a clinical perspective as GBM which is a rapidly fatal, incurable disease. One of the challenges of LGG is that it is a composite of different histologically and genetically defined tumors with differing natural prognoses with or without treatment [37, 39, 40, 42]. Within the WHO II histologically defined glioma categories, diffuse astrocytoma, oligodendroglioma, and oligoastrocytoma all have different outcomes [53]. Additionally, and as stated previously, genetic constitution can, even within the same subset of tumors (e.g., 1p/19q LOH in oligodendrogliomas, diffuse astrocytomas with or without 1p deletions, and oligodendrogliomas with or without IDH1 mutations), influence outcome [37, 39, 40, 42]. This is one particular problem with a review such as this.

In most cases, the WHO II tumors in the various series studied, undergoing proteomic analysis had nonspecific diagnostic breakdowns, and except for the specific cases of oligodendrogliomas, no subdivision due to genotypes. It is perhaps therefore surprising given this heterogeneity that the review identified powerful functional networks that potentially underlie the transition, firstly from normal to neoplastic tissue (LGG vs. control) and, secondly, pathways involved in the transition of LGGs to HGGs. To recapitulate, the "LGG vs. control" network presented (Fig. 9.2) revealed a dysregulation of apoptosis and a general response of cellular stress, and the "LGG vs. HGG" network (Fig. 9.3) revealed a general increase in cell proliferation (in HGGs) together with a failure of multiple key subcellular systems. The two distinct stages inferred by these networks (in the progression from healthy brain tissue to HGG) are highly fitting and perhaps predictive in terms of universal tumor biology initiation and subsequent increase in malignancy, but their precise definition may also be valuable for identifying therapeutic targets. The distinct and appropriate nature of these two networks also provides confidence in the utility of proteomics for elucidating the pathophysiological mechanisms underlying gliomas.

TP53 occurred as a hub protein in our IPA network from the "LGG vs. control" meta-analysis and in the IPA network presented in Anagnostopoulos' paper [15]. TP53 has been identified as playing a crucial role in LGG pathophysiology [37]. It is the first detectable genetic mutation in over 60 % of low-grade astrocytomas that transform into GBM [54]. The p53 gene is commonly known as a tumor suppressor gene as it plays a key role in response to DNA damage and triggers apoptosis of cells and inhibits further differentiation of cells exhibiting aberrant mitosis [55]. Our "LGG vs. control" network also notably contained GFAP, a protein considered fundamental in glioma pathophysiology and diagnosis [56]. Furthermore, the network comprised a number of heat shock proteins (HSPA8, HSPB1, HSPA9, HSPD1, HSPA5, and CRYAB). CRYAB and HSPB1 have been shown to be dysregulated in multiple cancer types [57–60] and likely reflect a general cellular stress response to tumorigenesis. Two less common and potentially interesting proteins in the network are prohibitin (PHB; upregulated in LGG vs. control) and phosphoethanolamine-binding protein 1 (PEBP1; downregulated in LGG vs. control brain). An increase in PHB expression has been reported in bladder, prostate, and thyroid cancers [61–63], showing the protein to be widely involved in tumorigenesis. PHB has also been shown to play a role in Ras-Raf signalling, a major pathway involved in malignant transformation [64, 65]. Likewise, PEBP1 has been highlighted in thyroid carcinomas and breast metastases [66, 67]. PEBP1 is a tumor suppressor that inhibits both the Raf-MEK-ERK and NF-kappaB pathways [68]. Recently, PEBP1 (also known as RKIP, Raf kinase inhibitory protein) has also been shown to endow significant resistance to cancer therapy when its expression is reduced or lost [68].

The HGG vs. LGG network also contained some well-described dysregulated proteins, (viz., EGFR and GFAP) but also some novel features such as the interacting protein cluster of PRKACA, STMN1, and AKAP9. PRKACA has been found downregulated in HGGs, whereas

STMN1 and AKAP9 are upregulated. PRKACA is a cAMP-dependent protein kinase which has previously been linked to neoplasm; however, there is little literature about STMN1 and AKAP9 in connection to cancer, and interaction between these three proteins in a small oncogenic network has not previously been recorded. PRKACA is usually reported as upregulated in invasive cancers and has been shown to integrate with AKAP3 to promote cell invasion of ovarian cancer cells [69]. Why PRKACA is more abundant in low-grade astrocytomas compared to HGGs is not clear, but it would be interesting to see whether AKAP9 has the same pro-invasive effects as AKAP3. Although AKAP9 and STMN1 are not directly linked, they both have a role in cellular organization with STMN1 regulating the microfilament system promoting MF disassembly and AKAP9 providing scaffolding for Golgi stability through microtubule organization [70]. Inhibition of STMN1 has already been suggested as a treatment target in laryngeal squamous cell carcinoma and in cervical cancer to induce apoptosis [71, 72]; the same may be true for HGG. Inhibition of AKAP9 may also have a pro-apoptotic effect.

LGG research has some large challenges ahead of it. There is very little overlap between the protein lists identified in this systematic review of the LGG proteomic literature. This may be due to different control samples or different techniques and reagents, but the absence of overlap highlights the lack of reproducibility of results. It is thought that even if the same lab runs the same sample through an LC/MS machine, up to 30 % of the proteins will differ [73]. Proteomic techniques are developing rapidly; however, the focus up until now seems to have been on speed and quantity rather than reliability, rigor, and reproducibility. In addition, many studies have just produced a continually growing list of proteins. The variability between both the strength of the findings and studies does not assist in either fully understanding how these proteins function or how they interact. Another challenge for proteomics is that of protein size. Small proteins are more difficult to identify; for instance, LC/MS generally only detects proteins between 30 and 100 kDa, and proteins expressing at low levels can still be overshadowed by those expressing at high levels. The Human Proteome Project is responding to many of these problems. It has already standardized many of the techniques used, and it is now developing three key areas to further tackle this problem: (1) identify one protein per gene, this will strengthen links between genomics and proteomics; (2) identify one antibody for each protein identified, this will respond to problems with misidentification and allow proteins to be tagged and their cellular location noted; and (3) identify which proteins talk to each other so that proteins can be visualized within their interaction networks [73].

Proteomic research that is focused on both histological and genetic subtyping of LGGs may help researchers to accurately subdivide this heterogeneous group and discover which patients would benefit the most from aggressive surgery, chemotherapy, radiotherapy, observation, or a combination. The findings from this review strongly suggest that an understanding of the proteomic differences between LGGs and HGGs will also provide insight into the pathophysiology of the evolution of some LGGs to HGG and the fundamental differences between primary and secondary glioblastoma. Many researchers have commenced trying to understand GBM through proteomics and in their enthusiasm have possibly missed out this potentially important step. As described at the outset of this review, proteomics is the natural sequitur to genomic research. It was hoped that the Human Genome Project would reveal the secrets behind the way humans worked and why they went wrong. The problem has been however that each gene can be spliced in multiple ways, regulated by epigenetic phenomenon, and protein expression can be modified by several mechanisms completely independent of genomics. The disassociation between genetic and proteomic findings has been well demonstrated in gliomas [1]. Proteomics thus at first glance appears valuable since it can evaluate proteins in all their splice forms and variants. However, it is not enough just to elucidate the proteins present and what their form is. It is vital to understand how they interact with each other. Until we can

picture proteins in their complex networks, we will struggle to predict what effect the upregulation or downregulation of that protein will have. This begs the question: "What is the next step for proteomics?" Are more studies looking at protein changes between control and sample tissues going to provide more answers? In some ways, yes; if a small group of proteins are continually found aberrantly expressed and we are able to correct or manipulate this expression, then we may be able to slow or halt tumor development. However, the research so far suggests that it is multiple small interactions that are the fundamental processes underlying disease initiation and progression.

The development of interactomes and metabolomes has the potential to identify protein function and metabolic interactions by association rather than direct knowledge. A recent study has evaluated some unique metabolic consequences of IDH1 mutations in gliomas [74]. The potential effect of these metabolic changes on protein function requires evaluation. There is a wealth of potential programs available online that allow researchers to see individual proteins in situ. The challenge remains to differentiate which interactions happen in cells and which are occurring because two proteins have been placed in close proximity in a cell [75]. As these areas develop, it will become easier to identify pathological interactions and generate pharmacological agents that will antagonize them. This could either be used as a treatment in itself or in oncology to increase the sensitivity of cells to chemotherapy.

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Human Low-Grade Glioma Cultures

10

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Abstract

Numerous diagnostic, prognostic, and therapeutic issues concerning low-grade gliomas still remain to be clarified. In this regard, in vitro and in vivo models of low-grade gliomas would represent a way to get insight into these crucial aspects. However, many of the in vitro and in vivo models assayed in the literature are focused on high-grade gliomas.

For this reason, in this chapter we reviewed the literature on low-grade glioma culture describing the results so far obtained, highlighting limitations and envisioning promising innovative directions that research is undertaking in this critical field.

Specifically, first, we critically presented the conventional methods adopted to study low-grade gliomas, such as continuous human tumor cell lines and short-term glioma cultures. Then, we discussed the culture methods utilized to isolate and in vitro expand glioma cancer stem cells from adult and pediatric tumors, underlining the need of optimizing new culture conditions because of the partial failure of the simple transfer to low-grade gliomas of the methods efficient in isolating glioma stem cells from high-grade gliomas. Finally, we presented the innovative, although still in its infancy, possibility to culture nonneoplastic stromal cells as a way to obtain cell lines representative of the biological behavior of the patient tumors.

In conclusion, the aim of this chapter was to explain why the culture of patient-derived cancer cells represents a unique opportunity to create in vitro and in vivo models closely mimicking the biological properties of the patient tumor, thus allowing a patient-based approach.

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Abbreviations

BCNU	1,3-bis(2-chloroethyl)-1-nitrosourea
bFGF	Basic fibroblast-derived growth factor
CAF	Cancer-associated fibroblast
CSC	Cancer stem cell
EGF	Epithelial-derived growth factor
GBM	Glioblastoma
GFAP	Glial fibrillary acidic protein
GSC	Glioma stem cell
LGG	Low-grade glioma
MASC	Multipotent adult stem cells
MGMT	O6–methylguanine–DNA methyltransferase
PDGF	Platelet-derived growth factor
TAF	Tumor-associated fibroblast
TMZ	Temozolomide

Introduction

Gliomas are the most frequent primitive tumors of the central nervous system and, although the cellular origin of gliomas is still unknown, experimental data in mice suggest an origin from neoplastically transformed neural stem or progenitor cells [1]. The term low-grade gliomas (*LGG*) includes all World Health Organization (WHO) grade I and II gliomas [2]. With respect to high-grade gliomas, LGG grow slowly but about 70 % of grade II gliomas evolve to anaplasia within 5–10 years [3]. Therefore, the natural course of LGG varies considerably and is highly influenced by treatment-independent factors, such as age, pretreatment performance score, tumor volume, contrast enhancement on CT/MRI, and tumor histology [2]. Therefore, the management of patients with low-grade gliomas (LGG) is a challenge, because: (1) There are no definitive criteria to classify a lesion as at high risk or low risk to relapse and/or to progress; (2) many of the potential adjuvant treatments can produce or contribute to chronic neurocognitive function impairment, particularly radiotherapy; these side effects are not justifiable in patients that are possibly at low risk of relapse/progression [2]; and (3) with the exception of temozolomide (TMZ), current therapies are mainly designed according to previously tested molecules against other types of cancer; in fact, novel drugs specifically designed to target LGG are not yet available.

Researches focused on other cancer types are currently exploiting these issues taking advantage of (1) wide genome analysis; (2) drug discovery approach; and (3) identification of putative novel therapeutic targets within the tumor, such as tumor-initiating cells, tumor-associated fibroblasts, and infiltrating mesenchymal stem cells (MSC).

For LGG all of these topics have been only incompletely explored. While a comprehensive genomic characterization defining human glioblastoma genes and core pathways is available [4–9], this extensive analysis is missing for LGG. What we know is that, genetically, the vast majority of LGG are mutated in IDH1, frequently deleted in 1p19q (oligodendroglioma), or mutated in p53 (astrocytoma) [10, 11]. The IDH1 mutation is inversely correlated with grade, tightly associated with a 1p19q co-deleted genotype and a MGMT methylated status, but mutually exclusive with EGFR amplification and loss of chromosome ten [10, 11]. Regarding tumor-initiating cells, they have been isolated mainly from high-grade gliomas [12, 13], while the MSC tropism for glioma has been mainly analyzed as drug delivery tool [14–16]. No extensive studies are present on LGG-associated activated stromal cells.

At this regard, it is important to notice that increasing evidences indicate that tumor–stromal cell interactions have a crucial role in tumor initiation and progression. These interactions modify cellular compartments, leading to the coevolution of tumor cells and their microenvironment [17]. The stromal elements of tumors are not only an integral part of cancer initiation, growth, and progression, but they hold prognostic, as well as response-predictive, information and abundant targeting opportunities within the tumor microenvironment [18]. It has recently demonstrated that bone marrow mesenchymal stem cells can originate tumor-associated stromal cells (named tumor-associated fibroblast (TAF) or cancer-associated fibroblast (CAF)) [19] and that glioma stromal mesenchymal stem cells are present in a murine orthotopic glioma model [20]. Accordingly, utilizing a method optimized in our laboratory for the isolation of multipotent adult stem cells (MASCs) from normal human tissues [21, 22], we have isolated from $n=56$ human-derived LGG a population of genetically normal mesenchymal stem cells possessing all the features of activated stromal elements [23–25].

In this chapter we will explain why the culture of patient-derived cancer cells represents a unique opportunity to create in vitro and in vivo models closely mimicking the biological properties of the patient tumor, thus allowing a patient-based approach. Moreover, we will review the literature regarding low-grade glioma culture describing the results so far obtained, enlightening limitations and envisioning promising innovative directions that research is undertaking in this critical field.

Reasons Why It Is Important to Develop Novel In Vitro Models of Low-Grade Glioma

Ideally, the possibility to obtain an in vitro culture model representative of the human tumor in situ would offer the unique opportunity to study the biological features of the tumor, to hypothesized novel diagnostic, prognostic and predictive markers, to allow drug-screening strategies, and it is prerequisite for developing in vivo models strictly resembling the tumor in situ.

At this regard, *continuous human tumor cell lines* have been the historical standard both for exploring the biology of human tumors and as preclinical models for screening potential therapeutic agents [26, 27]. Additionally, for many years, the in vivo animal models of human tumors have been based on the in vivo injection of these continuous cell lines [27]. Differently from what happens for high-grade gliomas, very few continuous human tumor cell lines representative of low-grade gliomas are commercially available (Table 10.1).

Unfortunately, it has become increasingly clear the failure of both in vitro and in vivo preclinical screening models, based on continuous human tumor cell lines, in predicting the outcome of clinical trials [27]. It has then demonstrated that, frequently, the genetic aberrations found in the continuous human cell lines strongly diverge from the ones commonly characterizing the human tumors [26]. Moreover, even phenotypically, growth characteristics and key histological features of the primary human tumors do not correspond to those of tumor xenografts obtained by injecting in vivo continuous human tumor cell lines [26]. These observations suggested the necessity of extreme warning in transferring the relevance of aberrant signaling pathways within cell lines to human primary tumors [26] and underlined the urgent need for new and more biologically and clinical relevant in vitro model systems for studying tumor biology and conducting preclinical screening of drugs [28].

With this purpose, *short-term culture* has been obtained culturing freshly isolated cells as monolayers in serum-enriched medium [29, 30]. However, whether these cell lines can be considered truly representative of the original tumor has been questioned [26].

A fundamental step forward has been represented by the possibility to isolate and in vitro expand, from numerous solid tumors, including gliomas, the so-called cancer stem cells or

Table 10.1 In vitro model of human low-grade glioma

Tumor of origin	Culture condition	Cell line characterization	Study	Ref.
Continuous human cell lines				
Cell line Hs683: low-grade glioma from an adult patient	Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % FBS	ATCC web site: http://www.lgcstandards-atcc.org/LGCAdvancedCatalogueSearch/ProductDescription/tabid/1068/Default.aspx?ATCCNum=HTB-138&Template=cellBiology *Non-tumorigenic for ATCC has been indeed used in several* in vivo *assays*	Mechanisms responsible of glioma invasion (e.g., GDNF). Drug sensitivity assay. In vivo assays	[56, 60–63]
Cell line H4: low-grade neuroglioma from an adult patient	DMEM/F12 high-glucose medium with 10 % FCS, 100 μg/ml streptomycin, and 100 units of penicillin	ATCC web site: http://www.lgcstandards-atcc.org/LGCAdvancedCatalogueSearch/ProductDescription/tabid/1068/Default.aspx?ATCCNum=HTB-148&Template=cellBiology *Non-tumorigenic*	Mechanisms responsible of glioma invasion (e.g., uPAR production, TFPI-2) and angiogenesis (AEG-1). Drug sensitivity assay. In vivo assays	[58, 59, 64]
Cell lines Res186, Res199, Res 251, Res 259, Res 286, UW467, Res 196, Res 253, Res 254: low-grade gliomas from pediatric patients	DMEM/F12 containing 5 % iron-supplemented bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B	For some of them, it is available the immunophenotype, the genomic analysis, and MGMT activity	BCNU and TMZ sensitivity	[65–67]
Short-term culture				
$n=2$ oligodendroglioma	DMEM/Ham's F12 supplemented with 10 % FBS, L-glutamine, and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin)	Expression of GFAP (−) and synaptophysin (+)	Gene expression profile of cell lines vs. tumor biopsy	[75]
$n=6$ pediatric pilocytic astrocytomas	Hams F10 nutrient mix with 10 % FCS	None	Gene expression profile of cell lines vs. tumor biopsy	[29]
$n=7$ ependymoma and $n=6$ pilocytic astrocytomas: childhood brain tumors	Ham's F10, 25 mM Hepes, 10 % (v/v) selected FCS, and 50 units/ml penicillin and 50 μg/ml streptomycin	None	Drug sensitivity	[30]

Glioma stem cells				
Prospective isolation				
CD133+ Neurosphere obtained from $n=3$ pilocytic astrocytomas and $n=1$ grade II astrocytoma obtained from pediatric patients	Neurospheres were cultured in Ex vivo 15 (BioWhittaker) medium with N2 supplement, fibroblast growth factor-2 (20 ng/ml), epidermal growth factor (20 ng/ml), lymphocyte inhibitory factor (10 ng/ml), neural survival factor-1, and 60 mg/ml *N*-acetylcysteine [136]	Evaluation of neurosphere formation capacity, karyotype and multipotency.	Identification and purification of a cancer stem cell from human pediatric brain tumors of different phenotypes	[86]
CD133+ $n=1$ oligoastrocytoma and $n=3$ grade II astrocytoma samples obtained from adults; $n=2$ pediatric pilocytic astrocytomas		Flow-cytometrical characterization of CD133+ cells	Comparison of the immunophenotype of the CD133+ cells isolated from low- and high-grade gliomas	[87]
NG2+ $n=2$ WHO grade II oligodendrogliomas	Neurobasal medium-A (Invitrogen) supplemented with 0.5xB27 supplement, 0.5xN2 supplement, 20 ng/ml FGF-2, 20 ng/ml EGF, and 2 mM L-glutamine	In vitro: reduced sphere formation with respect to GSC from high-grade glioma; differentiation ability restricted to the oligodendroglial fate; sensitivity to TMZ In vivo*: tumorigenicity*	Demonstration of the possible origin of oligodendroglioma from oligodendroglial progenitor cells (OPC)	[88]
Neurospheres				
$n=3$ pilocytic astrocytomas and $n=1$ grade II astrocytoma obtained from pediatric patients	Ex Vivo 15 (BioWhittaker) medium with N2 supplement, fibroblast growth factor-2 (20 ng/ml), epidermal growth factor (20 ng/ml), lymphocyte inhibitory factor (10 ng/ml), neural survival factor-1, and 60 mg/ml *N*-acetylcysteine [136]	In vitro: evaluation of neurosphere formation capacity, karyotype, and multipotency	Identification and purification of a cancer stem cell from human pediatric brain tumors of different phenotypes	[86]
$n=13$ pilocytic astrocytomas, $n=1$ fibrillary astrocytoma, and $n=1$ grade II oligoastrocytoma of pediatric origin	NSA-H medium,10 ng/ml FGF, 20 ng/ml EGF, and 1 μg/ml heparin or Dulbecco's modified Eagle's: F-12 medium (1:1) containing the N2, G5 (containing FGF and EGF), and B27 supplements	In vitro: self-renewal of neurospheres; molecular profiling In vivo: tumorigenicity	Clinical relevance of tumor cells with stem-like properties in pediatric brain tumors of different origins and WHO grades	[99]

(continued)

Table 10.1 (continued)

Tumor of origin	Culture condition	Cell line characterization	Study	Ref.
$n=15$ pilocytic astrocytomas, $n=3$ other low-grade astrocytomas of pediatric origin	DMEM-F12 medium supplemented with 1–100 B27, 20 ng/ml bFGF, 50 ng/ml EGF, penicillin/streptomycin, L-glutamine, and 5 μg/ml heparin	In vitro: neurosphere formation	Potential associations between neurosphere formation and clinical outcome were analyzed retrospectively in $n=56$ pediatric tumors of different grades	[100]
$n=4$ grade II glioma and $n=3$ grade I glioma of adult origin	DMEM/F12 medium supplemented with B27, 20 ng/ml bFGF, 50 ng/ml EGF, penicillin/streptomycin, L-glutamine, and 5 ug/ml heparin	In vitro: no formation of self-renewing neurospheres	Study of the role of renewable neurosphere formation in cultured human glioma ($n=32$) in predicting patient death and tumor progression	[97]
Adherent cultures				
$n=2$ WHO grade II astrocytomas (only one of the two could be propagated)	ECM-coated flask medium: Phenol red-free Neurobasal A, 20 mM L-glutamine, 1 % (v/v) PSF solution, 20 ng/ml hEGF, 20 ng/ml hbFGF, 20 ng/ml heparin, 2 % (v/v) B27, and 1 % (v/v) N2 supplement	In vitro: expression of transcription factors typical of neural development	The study is mainly focused on the demonstration of the possibility to isolate GSC in adhesion from glioblastoma ($n=22$)	[35]

GSC glioma stem cells, *FCS* fetal calf serum, *FBS* fetal bovine serum, *PSF* penicillin/streptomycin/fungizon, *ECM* extracellular matrix, *TMZ* temozolomide, *bFGF* basic fibroblast growth factor, *EGF* epidermal growth factor, *BCNU* 1,3-bis(2-chloroethyl)-1-nitrosourea, *TFPI-2* tissue factor pathway inhibitor-2, *GDNF* glial cell-derived neurotrophic factor, *MGMT* O6-methylguanine-DNA methyltransferase, *GFAP* glial fibrillary acidic protein, *AEG-1* astrocyte-elevated gene-1

tumor-initiating cells [31]. These latter represent a rare fraction of tumor cells endowed with stem cell properties and therefore able to self-renew and, once injected into a proper murine model, to originate a tumor that exactly recapitulates the original tumor. Thus, the identification, in 2004, of brain tumor-initiating cells represented a groundbreaking scientific discovery for several reasons [12, 13]: (1) It provided insights into human brain tumor pathogenesis envisioning, within gliomas, the same hierarchy present in normal tissues [32]; (2) it established a previously unidentified cellular target for more effective cancer therapies [12, 13, 33]. In fact, therapies unable to specifically hit this rare population are predetermined to not succeed, and recurrences are unavoidable [31]. Additionally, the well-described intrinsic resistance of stem cells toward many drugs could be considered as a putative mechanism responsible of tumor drug resistance; (3) importantly, tumor xenografts originated by injecting cancer stem cells shared the same genetical landscape and the key histological properties of the original tumors, being a bona fide phenocopy of the patient's tumor. Therefore, the possibility to optimize a cellular system genetically and biologically similar to the original one would allow overcoming the crucial limitations presented by human continuous cell lines; (4) finally, the possibility to create a model system to study genotype, gene expression profile, and in vitro and in vivo biological characteristics of different tumors makes possible to move toward a concept of personalized therapy for individual tumors.

This latter fascinated possibility requires isolating stem cells from each tumor patient, to expand them, at a number adequate for drug-screening assays, within an interval of time clinically acceptable. Unfortunately, the two classical methods of isolation of glioma stem cells (GSC) do not completely fulfill these criteria. The fraction of cells expressing the most common GSC marker, CD133, although extremely variable, is usually low; conversely, neurosphere formation is effective only in a fraction of high-grade glioma samples and is time consuming [34]. Modifications of the original protocols [35] and optimization of novel procedures [33] apt to overcome these restrictions are now under investigations. However, as we will discuss in the next paragraphs, it must be underlined that most of the evidences regarding the in vitro and in vivo properties of glioma-initiating cells have been reached using GSC isolated mainly from high-grade gliomas, while only few evidences are available for LGG (Table 10.1).

Finally, since 2000, Hanahan and Weinberg have postulated that tumors are not simply constituted by proliferating cancer cells [36], but they resulted composed of various distinct cell types involved in heterotypic interactions with one another [37]. Importantly, this *tumor-associated stroma*, although constituted by recruited normal cells, is not a passive spectator, but it plays an active role in tumorigenesis; as such, these stromal cells contribute to the development and expression of certain hallmark properties [37], such as sustaining tumor proliferation, inducing angiogenesis, avoiding immune destruction, deregulating cellular energetics, and inducing invasion and metastasis [38–40]. Importantly, many authors pointed to a possible prognostic as well as predictive function of the stromal elements of tumors, and novel targeting opportunities within the tumor microenvironment are under investigation [18, 41, 42]. Therefore, the possibility to create an in vitro model representative of the tumor stroma can allow gaining insight into tumor biology and represents another key element for a patient-based approach. As we will discuss later, this stroma component has been studied essentially in solid tumor of epithelial origin, where cancer-associated fibroblasts (CAF) represent the most abundant cell types in the tumor stroma. Although the biological origin of CAF is still undetermined, evidences point to a possible role played by either bone marrow-derived or resident mesenchymal stem cells [43, 44]. Regarding gliomas, xenotransplantation experiments employing human glioma cell lines have suggested that astrocytes in the vicinity of glioma cells can be activated and facilitate tumor invasiveness [45]. In addition, genetic fate-mapping studies have shown that reactive glia could acquire a stem cell potential outside the two major neural stem cell niches in adult

mammalians [46, 47]. Similarly, PDGF-induced gliomas arising in both adult and neonatal rats have been shown to contain normal stem and progenitor cells "recruited" into glioma mass and induced to proliferate, supporting the hypothesis that proliferative stem-like portions of the tumor can arise from normal progenitors [48]. However, precise nature and specific functional characteristics of these "recruited" stem or progenitor cells have not been described in humans [48]. In this regard, the possible role played by factors other than cancer stem cells in glioblastoma chemoresistance is becoming apparent [49].

In conclusion, low-grade glioma cell cultures could represent an invaluable tool to get insight into the biological features of the tumor; to derive information useful to identify novel diagnostic, prognostic, and predictive markers; and to allow drug-screening strategies. In this regard, the possibilities to obtain cell culture from each single patient are a fundamental prerequisite for the so-called personalized medicine, aimed at treating the right patient with the right drug delivered in the right dose and at the right time. Importantly, since LGG is a disease characterized by long survival rates (although often leading ultimately to death), isolating and storing in liquid nitrogen LGG cell lines gives the opportunity to extend over time the potential direct benefits to the patients from which cell lines have been derived. For example, as soon as new drugs are developed, they can be tested in the stored cell lines giving information that still are useful to treat exactly the same patients from which the cell lines were obtained.

In Vitro Model of Glioma

Several in vitro models of high-grade gliomas have been optimized. Specifically, numerous continuous cell lines are available as well as methods aimed at obtaining short-term culture. Besides, nowadays isolation of glioblastoma (GBM) stem cells can be accomplished adopting at least three different protocols: prospective isolation of definite cell subpopulations on the basis of specific antigens [12, 50–54], growth of cells as neurospheres [13, 35], and growth of cells in adhesion in selective medium [33]. Unfortunately, the same wealth of methods has not been systematically applied to low-grade gliomas. Moreover, there are very few studies on the supporting stroma of both low- and high-grade gliomas.

Immortalized Tumorigenic Cell Lines

Established cell lines have the advantage of being always available, but they are generally considered a poor representation of primary tumor biology, since they present a multitude of genomic and gene expression changes not observed in primary tissues, although they conserve some discrete features of glioma biology [26, 55].

Unfortunately, while numerous cell lines obtained from high-grade gliomas are available (e.g., A172, LN229, SF268, U87MG, U118MG, and U138MG), only few cell lines representative of low-grade glioma are commercially available. Specifically, Hs683 is a cell line derived from a low-grade glioma, while H4 was obtained from a low-grade neuroglioma (see Table 10.1). Importantly, H4 and H683 were both obtained from adult patients. In vitro, they have been assayed in comparison with high-grade glioma-derived cell lines, in order to uncover mechanisms responsible of the acquirement of properties related to the malignant progression of human gliomas, such as cell migration and vascularization. For example, they have been used to define the role played by glial cell-derived neurotrophic factor (GDNF) [56], tissue factor pathway inhibitor-2 (TFPI-2) [57], and the urokinase-type plasminogen activator receptor (uPAR) [58] in inducing cell invasion, as well as the role of astrocyte-elevated gene-1 (AEG-1) in angiogenesis [59].

Although considered non-tumorigenic by ATCC (http://www.lgcstandards-atcc.org/LGCAdvancedCatalogueSearch/ProductDescription/tabid/1068/Default.aspx?ATCCNum=HTB-138&Template=cellBiology), Hs683 has been

described as tumorigenic in vivo by Lefranc group [60–63]. In fact, when orthotopically injected into immunodeficient mice, Hs683 gave rise to a highly invasive oligodendroglial tumors [60–63]. The oligodendroglial origin of the Hs683 model is supported by several evidences (reviewed in [61]). Specifically, Hs683 tumor cells (1) are 1p19q co-deleted; (2) are sensitive to pro-apoptotic chemotherapy and to TMZ under in vivo orthotopic brain xenograft conditions; (3) display high levels of expression of integrin β4 as human biopsy oligodendrogliomas do; (4) do not express the human 1p-distal ATAD 3B gene, which is highly expressed in astroglioma cells; and (5) contain only one Notch2 gene copy per diploid genome as seen in oligodendrogliomas. Lastly, BEX2 (the brain-expressed X-linked gene) interferes with Hs683 oligodendroglioma cell biology in a manner that markedly differs from what is observed in astrocytic tumors. However, the highly invasive nature of the in vivo xenograft induced authors to consider the in vivo Hs683 oligodendroglioma model as a model of GBM displaying an oligodendroglial origin and/or component [61]. Conversely, H4 are not tumorigenic in vivo. However, they could gain in vivo tumorigenicity by ectopic expression of Fos-related antigen 1 (FRA-1) gene, suggesting a possible role of this latter in the maintenance/progression of malignant gliomas [64].

Several cell lines, not commercially available, were instead obtained from low-grade gliomas of pediatric origin [65–67]. Although pediatric gliomas share some morphological similarities with their adult counterparts, they appear to be clinically and biologically different; therefore, there is great interest in determining whether cell lines could be useful in distinguishing these differences [67]. However, this information has been obtained only for high-grade gliomas [67]. Importantly, these cell lines have been exploited as models for preclinical drug development [67] or for drug assays aimed at defining sensitivity to common drugs such as BCNU and TMZ [65–67]. However, there is always more concern in translating the results obtained from tumor cell lines to in vivo tumor. For example, in a recent paper dealing with the effect of aberrant p53 function on TMZ sensitivity, authors established that altered p53 expression or function had only minor effect on brain tumor-initiating cells isolated from GBM, while it determined an increased TMZ sensitivity in traditional immortalized glioma cell lines [68]. These results are somehow questioning the usefulness of established glioma cell lines in drug-screening strategies. However, although results derived from serum-cultured glioma cell lines may be biased by multiple new mutations induced during long-term culture in serum-containing medium [26, 55], several authors have described stem cell-like cells within these cell lines [69, 70]. Therefore, experimental data collected in the "pre-GSC era" may provide some clues to the effects of TMZ or other drugs on GSC and their mechanisms of action [61, 69–74].

In summary, very few continuous cell lines representative of low-grade gliomas, either of adult or pediatric origin, are available. They are not tumorigenic in vivo, and they are mainly used to identify factors responsible of malignant progression or in drug-screening strategies. Their value is questioned by the fact that part of their gene mutation setting has been generated in culture, and therefore, it is not really representative of the tumor tissue.

Short-Term Cell Lines

Short-term cell lines are finite cell lines obtained from culturing glioma cells under serum-containing culture conditions. Since these cell lines are not continuous, they are destined to undergo senescence after a certain number of passages, and therefore, their use is limited over time.

The use of derived short-term cell cultures rather than established cell lines in the study of any tumor may reduce differences between the tumor of origin and the cultured cells [29]. In fact, although culture is expected to influence gene expression, short-term cultures at low passage are less expected to have acquired additional DNA alterations [29]. At this regard, Potter et al. investigated gene expression profile of pediatric pilocytic astrocytoma ($n=6$) and GBM ($n=3$) biopsy

and derived short-term cell culture samples establishing that cell culture influenced approximately 30–35 % of global gene expression. However, considering the derived short-term cell cultures at the passage (P) ten and paired biopsy samples, they identified a molecular profile that could distinguish pediatric pilocytic astrocytoma and adult GBM, regardless of the sample source, confirming that derived astrocytoma short-term cell cultures did retain gene expression resemblances to the tumor in situ. However, these similarities were not revealed by Mehrian Shai et al. who compared cell lines obtained from $n=2$ oligodendroglioma and $n=1$ GBM with the respective tissue biopsies [75]. In another paper, Darling group examined the range of sensitivity of a panel of short-term cultures derived from different types of malignant childhood brain tumors including medulloblastoma, ependymoma, and GBM to three cytotoxic drugs—lomustine, vincristine, and procarbazine—showing a different spectrum of sensitivity on the basis of the histotype of origin of the cell lines [30].

The main concerns regarding short-term cultures are that, besides the low number of short-term cultures obtained from LGG described in literature, cell lines have been poorly characterized. Specifically, it has not established, for example, the presence of DNA alteration. Moreover, it has been shown that the phenotype of the cultured cells is changing with passages [76]. Specifically, while in primary cell culture 80–100 % of neoplastic astrocytes expressed GFAP and were negative for fibronectin and actin, by the fifth to sixth passage in vitro, GFAP immunoreactivity was lost while fibronectin and actin were prominently expressed, thus suggesting either an overgrowth of undifferentiated cells or dedifferentiation of tumor astrocytes [76].

At this regard, more information is available for high-grade gliomas. Specifically, Lee et al. demonstrated that GBM tumor cells grown under serum-containing culture conditions were characterized by the outgrowth of cells that were different, both genetically and biologically, from their parental tumors; moreover, cell lines were not tumorigenic at low passages, while at high passages, they gave rise to tumors that did not resemble the original one [26]. Conversely, when the same primary human glioblastoma cells were propagated in vitro under conditions optimal for the expansion of glioma stem cells (GSC), obtained cell lines closely mimic the genotype, gene expression profile, and biology of their parental primary tumors [26].

In conclusion, data obtained analyzing short-term LGG cell lines provides some validity for the use of cultured cells in research for functional studies. However, the scant characterization of the LGG-derived cell lines and the data attaining GBM-derived short-term cell lines highlight the need to verify the relevance of standard cancer cell lines for studying the biology of human cancer and for screening new therapeutic agents.

Glioma-Derived Tumor-Initiating Cells

Vescovi gave a functional definition of brain tumor stem cells. Specifically, brain tumor cells could be considered as stem cells if they show (1) cancer-initiating ability upon orthotopic implantation, (2) extensive self-renewal capacity either ex vivo or in vivo, (3) karyotypic or genetic alterations, (4) aberrant differentiation properties, (5) capacity to generate non-tumorigenic end cells, and (6) multilineage differentiation capacity [77]. Because this subpopulation of glioma cells, generally called glioma stem cells (GSC), may play an extremely critical role in the initiation and recurrence of gliomas, plenty of studies focusing on GSC have been published. At the present, a well-accepted evidence is that glioma stem-like cells are more representative of their parent tumors than continuous cell lines, and therefore, they could represent a more reliable model for understanding the biology of primary tumors, for screening new therapeutic agents, and for guiding personalized therapy [26].

As previously mentioned, several methods have been applied to isolate GSC either based on the prospective isolation of specific cell subpopulation or on selective growth conditions.

Prospective Isolation of Definite Cell Subpopulations on the Basis of Specific Antigens

Glioma stem cells (GSC) were among the first cells to be defined as a small population of cells expressing the cell surface marker CD133. CD133, formerly known as PROML-1 or AC133, was originally discovered as the equivalent to mouse prominin, a pentaspan transmembrane glycoprotein of murine neuroepithelial stem cells located in plasma membrane protrusions [34, 78]. Although no interacting proteins are known, a role in cell polarity and cell migration was suggested due to its specific localization [79]. Whereas CD133 is expressed in a variety of human tissues, the CD133 antigen with the glycosylated epitope AC133 is mainly restricted to stem cells [34, 78]. When isolated from human brain tumors, CD133+ displays stem cell properties in vitro, such as enhanced capacity for proliferation, self-renewal, differentiation, and neurosphere-like growth [12]. More importantly, CD133+, but not CD133−, tumor cells were able to reconstitute, when injected in vivo into immunodeficient nude mice, a tumor characterized by the same phenotype of the patient's original tumor [12, 13]. In fact, injection of as few as 100 CD133+ cells produced a tumor that could be serially transplanted, whereas injection of 10^5 CD133− cells failed to produce any tumor [12]. Importantly, the small fraction of CD133+ cells seemed to be preferentially resistant to chemotherapeutic agents and radiation and expressed higher levels of mRNA for the ABC transporter BCRP1, the O6–methylguanine–DNA methyltransferase, markers associated with neural precursors, and negative regulators of apoptosis and could thus be responsible for posttreatment recurrence [80, 81].

However, recent reports indicate that this initially proposed model may represent an oversimplification and stem cell specificity of the epitope detected by the antibody AC133 (i.e., glycosylated prominin, CD133) [82] has been questioned [34]. GBM cells may acquire CD133 after xenotransplanation [83]; conversely, CD133+ and CD133− cells may have similar tumorigenic potential [32, 84]. In addition, CD133 does not appear to be essential for stem cell-like properties, as testified by the identification of subgroups of GBM derived by CD133− GSC [50, 52, 85]. Thus, stem cell-specific markers other than CD133, for example, CD15/SSEA-1 and integrin α6, have been described, but there is not yet consensus on the optimal markers for GSC in GBM [51–54].

Regarding human low-grade glioma, it is important to underline that the possibility to isolate CD133-positive cells from low-grade gliomas or low-grade glioma-derived cultures has been confirmed by independent groups (Table 10.1). For example, Singh in 2003 demonstrated the possibility to isolate CD133-positive cells from neurospheres expanded from pilocytic astrocytoma ($n=3$) and grade II astrocytoma ($n=1$) obtained from pediatric patients [86]. More recently, Rebetz et al. flow-cytometrically analyzed oligoastrocytoma ($n=1$) and grade II astrocytoma ($n=3$) samples obtained from adults, as well as pediatric pilocytic astrocytoma ($n=2$) [87]. Importantly, they found that low-grade gliomas, with respect to high-grade gliomas, were characterized by a lesser fraction of CD133+ cells and that most of these latter co-expressed the endothelial marker CD31, possibly excluding their tumor origin [87]. Conversely, CD133+ cells isolated from high-grade gliomas did not express endothelial markers but co-expressed glial and neural markers, probably reflecting a more primitive nature of the cells. Indeed, the tumorigenic properties of CD133-positive cells isolated from low-grade gliomas have never been tested, being the paper claiming the tumorigenic properties of the CD133+ cells focused on cells obtained from high-grade glioma samples [12].

Nonetheless, the proportion of CD133+ cells in a cohort of 95 patients with gliomas, as well as their topological organization in clusters, was a significant prognostic factor for adverse progression-free survival and overall survival independent of tumor grade, extent of resection, and patient age [78]. Furthermore, the proportion of CD133+ cells resulted as an independent risk

factor for tumor regrowth and time to malignant progression in WHO grade 2 and 3 tumors [78].

More recently, it has been evaluated the ability of the oligodendroglial marker NG2 to identify a tumorigenic population within human primary grade II oligodendroglioma samples ($n=2$) [88]. Specifically, Persson et al. fractioned acutely isolated oligodendroglioma cells followed by orthotopic grafting of 1,000 NG2+ or NG2− cells, showing the formation of tumors after 5–8 months only in the mice injected with NG2+ cells.

Altogether, the reported evidences indicate that (1) although debated, CD133 remain a marker able to identify, at least in high-grade gliomas, a population of tumor-initiating cells; (2) it is missing a demonstration of the tumor-initiating potential of the CD133+ cells isolated from low-grade gliomas; nonetheless, the expression of this marker at tissue level seems to possess some clinical relevance; and (3) it is deemed desirable to investigate novel markers, and, at this regard, NG2 emerges as particularly promising, at least for oligodendrogliomas.

Neurosphere Assay

Neurosphere assays were initially employed by Reynolds and Weiss to isolate neural stem cells from the mouse striatum [89] and were subsequently used to effectively enrich tumor-initiating cells from brain tumors [13, 35, 86, 90–92], as well as from other solid tumors [93, 94].

Neurosphere assays are carried out, culturing glioma-derived cells on nonadherent plates and in selective serum-free media usually added with mitogens including epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), or both [34]. This culture condition would favor the survival and expansion of stem-like cells responsive to these cytokines and able to continually divide and form multipotent clonal spheres called neurospheres, while the more differentiated cells, incapable of self-renewal, and multipotency, are lost with serial passages [34]. In fact, serum factors and adherence both promote differentiation. However, within primary neurosphere, less than 1 % of cells are bona fide stem cells [34], that is, cells that are: (1) able to self-renew (i.e., to give rise to new sphere, even upon several passages), (2) clonogenic (i.e., able to give rise to a sphere when seeded as single cell), and (3) multipotent (i.e., able to differentiate into the primary cell types of the tissue from which they were obtained). The remaining cells are constituted by differentiated progeny or by progenitor cells endowed with a limited self-renewal capacity. For this reason, it is necessary to ensure that bona fide stem cells have been isolated by at least demonstrating self-renewal, clonogenicity, and multipotency over an extended period of time (more than six passages) [95, 96].

Neurospheres were initially isolated from both adult [91] and pediatric tumors [86, 90]. Authors demonstrated that brain tumor cells were able to produce proliferating neurospheres that could be passaged at clonal density and differentiated into cells of both neuronal and glial lineage. However, with respect to neural stem cells, tumor neurospheres were characterized by an impaired differentiation capacity mainly favoring the differentiation along the phenotype of the tumor of origin [86, 90] or characterized by generation of cells co-expressing glial and neuronal markers [86, 90, 91]. Importantly, neurospheres expressed many genes characteristic of NSC-derived spheres, such as CD133 [86, 90].

Subsequently, Galli et al. demonstrated that GBM-derived neurospheres were characterized by genetic aberration and were able, once injected both subcutaneously and orthotopically into immunocompromised animal, to generate tumor xenograft histologically resembling the original tumor [13]. Importantly, tumor xenograft could be serially transplanted thus confirming the in vivo self-renewal and tumorigenic capacity of neurospheres [13]. Many papers have subsequently confirmed the results obtained by Galli [13–34] and the superiority of the serum-free culture method above the standard serum-supplemented culture conditions [26]. Specifically, GSC cultured in serum-free medium maintained the genetic aberration characteristics of the parental tumor and, once injected into the mouse, gave rise to xenografts histological resembling the original tumors. Conversely, cultures in

serum-supplemented medium are characterized by the progressive loss of cells bearing the same genetic aberration of the primary tumor and by the appearance, at higher passages, of cells with new mutations. Accordingly, in vivo injection of these cells at low passages is characterized, with respect to those cultured in serum-free conditions, by a reduced tumor formation, while the tumors formed by high-passage cultures are not resembling the original tumor but rather the tumor that are usually formed upon injection of continuous cell lines [26].

Most importantly, in vivo studies have shown that neurosphere formation is a significant predictor of clinical outcome in glioma patients, independent of Ki67 proliferation index, and is a robust, independent predictor of glioma tumor progression [97].

However, neurosphere assays, in common with other in vitro assays, are associated with some limitations [34]. Specifically, with some exception [98], the efficiency of neurosphere assays for producing GSC lines is considered rather low, there are no standard protocols allowing comparison of results, neurospheres are heterogeneous cell cluster constituted in majority by differentiated cells, and because of physical and geometrical reasons, within the spheres there is a gradient of nutrients, oxygen, and growth factors, and all of these latter factors can significantly influence stem cell proliferation, differentiation, and death [34].

Moreover, this method has been shown to be effective in isolating GSC from GBM, while the efficiency in isolating tumor-initiating cells from medulloblastoma has been questioned from different authors [13, 90]. In fact, very few data are available regarding low-grade gliomas. Specifically, as previously mentioned, neurospheres were obtained from $n=3$ pilocytic astrocytomas and $n=1$ grade II astrocytoma low-grade gliomas of pediatric origin by Singh [86]. Comparing pilocytic astrocytomas with medulloblastoma and GBM, authors established that (1) pilocytic astrocytomas were characterized by a ten-time lower fraction of tumor cells able to grow as neurospheres (about 1 %); (2) cells derived from the low-grade gliomas and grown as neurospheres proliferated slower; and (3) expressed less CD133. It was then demonstrated that the capacity for tumor self-renewal was restricted to the CD133+ fraction: While CD133+ cells possessed proliferative capacity, CD133-cells did not proliferate. However, in vivo tumorigenicity as well as presence of genetic aberration was not assessed in the neurospheres obtained from low-grade gliomas [12, 86].

More recently, Thirant et al. extensively studied and characterized neurospheres obtained from 55 pediatric tumors of different origin [99]. They observed a strong association between the type of self-renewal properties exhibited by neurospheres, that is, the ability of the neurospheres to be serially propagated, and the histopathological subtypes. Specifically, they distinguished spheres with limited self-renewal ability, like neural progenitors, that yielded secondary spheres for less than seven times and spheres with extended self-renewal ability, like neural stem cells, that yielded secondary spheres for more than seven times. All medulloblastoma-derived as well as most of the low-grade glioma-derived oncospheres exhibited the limited self-renewal abilities of progenitor-like cells, whereas high-grade gliomas yielded essentially cells with extended self-renewal properties [99]. Importantly, there was an association between isolation of cells with long-term self-renewal and patient mortality. Moreover, neurospheres with extended self-renewal, even if obtained from a low-grade glioma, were able, once injected into immunocompromised mice, to originate a highly infiltrative tumor, histological and molecular phenocopy of the original one [99]. Conversely, non-self-renewing cells did not form tumors.

Such a high yield in obtaining self-renewing neurospheres from pediatric low-grade gliomas was not reported by Panosyan et al. [100]. In this case, only 17 % of low-grade glial tumors (3 out of 18) formed renewable neurospheres, whereas 50 % of high-grade glial tumors and 57 % of embryonic tumors formed renewable neurospheres. Importantly, when evaluating tumor progression, in the glioma tumor subpopulation, neurosphere formation displayed significant predictive value, even when adjusted for grade of

glial tumors. This property of neurosphere formation capacity to predict clinically relevant disease severity, independent of glioma grade, indicated that the neurosphere model possessed distinct prognostic value [100].

In the literature there are very few evidences regarding the possibility to isolate primary neurosphere from low-grade gliomas obtained from adult patients. Specifically, by applying a slightly modified protocol, named Oxford protocol (see below), to $n=2$ adult grade II gliomas, only one of the two cell lines could be propagated, but, importantly, in vivo tumorigenicity and genetic aberration were not investigated [35]. Conversely, Laks et al. in the attempt to study the role of renewable neurosphere formation in cultured human glioma ($n=32$) in predicting patient death and tumor progression failed to obtain neurospheres from all the LGG samples analyzed ($n=4$ grade II glioma and $n=3$ grade I glioma of adult origin) [97].

In conclusion, although the growth of GSC in suspension in serum-free medium has been widely accepted for enriching cell culture in GBM-derived cells characterized both by stem cell properties and tumor-initiating capacity, these evidences are unfortunately lacking for low-grade gliomas obtained from adult patients. Conversely, some evidences exist for pediatric tumors, where in a variable percentage of cases, it was possible to isolate neurospheres, although usually endowed with a limited self-renewal capacity.

Glioma Stem Cell Lines Expanded in Adherent Culture

Several evidences suggest that GSC are more representative of their parent tumors when cultured in a defined serum-free medium added with EGF and bFGF [26, 101]. In spite of this, culturing tumor cells as neurospheres present some drawbacks: the efficiency of the method is of about 50 % for high-grade gliomas [13, 85]; the sphere environment is characterized by spontaneous differentiation and cell death; routine culture and experimental manipulation such as transfection, immunostaining, and generation of single cell suspensions for flow cytometry are not trivial, and therefore, they are not ideally suited for high-content genetic and chemical screening [33]. Maintaining cells in monolayer culture would allow to ideally overcome many of the problems related to the growth of GSC in suspension.

With this aim, two major protocols have been optimized [33, 35].

In the so-called Cambridge protocol, authors utilized a serum-free medium added with EGF and bFGF in a two-phase method [35]. During the first phase, named *derivation phase*, dissociated surgical specimens were allowed to form primary spheroids. These spheroids were not dissociated but transferred onto extracellular matrix (ECM)-coated flasks, where they formed a primary monolayer culture that could be passaged and subsequently propagated (*propagation phase*) [35]. Applying this protocol, it was possible to derivate cell lines from all the tested samples ($n=24$ glioma samples) and 92 % of these could be successfully propagated. Importantly, authors analyzed $n=7$ propagated cell lines obtained from GBM samples, and they verified that these cells expressed, although variably, nestin, CD133, A2B5, NG2, and Olig2 and shared the same genomic abnormalities of their parent tumors. Importantly, when injected in vivo, they generated tumors that could be serially transplanted. Regarding low-grade gliomas, only one of the two obtained cell lines could be propagated (50 % efficiency). Although this cell line was shown to express neural transcription factors, it was assayed neither its genomic setting nor its in vivo tumorigenic capacity.

The second method of culture in adhesion has been described by Pollard et al. [33]. In this case authors demonstrated the possibility to expand in vitro pure populations of cancer stem cells using adherent culture methods previously optimized for fetal and human neural stem cells [102, 103]. Specifically, cells were isolated from $n=6$ high-grade glioma samples and cultured in a serum-free medium added with EGF and bFGF onto laminin-coated flasks. Cell lines were obtained from all the specimens and could be expanded for more than 20 passages in culture without growth modifications [33]. These cells

variably expressed neural stem cell markers such as vimentin, Sox-2, nestin, CD44, 3CB2, and CD133 and were characterized by the capacity to differentiate into astrocyte-like, oligoastrocyte-like, and neuronal-like cells, although at different extent. When intracranial transplanted into immunocompromised animals, all tested GSC lines were able to originate highly vascularized tumors strictly resembling the original human GBM. Like for the CD133+ cells [12], as few as 100–1,000 cells were sufficient to generate a tumor and the tumor xenografts could be serially transplanted. When analyzed for genomic aberrations, cell lines displayed genomic alterations characteristic of GBM. Importantly, GSC displayed gross chromosome instability only at high passages (passage 50). What is amazing in this paper is the fact that each cell line was extremely different from the others in terms of phenotype, differentiation ability, gene expression profile, and in vivo tumorigenicity. Importantly, this heterogeneity was strictly related to the histological characteristics of the tumor of origin, thus strongly supporting the notion that GSC cultured in adhesion could represent an invaluable tool for personalized approaches. In fact, this method resulted to be not only extremely efficient but allowed high-content chemical screening. In fact, a proof-of-principle live cell imaging-based chemical screen was able to establish both drugs effective on all the GSC as well as drugs selectively active on specific GSC lines. Unfortunately, this method has not been applied to low-grade gliomas.

In conclusion, culture of GSC in adhesion in serum-free conditions seems to overcome many of the problems related to the growth of GSC as neurosphere. However, additional independent studies are required before to switch from neurosphere to adhesion culture as a gold-standard method for GSC culture [98].

Non-Stem Cell Origin for Low-Grade Gliomas

The lack of evidences in literature on the isolation and culture of tumor-initiating cells from low-grade gliomas is somehow unexpected. Low-grade gliomas represent a challenging clinical problem, and since surgical removal is a key step in their treatment, biological samples are easily available. Therefore, the lack of data are probably related to the failure of the described methods in isolating low-grade glioma tumor-initiating cells, rather than by the absence of specific interest in this issue. Indeed, in the last years, we have systematically cultured high-grade and low-grade glioma samples in serum-free media added with EGF and bFGF, both in suspension [12] and in adhesion [33]. The first method was effective in isolating neurosphere that could be serially transplanted in about 60 % of the high-grade gliomas but in none of the low-grade gliomas (data not shown). Conversely, both low- and high-grade glioma cells were able to grow in adhesion. However, when cells were genetically evaluated, only those obtained from high-grade gliomas were genetically abnormal (Fig. 10.1, unpublished data).

This inability to isolate tumor-initiating cells can be due either to the lack of stem cells within the tumor or by the inadequacy of the methods, so far optimized, in isolating and expanding this rare cell population. Indeed, the growth in serum-free medium added with EGF and bFGF, either in suspension or in adhesion, has been derived from neural stem cell study, and it remains unclear whether tumor stem cells in low-grade gliomas derive from the transformation of normal human neural stem cells or whether their presence therein reflects the result of the transformation and dedifferentiation of a more mature brain cell, which reacquired stem cell properties such as self-renewal [31, 104].

Indeed, based on information from patients, developmental biology, and experimental glioma models, the most putative target cells include not only neural stem cells but also astrocytes and oligodendrocyte precursor cells [104]; importantly, depending on the initiating cell type, different culture conditions could be required [88].

The issue is even more complex if we consider that very recently it was shown, in an experimental model of glioma, that the cell of mutation and the cell of origin could be different cell types [105]. When p53 and NF1 were homozygously mutated in neural stem cells (NSC) using the

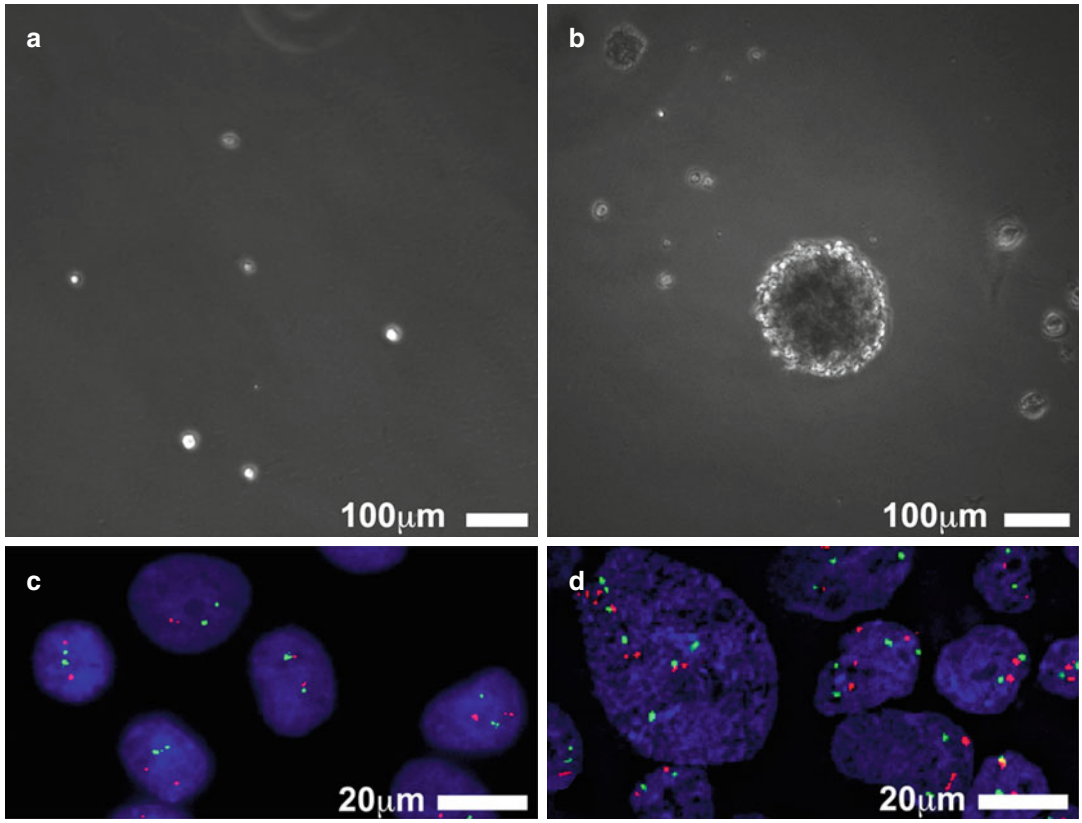

Fig. 10.1 Neurosphere formation. Phase-contrast pictures. While low-grade glioma failed to grow when cultured in suspension in serum-free medium (**a**), the high-grade glioma sample was able to form neurospheres (**b**). Growth in adhesion culture in serum-free medium. Fluorescence in situ hybridization using LSI 1q25 spectrum green probe (*green dots*) and LSI 1p36 orange probe (*red dots*) (Vysis). While low-grade glioma-derived cells did not show 1p deletion (**c**), the high-grade glioma cells displayed polyploidy (**d**). Nuclei are depicted by the blue fluorescence of DAPI staining

MADM (mosaic analysis with double markers) mouse model, malignant transformation generating gliomas occurred only in oligodendroglial progenitor cells (OPC) [105]. Whether this means that OPC are more prone to oncogenic transformation than other glial cell types or if OPC are more susceptible to this particular combination of genetic alterations is yet not defined. Nonetheless, other accumulated evidences strongly support OPC as cell of origin for glioma [104].

OPC can be identified by co-expression of platelet-derived growth factor receptor α (PDGFR-α), transcription factor Sox10 and Olig2, and the neuroglial chondroitin sulfate proteoglycan 4 (NG2) [88]. OPC is the major dividing cell population in the adult brain and gives rise to oligodendrocytes. The broad distribution of OPC in the subventricular zone, white matter, and gray matter, together with their proliferative ability, makes them a susceptible target to oncogenic transformation [104]. In support of this, the PDGFR-α signaling pathway involved in normal development of oligodendrocytes by controlling proliferation and migration of OPC is also commonly altered in both low- and high-grade gliomas [106, 107]. Importantly, in 2000 Kondo demonstrated that OPC are not irreversibly committed precursor cells that could only give rise to oligodendrocytes in vivo and oligodendrocytes and type 2 astrocytes in culture [108], but they can be converted to immature multipotent cells in vitro that in turn can differentiate into neurons, type 1 astrocytes, and oligodendrocytes [109]. Further, it has been shown that OPC upon a combination of Ras activation and p53 depletion can become glioma-initiating cells and able to induce

with high efficiency secondary tumors [110]. Interestingly, the gene expression profile of the transformed OPC showed that these cells had undergone global reprogramming and were more similar to NSC than OPC [110].

Evidences that murine oligodendroglioma arises from NG2-expressing cells in white matter regions, rather than NSC, have been recently given by Weiss's group that linked the therapy-responsive nature of this tumor to a progenitor origin [88]. Specifically, authors demonstrated that NG2+ oligodendroglioma cells expressed genes and proteins associated with OPC rather than NSC; that NG2+ oligodendroglioma cells showed limited sphere formation, consistent with a progenitor population; and that NG2+ oligodendroglioma cells were lineage restricted, being able to differentiate only into oligodendroglial derivatives. Importantly, NG2-expressing cells, from both mouse and human oligodendroglioma, displayed high in vivo tumorigenicity and were sensitive to both alkylating and differentiating agents [88]. Finally, they showed that these oligodendroglioma cells were neither chemoresistant nor quiescent and suggested that a progenitor origin for these cells could explain their chemosensitivity. Similarly, to analyze if OPC could serve as cell of origin for glioma, Lindberg et al. developed a new tv-a transgenic mouse line, Ctv-a, in which viral infection could be targeted to OPC expressing 2′, 3′-cyclic nucleotide 3′-phosphodiesterase (CNP) [111]. CNP is a highly specific marker that in the central nervous system is only expressed late in OPC development and in mature oligodendrocytes. With the Ctv-a mouse, authors could induce low-grade oligodendroglioma with RCAS-PDGF-B showing that the cell of origin for oligodendroglioma may be a committed glial progenitor cell [111].

Regarding mechanism of transformation, Sugiarto et al. have shown that the neoplastic transformation of OPC is associated with a loss of asymmetric division [112]. In fact, while asymmetric division of human NG2+ cells was prevalent in nonneoplastic tissue, it resulted in decrease in oligodendrogliomas, where regulators of asymmetric cell division were misexpressed [112].

In conclusion, several experimental evidences point to the fact that the cell of origin of low-grade gliomas has not been unequivocally identified. Nevertheless, establishing the cellular origin can be essential to give a more accurate representation of the biological properties of a particular glioma and thereby a better prediction of response to different therapeutic strategies [104]. For this reason, and considering that current protocols have been exclusively developed for neural stem cells, it is essential to develop novel in vitro protocols specifically aimed at isolating cells of different origin, possibly allowing the development of more efficient, reliable, and patient-based in vitro models of low-grade gliomas. This would be essential to study the cell of origin for glioma; to investigate how the originating cell type affects glioma initiation, progression, recurrence, and response to therapy; and to identify biomarkers differentiating cellular origin that could be of predictive and therapeutic importance [104].

Tumor-Associated Parenchymal Cell Lines

Since 2000, Hanahan and Weinberg have postulated that tumors are not simply constituted by proliferating cancer cells [36] but composed of multiple distinct cell types involved in heterotypic interactions with one another [37]. Importantly, this *tumor-associated stroma*, although constituted by recruited normal cells, is not a passive spectator, but it plays an active role in tumorigenesis; as such, these stromal cells contribute to the development and expression of certain hallmark properties [37], such as sustaining tumor proliferation, inducing angiogenesis, avoiding immune destruction, deregulating cellular energetics, and inducing invasion and metastasis [38–40]. Importantly, many authors pointed to a possible prognostic as well as predictive function of the stromal elements of tumors, and novel targeting opportunities within the tumor microenvironment are under investigation [18, 41, 42]. Therefore, the possibility to create an in vitro model representative of the tumor stroma can allow gaining insight into tumor biology and represent another key element in a patient-based approach.

Nowadays this stroma component has been studied mainly in solid tumor of epithelial origin;

specifically, authors focused their attention on the so-called cancer-associated fibroblast (CAF) or tumor-associated fibroblasts (TAF) that represent the most abundant cell types in the tumor stroma [17, 18, 113]. Although the biological origin of CAF is still undetermined, evidences point to a possible role played by either bone marrow-derived or resident mesenchymal stem cells [43, 44].

Regarding gliomas, it is becoming apparent the role played by tumor-associated parenchymal cells, such as vascular cells, microglia, peripheral immune cells, activated astrocytes, and neural precursor cells, in defining many of the key features of brain tumors and in controlling the course of pathology [45]. For example, the tumor vasculature not only supports gliomas but also provides a specialized niche, named perivascular niche [114–116], fundamental for GSC self-renewal [117]. Microglial cells, which can contribute up to 30 % of a brain tumor mass, promote glioma migration and tumor growth [118, 119]. Nonneoplastic astrocytes can be converted into a reactive phenotype by the glioma microenvironment [120, 121] and can then secrete a number of factors which influence tumor proliferation and invasion, for example, via activation of pro-MMP2 [122], production of SDF-1 [123], neurotrophic factors [124], and astrocyte-elevated gene-1 (AEG-1) [125]. Importantly, tumor microenvironment not only profoundly controls tumor biology but also can interfere with therapy. For example, recent data suggests that GSC are neither resistant nor susceptible to chemotherapy per se [49]. Together with detoxifying proteins such as O6-methylguanine-DNA-methyltransferase (MGMT), which confers a strong intrinsic resistance to GSC in all studies [126, 127], extrinsic factors may also contribute to the resistance of GSC to temozolomide (TMZ). These may include TMZ concentrations in the brain parenchyma, TMZ dosing schemes, hypoxic microenvironments, niche factors, and the reacquisition of stem cell properties by non-stem cells [49]. It is therefore necessary to consider these factors in order to overcome chemoresistance in the patient [49].

A special issue regards stem cells, other than GSC, present in the brain tumor. For example, the young brain may have the capacity to inhibit gliomagenesis by the endogenous neural stem and progenitor cells that migrate toward primary brain tumors [128–130] and secrete tumor suppressive factors [129–133]. In addition, genetic fate-mapping studies have shown that reactive glia could acquire a stem cell potential outside the two major neural stem cell niches in adult mammalians [46, 47]. Similarly, PDGF-induced gliomas arising in both adult and neonatal rats have been shown to contain normal stem and progenitor cells "recruited" into glioma mass and induced to proliferate, supporting the hypothesis that proliferative stem-like portions of the tumor can arise from normal progenitors [48]. However, the precise nature and specific functional characteristics of these "recruited" stem or progenitor cells have not been described in humans [48].

Recently, utilizing a method optimized in our laboratory for the isolation of multipotent adult stem cells (MASCs) from normal human tissues [21, 22], we have demonstrated that human neoplastic livers possess a population of MASC with properties of tumor-associated fibroblasts and that these cells could originate from resident mesenchymal cells, possibly through a paracrine mechanism [44].

Importantly, when the same protocol was applied with minor modifications to human gliomas, it was possible to isolate MASC with aberrant growth properties from both low-grade ($n=56$) and high-grade ($n=71$) human gliomas [23–25]. The method is characterized by an efficiency of about 100 % for both low- and high-grade gliomas allowing to obtain several million of proliferating cells in few weeks [23–25]. Unpublished data from our laboratory demonstrate that these cells are characterized by a mesenchymal cell surface immunophenotype; expression of pluripotent state-specific transcription factors Oct-4, Sox-2, and NANOG; clonogenicity; and multipotency, being able to differentiate into all three neural lineages (Fig. 10.2). Significantly,

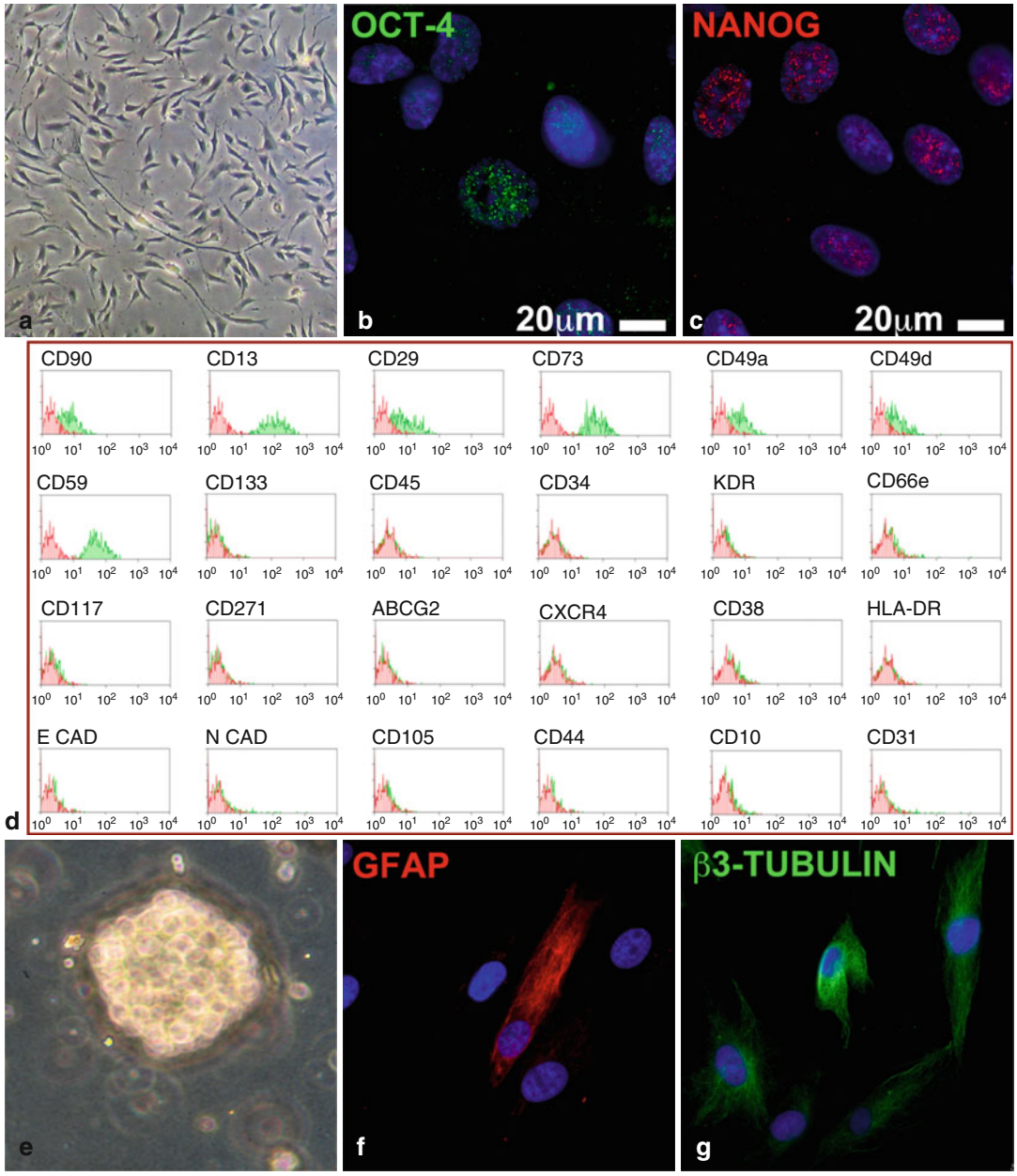

Fig. 10.2 Multipotent adult stem cells (MASC) obtained from LGG. (**a**) Phase-contrast image of a cell line at the third passage in culture. (**b–c**) Pluripotent state-specific transcription factor expression. Cells at the third passage in culture express Oct-4 (*green* fluorescence; **b**) and Nanog (*red* fluorescence; **c**). (**d**) Surface immunophenotype. LGG-derived cell lines displayed a mesenchymal stem cell immunophenotype as assessed by flow cytometry. (**e**) Growth in soft agar. Phase-contrast image showing a colony of LGG cells grown in the absence of anchorage. (**f–g**) Multipotency. When cultured in differentiation-inducing conditions, cells express GFAP (*red* fluorescence; **f**) and beta3-tubulin (*green* fluorescence; **g**). In (**b–c** and **e–f**), nuclei are depicted by the *blue* fluorescence of DAPI staining

MASCs show anchorage-independent growth and the ability to significantly modify clonogenicity and migratory properties of two GBM cell lines, U87MG and A172. In spite of this, at a genetic level, MASCs do not present genetic mutations, including those characterizing the respective tumor tissue, as observed by a whole genome SNP analysis. Interestingly, MASCs display a protein expression pattern characteristic of reactive astrocytes, suggesting that these cells represented an activated component of the glioma microenvironment. The importance of the latter in glioma behavior is supported by the fact that some of the in vitro properties of MASC (e.g., population doubling time) significantly correlated with relevant patient's prognostic data (i.e., survival in high-grade glioma and time of relapse in recurrent low-grade glioma), suggesting that isolated stromal cell lines could represent an in vitro model mirroring the in vivo behavior of the original tumor.

Altogether these results suggest that cells other that tumor cells can be efficiently cultured from LGG. These cells are endowed with aberrant growth properties and could be used to identify novel prognostic and/or predictive factors. Notably, the use of nonneoplastic stromal cells as novel therapeutic target is encouraging since stromal cells are genetically more stable and therefore less prone to drug resistance development. This pioneering field requires further work to confirm the obtained data and to better define the cell of origin of these cell lines.

Critical Overview of the Methods Applied to Obtain Low-Grade Glioma Culture and Future Perspectives

Low-grade glioma cell cultures could represent an invaluable tool to get insight into the biological features of the tumor; to derive information useful to identify novel diagnostic, prognostic, and predictive markers; and to allow drug-screening strategies. As previously explained, the possibility to obtain cell culture from each single patient is also a fundamental prerequisite of the so-called personalized medicine [134], and cell lines are instrumental for developing in vivo model of disease strictly resembling the human tumor.

Nevertheless, considering Table 10.1 that summarizes the methods described in the literature to obtain low-grade glioma culture, several concerns arise. Specifically:

1. As previously mentioned, what is unexpected is that, with respect to high-grade gliomas, very few in vitro LGG models are nowadays available. One possible explanation is that up to now it has been simply transferred to LGG the methods optimized for GBM. In fact, the possible different origin of LGG, with respect to GBM, can require the development of novel in vitro protocols specifically aimed at isolating cells of different origin, thus allowing the development of more efficient, reliable, and patient-based in vitro models of LGG.
2. LGG cell lines used in drug-screening assays were usually scarcely characterized and grown under serum-enriched conditions. For GBM it has been demonstrated the superiority of growing cells in serum-free conditions in order to obtain cell lines in vitro and in vivo representative of the tumor of origin. Again, the low efficiency in obtaining cell culture from LGG cells, when cultured under serum-free conditions (either as neurospheres or in adhesion), highlights the importance of developing novel culture methods.
3. The cancer stem cell (CSC) hypothesis states that CSC able to escape multimodal therapy drives tumor relapses. Possible explanations for treatment failure include insufficient drug delivery or the fact that the treatment targets only more differentiated tumor cells (the tumor bulk), while sparing the small subset of CSC, or that CSC develop mechanisms to overcome chemotherapy-induced cell death [80, 135]. Therefore, only therapies that efficiently hit the CSC fraction of a tumor are able to induce long-term responses and thereby halt tumor progression. Unfortunately, glioma stem cell culture from LGG is not yet an efficient method. The prospective isolation of putative GSC from freshly isolated glioma cells, for example, on the basis of CD133 or NG2, could be promising. In this regard, the

number of cells that can be isolated is at the moment quantitatively insufficient to apply high-throughput methods such as proteomics. Perhaps, the development of novel strategies based on nanotechnology would allow in the future to obtain data from freshly isolated cells without requirement of cell expansion.

4. Most of the successful LGG cultures have been obtained starting from pediatric tumors though pediatric and adult LGG are considered biologically and clinically distinct diseases and therefore results obtained in one setting cannot be simply applied to the other.
5. LGG study can take advantage of the study of components other than tumorigenic cells, such as the tumor-supporting stromal components. Preliminary results show that the efficiency in isolating these cells is very high and that cell lines obtained can be used to identify novel prognostic and predictive markers. Then again, more definitive results must be acquired to confirm these promising results.

Conclusion

LGG cultures represent a very promising tool in LGG study and personalized medicine approaches. For this reason further efforts are required to ensure that LGG culture could become efficient, reliable, and patient-based in vitro models of low-grade gliomas.

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Animal Models for Low-Grade Gliomas

11

Rolf Bjerkvig, Sébastien Bougnaud, and Simone P. Niclou

Abstract

The establishment of animal models for human brain tumors is based on the realization that clear mechanistic information with a functional focus is difficult to obtain in human studies and from the fact that in vitro tumor models do not reflect the physiological complexity of tumors grown in vivo. Animal models for low-grade gliomas have mainly appeared during the last two decades with the development of genetically engineered mice where in particular the platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) systems seem to be important in tumor formation. Also, with the advent of neurosphere culture techniques used to propagate neural stem cells, recent developments indicate that low-grade gliomas can be initiated as xenografts in immunodeficient animals. A major challenge of these models is the time required to tumor development. Nevertheless, it is expected that animal modeling for low-grade gliomas will provide important new insight into the etiology of low-grade tumor development in the years to come.

Keywords

Genetically modified mice • Xenografts • Oligodendroglioma • PDGF EGFR • IDH1 • IDH2

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Introduction

A substantial number of animal models have been developed during the past 60 years for malignant brain tumors. The success of establishing high-grade primary brain tumors has led to a comprehensive understanding of driver as well as bystander mutations involved in tumor development. The rationale for establishing animal models for brain tumors is based on the realization that detailed information with a mechanistic functional focus is difficult to obtain in human

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studies and from the fact that in vitro tumor models do not reflect the physiological complexity of tumors growing in vivo. Evaluation of therapeutic response to novel drug candidates is more reliable when it is based on orthotopic brain tumor models. In general brain tumor animal models have been divided into three categories: (1) chemically induced models, (2) genetically engineered mouse (GEM) models, and (3) xenograft models.

Chemical induction of brain tumors gained considerable attention in the 1970s using different rat strains for tumor development [1]. Murine [2], canine [3], and feline [4] models do also exist, but these have gained less popularity based on the fact that model standardization is difficult. In particular in the rat local, oral, intravenous, or transplacental administration of the potent neurocarcinogen *N*-ethyl-*N*-nitrosourea (ENU) to adult or pregnant animals has led to brain tumor formation. These models enable investigation of the sequential development of brain and spinal neoplasms. Although some oligodendroglioma- and Schwannoma-like neoplasms also occur in ENU-induced tumors [5], most of the malignant lesions are pleomorphic and can be more accurately characterized as gliosarcomas bearing little resemblance to low-grade gliomas.

During the last 15 years, advances in molecular biology, including development of gene expression arrays, determination of mutational tumor profiles, and more recently next-generation sequencing, has led to an in-depth characterization of genomic alterations in gliomas. Following this knowledge, there has also been a considerable advancement in the development of numerous GEM models that have been used to study underlying mechanisms of tumor development. In particular GEM models have led to a better understanding of the genetic mutations and genomic alterations that underlie the initiation and progression of several glioma subtype [6], including gliomas of lower grade.

The third major model system involves xenografting tumor material, either in the form of cell lines or of patient biopsies into the brain of mice or rats. These models have proven to be highly valuable in the search for mechanisms that determine tumor growth and progression and in cancer drug development [7]. In particular, with the advent of immunodeficient animals, important insights have been obtained related to the growth of human tumors within the central nervous system. Although most of these xenografts have focused on malignant gliomas, tumor lesions may evolve that resemble low-grade gliomas.

In the following sections, we will discuss in more detail the GEM and xenograft models that can give rise to malignant lesions that resemble low-grade gliomas.

Genetically Engineered Models for Low-Grade Gliomas

An increased knowledge of genomic alterations present in primary brain tumors has led to the development of highly characterized GEM models based on known genomic changes seen in human tumors. GEM models frequently reflect the histopathology, etiology, and biology of human tumors, and they have proven to represent important experimental tools to determine the tumor-initiating capacity of specific mutations as well as to follow the temporal appearance of additional mutations as a consequence of tumor initiation. By crossing different transgenic strains, the effect of a combination of genetic lesions can be analyzed. The models have also been used to study new therapeutic strategies [8].

During the last years, a number of extensive reviews have been published on GEM modeling of human brain tumors [6, 9] including gliomas of lower grade (Table 11.1). Of interest here is the fact that many single mutations have turned out to give rise to low-grade gliomas, while the combination of genetic lesions often leads to more malignant tumors. The most important insight into low-grade glioma development in animals has been provided in GEM models affecting the platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) signaling pathways (Table 11.1).

PDGF-Driven Gliomas

It was early demonstrated that an activation of the PDGF receptor can induce extensive proliferation of immature glial precursors but blocks their differentiation into mature oligodendrocytes

Table 11.1 Overview of transgenic rodents that may serve as models for low-grade gliomas

Genes and species	Additional gene	Model system (promoter)	Histology and grade	Reference
PDGFB overexpression Mice		Retroviral vector	*Human GBM-like* (*IV*): mitotic figures, hemorrhagic areas, and pseudopalisading necrosis *Primitive NET* *Some O, MD, and SP*	Uhrbom et al. [21]
PDGFB overexpression Mice		RCAS/tv-a (nestin)	*60 % O* (*II*): diffusely infiltrative neoplasm, small tumor cells, perinuclear halo, secondary structure of Scherer	Dai et al. [26]
		RCAS/tv-a (GFAP)	*70 % O* (*II*) *Gliomas of mixed histology* (*II*): one component O and one OA more pleomorphic and atypic	
	Ink4a-Arf deletion Ink4a-Arf$^{-/-}$ transgenic mice	RCAS/tv-a (nestin)	*50 % AO* (*III*): dense cellularity, mitotic features, cellular and nuclear polymorphism, tumor necrosis	
	Ink4a-Arf deletion Ink4a-Arf$^{-/-}$ transgenic mice	RCAS/tv-a (GFAP)	*70 % AO* (*III*): dense cellularity, mitotic features, cellular and nuclear polymorphism, tumor necrosis	
PDGFB overexpression Mice		RCAS/tv-a (nestin)	*78 % O* (*II*): small tumor cells with regular round nuclei, some perinuclear halo, secondary structure of Scherer *22 % AO* (*III*): high tumor cell density with mitotic figures, cellular and nuclear polymorphism, microvascular proliferation, and pseudopalisading necrosis	Tchougounova et al. [27]
	Ink4a-Arf deletion Ink4a-Arf$^{-/-}$ transgenic mice	RCAS/tv-a (nestin)	*75 % AO* (*III*) *25 % O* (*II*)	
		RCAS/tv-a (GFAP)	*O and OA* (*II*): mixed histology	
	Ink4a-Arf deletion Ink4a-Arf$^{-/-}$ transgenic mice	RCAS/tv-a (GFAP)	*74 % AO and AOA* (*III*) *26 % O and OA(II)*	

(continued)

Table 11.1 (continued)

Genes and species	Additional gene	Model system (promoter)	Histology	Reference
PDGFB overexpression		RCAS/tv-a (nestin)	*Low-grade glioma* (*II*): round nuclei, infiltrative, O and OA	Hambardzumyan et al. [29]
Intracranial injection of adult mice				
	Ink4a-Arf deletion	RCAS/tv-a (nestin)	*GBM* (*IV*): necrosis, high cellularity, microvascular proliferation	
		Ink4a-Arf$^{-/-}$ transgenic mice		
		RCAS/tv-a (GFAP)	*Low-grade glioma* (*II*): mixed histology O and A	
	Ink4a-Arf deletion	RCAS/tv-a (GFAP)	*GBM (IV)*: mixed histology O and A	
		Ink4a-Arf$^{-/-}$ transgenic mice		
	PTEN deletion	RCAS/tv-a (nestin)	*GBM* (*IV*): necrosis, high cellularity, microvascular proliferation	
		PTEN$^{-/-}$ transgenic mice		
PBGFB overexpression		RCAS/tv-a (CNP)	*O II*: isomorphic cells, round nuclei and perinuclear halo	Lindberg et al. [28]
Mice			*Some OA* (*II*): mixed histology	
PDGFB overexpression	P53 deletion	Transgenic mice (GFAP)	*GBM* (*IV*): palisading necrosis, microvascular proliferations	Hede et al. [31]
Mice			*8 % O* (*II*): diffuse tumor border, spread of tumor cells on surrounding brain substance	
V12HA-ras overexpression		V12HA-ras (multiple copy integration) transgenic mice	*GBM* (*IV*): multifocal, diffusely hypercellular, pseudopalisading necrosis, factor VIII positive blood cells, multitude mitotic figures, increase VEGF around perinecrotic areas	Ding et al. [70]
		V12HA-ras$^{+/-}$ (single copy) transgenic mice	*80 % A* (*II*): infiltrative, solitary focus of astrocytoma-like lesions	
Mice and serial transplantation in adult mice			*20 % AA* (*III*): more cellularity, nuclear atypia, mitotic figures, factor VIII, VEGF, and microglial reaction	
		V12HA-ras$^{+/+}$ (double copy) transgenic mice	*AA* (*III*)	

EGFRwt and EGFR vIII overexpression Mice		EGFR vIII transgenic mice	No tumor	Ding et al. [34]
		EGFR wt transgenic mice	No tumor	
	ras overexpression	V12HA-ras$^{+/-}$ transgenic mice	*80 % A* (*II*): low-grade infiltrative, solitary focus of astrocytoma-like lesions, GFAP positive transgene expression with cellular atypia *20 % A* (*III*): more cellularity, nuclear atypia, mitotic figures, factor VIII, VEGF, and microglial reaction	
	ras overexpression	V12HA-ras$^{+/-}$ and EGFR vIII double transgenic	*O* (*II*) *Some mixed OA*: GFAP and nestin immunopositive	
v-erb (*EGFR*) *overexpression* Mice		v-erb (S100β) transgenic mice	*O* (*II*): monomorphic fields of cells, round homogenous nuclei, perinuclear halo, invasion of the white matter tracts	Weiss et al. [32]
	Ink4a-Arf deletion	v-erb (S100β) and Ink4/arf$^{+/-}$ double transgenic	*50 % AO* (*III*): increase cellularity, endothelial proliferation, necrosis, nuclear atypia, disrupted blood brain barrier (MRI) *50 % O* (*II*)	
	Ink4a-Arf deletion	v-erb (S100β) and Ink4/arf$^{-/-}$ (double transgenic)	AO (*III*)	
	P53 deletion	v-erb (S100β) and p53$^{+/-}$ (double transgenic)	AO (*III*)	
v-erb (*EGFR*) *overexpression* Rat		v-erb (S100β) (transgenic rat)	Cerebellar localization *O* (*II*): isomorphic cells, round nuclei and perinuclear halo *Some MG:* fibrillary and epithelioid tumor cells with eosinophilic cytoplasm	Ohgaki et al. [35]

CNP 2′,3′-cyclic nucleotide 3′-phosphodiesterase, *O* oligodendroglioma, *SP* spongioblastoma, *MD* medulloblastoma, *NET* neuroectodermal tumor, *OA* oligoastrocytoma, *A* astrocytoma, *AA* anaplastic astrocytoma, *GBM* glioblastoma multiform, *MG* malignant glioma undefined, *AO* anaplastic oligodendroglioma, *(II)* WHO classification of brain tumors (grade II), *(III)* WHO classification of brain tumors (grade III), *(VI)* WHO classification of brain tumors (grade IV), *RCAS/tv-a* replication competent avian leukosis virus splice acceptor/the receptor for avian leukosis subgroup-a, *V^{12}HA-ras* constitutively active human ras, *v-erb* EGFR constitutively active

and astrocytes [10–12]. In particular the PDGF receptor α isoform is more expressed in glial progenitors compared to mature glial cells [13, 14]. These findings, together with the observations that PDGF and its receptors are expressed in oligodendroglioma [15–17] and that the PDGF receptor α gene is amplified in high-grade oligodendrogliomas [18], led early to the hypothesis that PDGF may be an important factor in low-grade glioma development. The observation that PDGF and its receptors are co-expressed on human glioma cell lines as well as on human glioma biopsies including oligodendrogliomas led to suggest the existence of autocrine signaling loops between PDGF and its receptors [19, 20]. The first indication that abnormal PDGF signaling can contribute to glioma etiology came from studies overexpressing PDGF-B using a retroviral vector system which led to primary brain tumors in mice showing multiple histological appearances [21]. The variety of histological appearances observed can most likely be explained by the type of retroviral vector system used that has the capacity to transform multiple cell lineages. Since these initial observations, the PDGF-driven tumor models have been extensively improved through the use of cell type-specific gene transfer of PDGF using the *r*eplication *c*ompetent *A*LV *s*plice acceptor (RCAS)/tv-a system [22, 23]. This system involves the *a*vian *l*eucosis *v*irus (ALV)-based RCAS viral vector and transgenic mice that express the ALV receptor tv-a from cell type-specific promoters [24, 25]. The system has been used to produce an RCAS vector that encodes the PDGF-B chain that subsequently was used to infect mice expressing tv-a receptors in astrocytes, either through a glial fibrillary acidic protein (GFAP) promoter or a nestin promoter (to infect glial precursors). In vivo PDGF-B gene transfer in GFAP positive and nestin positive cells gave rise to oligoastrocytomas and oligodendrogliomas, respectively [26]. Interestingly, loss of $p16^{Ink4a}$ and $p19^{Arf}$ was not required for PDGF-induced tumor formation, but by introducing these mutations to the cells, this led to tumors of higher malignancy [26].

Following up on the function of the *Ink4a-Arf* locus in glioma progression, it was later shown in animal models that *Ink4a* loss in particular contributes to tumor initiation from astrocytes, whereas *Arf-loss* causes tumor progression from both astrocytes and progenitor cells [27]. Moreover, the mechanisms of PDGF-driven oncogenesis seem to involve the activation of the mitogen-activated protein kinase (MAPK) pathway via upregulation of extracellular signal-regulated kinase (Erk), whereas tumor progression seems to occur upon loss of $p19^{Arf}$ causing upregulation of cyclin D1 and activation of the phosphoinositide 3-kinase (P13K) pathway [27]. The RCAS/tv-a model has also been used to generate mice where PDGF tumor induction is restricted to myelinating oligodendrocyte progenitor cells (OPCs), a committed glial progenitor cell. OPCs induced tumors that resembled human WHO grade II oligodendrogliomas suggesting that these cells may be at the origin of oligodendroglial tumors [28]. Interestingly, using the RCAS/tv-a model, it was also shown that tumor initiation could occur in different brain regions including the subventricular zone, the cortex, and the cerebellum [29].

To determine whether PDGF could induce spinal cord gliomas, mice have also been generated that express PDGF-B under the control of a tetracycline-responsive element (TRE/PDGF-B). When these mice were crossed with transgenic mice expressing the tetracycline transcriptional activator (tet-off) regulated by GFAP, GFAP promoter showed a stronger activity in the spinal cord which resulted in spinal cord neoplasms resembling human mixed oligoastrocytomas [30].

Transgenic mice have also been generated overexpressing human PDGF-B in brain, under control of the human GFAP promoter. In contrast to retroviral induced PDGF expression, these mice showed no phenotype, but on a *p53* null background, a majority of them developed brain tumors displaying tumor lesions throughout the brain that resembled high-grade oligodendroglioma or glioblastoma [31].

EGFR-Driven Gliomas

A large proportion of human gliomas show amplification, overexpression, or mutations of the EGF receptor (EGFR). To define a functional role of the receptor in tumor initiation and development, several GEM models have been developed including both transgenic mice and rats (Table 11.1). In the context of low-grade gliomas, in particular, oligodendrogliomas frequently overexpress EGFR, whereas, as mentioned above, the additional loss of *Ink4a/Arf* is found mostly in high-grade tumors. Transgenic mice have been generated expressing the activated viral form of EGFR (v-erbB) under control of the S100β promoter [32]. These mice have been shown to develop low-grade oligodendrogliomas, whereas animals heterozygous for *Ink4a/Arf* or *p53* developed high-grade tumors. Interestingly this model indicates that the tumors established share similarities to oligodendrocyte progenitors and that progenitors rather than stem cells drive tumor formation [33]. These observations further indicate that also overexpression of EGFR, an observation of uncertain significance in human oligodendroglioma, can initiate low-grade oligodendrogliomas in mice.

Transgenic mice have also been developed expressing both wild-type (wt) and mutant (EGFRvIII) human EGFR molecules using the human GFAP promoter. Both transgenic strains showed increased numbers of astrocytes compared to the normal brain but showed no evidence of tumor formation. However, when mice expressing both activated RAS (v(12)ha-ras) and EGFR were developed, v(12)ha-ras/EGFRvIII, but not v(12)ha-ras/EGFRwt double transgenic mice, had decreased survival with 50 % of the mice dying from glioma within 2–4 weeks. Interestingly, v(12)ha-ras/EGFRvIII mice developed oligodendrogliomas and mixed oligoastrocytoma tumors, whereas fibrillary astrocytomas developed in v(12)ha-ras mice. In addition to the development of a spontaneous model of infiltrating oligodendroglioma, this study demonstrates that astrocyte-specific expression of EGFRvIII alone is insufficient for gliomagenesis but rather contributes to glioma progression [34].

Low-grade gliomas have also been established in transgenic rats expressing an activated form of EGFR (v-erbB) under transcriptional regulation of the S100β promoter. A relatively large proportion of both homozygous and heterozygous rats developed brain tumors with either a highly malignant infiltrating phenotype or a less invasive oligodendroglioma phenotype with typical S100 positive isomorphic cells with round to ovoid nuclei and perinuclear halos [35].

Isocitrate Dehydrogenase 1 (IDH1) or 2 (IDH2) Genes in Low-Grade Gliomas

During the last two decades, an unprecedented number of cancer-associated mutations have been identified. It was early acknowledged that several mutated genes encode proteins that cluster in specific signaling pathways, and different sets of mutated genes in different tumors might affect the same functional pathways or processes [36, 37]. Such pathway-mapping approaches have recently led to large-scale tumor-sequencing results, which, along with a list of mutations and mutated genes, have generated lists of pathways enriched in mutations [38]. In the context of gliomas, the sequence-based genome-wide analysis of 22 human glioblastoma samples identified a novel mutation in a gene coding for isocitrate dehydrogenase 1 (IDH1) in a subgroup of glioblastoma samples. The mutation was most frequent in younger patients with secondary glioblastomas, and interestingly the presence of a mutated IDH1 allele was associated with increased overall survival [39]. Subsequent studies revealed that IDH1 mutations are very common in low-grade gliomas (70 % of all grade II and III glioma harbor the mutation), in oligodendrogliomas (70 %), and in secondary glioblastomas (88 %), but are fairly rare in primary glioblastomas (7 %) [40, 41]. Mutations in the homologous mitochondrial gene, IDH2, have also been identified in gliomas but at a lower frequency.

Based on gene expression profiles, The Cancer Genome Atlas Network recently described genomic abnormalities in glioblastoma where a specific

genomic classification divided the tumors into proneural, neural, classical, and mesenchymal subtypes. In particular, aberrations in gene expression of EGFR, NF1, and PDGFRα/IDH1 in the tumors define the classical, mesenchymal, and proneural subtypes, respectively [42]. Moreover, the gene signatures of normal brain cell types show a strong relationship between subtypes and different neural lineages where the progression of low-grade gliomas into glioblastomas is associated with the proneural phenotype. It is therefore feasible that glioblastomas in specific neural subtypes develop as the result of different causes or different cells of origin. In particular, the proneural phenotype associated with secondary glioblastomas frequently shows mutation of the *IDH1* or *IDH2* genes.

Mutant IDH1 has reduced activity toward its normal substrate isocitrate (less than 20 % of the activity of a wild-type homodimer) resulting in lower levels of the reaction product α-ketoglutarate (α-KG) [43]. It was later found that a gain-of-function activity of the mutant enzyme converts α-KG to 2-hydroxyglutarate (2HG), suggesting an oncogenic function of mutant IDH [44]. Increased 2HG levels were also detected in gliomas from patients harboring IDH1 mutations. Recent observations highlight a possible functional link between mutations in IDH1/IDH2 and a hypermethylation phenotype [45, 46] (reviewed in [47]).

The exact functional role of these mutations in glioma development is still unknown, but when it occurs, it appears to be an early mutational event, preceding 1p/19q loss in oligodendrogliomas and *p53* mutation in lower grade astrocytomas. Whether *IDH* mutations can initiate tumor development in a transgenic model or are necessary to sustain progression remains to be shown. If so, such models will represent excellent tools to study low-grade glioma development.

Xenograft Models for Low-Grade Gliomas

Another approach to investigate brain tumor development in vivo is by stereotactic implantation of tumor cells either as cell lines or as patient-derived tumor material into immunodeficient animals.

With regard to cancer cell lines which are (still) widely used, it has become clear that due to clonal selection and accumulation of genetic lesions in culture [48], they are poorly representative of the original patient tumor and animal models based on them largely diverge from patient gliomas at the histological, phenotypical, and genetic level. Although more laborious and time consuming, more and more laboratories have therefore turned to xenografts based on fresh patient-derived tumor material that are maintained by serial transplantations in rodents [49–51]. Such models can be expanded by direct intracranial implantation of tumor pieces or dissociated tumor cells or by the generation of three-dimensional tumor spheroids prior to implantation. Sometimes the serial expansion is performed via subcutaneous rather than orthotopic transplantation followed later on by a flank-to-intracranial transfer [49, 52–54]. Such models are invaluable to evaluate the effect of specific therapeutic interventions or novel drug candidates directly on patient tumor material in vivo [55]. These methods have been highly successful for the establishment of human glioblastomas in animals but less successful when low-grade gliomas are xenografted, although some oligoastrocytomas could also be expanded [49].

With the reemergence of the cancer stem-cell hypothesis, initially suggested more than 100 years ago [56, 57], new cell culture tools have been developed during the last 10 years for propagating cancer stemlike cells in serum-free growth media, supplemented with EGF, FGF-2, and insulin (neurobasal media) [58, 59]. The establishment of such spheres from gliomas has provided novel insight into tumor cells that show stem-cell characteristics and allowed the establishment of xenograft models after short-term cultures. The method has proven to be highly successful using a variety of tumors including pediatric gliomas and adult glioblastomas [60–64].

The culture of neurosphere-like cells from high-grade gliomas is relatively straightforward where proliferating spheres may occur within the first weeks of culture. However, neurospheres do not develop successfully from all human gliomas,

and some controversy exists as the success rate varies considerably from 10 to 100 % in different laboratories [58, 65, 66]. Growing neurospheres from low-grade gliomas are an even more frustrating endeavor. Nevertheless, using this technique, two oligodendroglioma cell lines were recently generated from 1p/19q co-deleted anaplastic oligodendrogliomas [63]. These cells proliferate slowly, but one of the lines (BT088) showed the ability to form oligodendrogliomas in immunocompromised mice. The other cell line (BT054) that harbored the IDH1 mutation showed no signs of tumor development in the animals. However, follow-up studies using the neurosphere culture method have shown that a neurosphere cell line could be established from an IDH1-mutant anaplastic oligoastrocytoma and that an orthotopic xenograft system allowed its expansion in mice [67]. These results clearly indicate that brain tumor stem cell lines with an endogenous mutation in IDH1 can develop a tumor-initiating capacity and provide a model to study the effect of 2-HG production in vivo.

At present there are very few orthotopic xenograft models for low-grade gliomas. Ideally, an animal xenograft model should reflect the mutations and the genomic abnormalities seen in humans and should display a similar disease course as in humans. A major problem in this context could be that if a human disease course is reflected, tumor initiation and a slow-growing tumor will not appear during the relatively short life span of mice and rats. This may in particular represent a problem for the establishment of low-grade gliomas harboring IDH1/2 mutations. Yet, with the advent of neurosphere cultures for the propagation of low-grade gliomas, also xenograft models appear to show promise.

In this context, both for GEM models and xenografts, a major challenge will be to identify and delineate initiating mutations, the key mutations that initiate the tumor development, from driver or bystander mutations that may play a central role in tumor progression from low- to high-grade glioma.

Conclusions

Compared to various GEM models, there has been considerably less success in the establishment of xenograft animal models for low-grade gliomas. In GEM models, there are strong indications that tumor development toward, in particular, oligodendrogliomas is either driven by alterations in PDGF or EGFR signaling, where other bystander mutations as for instance *Ink4a/Arf* or Ras contribute to the malignancy grade. Lessons also learned from GEM models are that in particular in oligodendroglioma development in animals, OPCs carrying tumor-initiating mutations, but not neural stem cells, represent most likely the cellular origin of these tumors [28, 33]. In particular tumor cells expressing the OPC marker NG2 have been shown to have high tumor-initiating potential. Support for these observations has also arrived through recent work indicating that differentiation-defective $NG2^+$ cells represent the predominant cell type in ENU-induced low-grade oligodendrogliomas in rats [68] as well as in highly malignant gliomas generated in adult rats [69].

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Part III

Clinical Aspects and Diagnostic Imaging

Clinical Presentation in Diffuse Low-Grade Gliomas

12

Juan Torres-Reveron, Joseph M. Piepmeier, and Kevin P. Becker

Abstract

Diffuse low-grade gliomas (DLGG) are primary brain tumors that typically arise in adults. Seizures are the most common presenting symptom. Management decisions are determined by tumor size, location, imaging characteristics, and histopathological findings. Most DLGG ultimately progress to high-grade lesions, and the events leading to this transition are the subject of active investigation. MRI is the standard imaging modality for assessing the progression of these tumors. New advances in imaging technologies now allow for multimodal evaluation of their phenotype based on metabolism and improved prediction regarding anaplastic transformation. A typical case is presented along with discussion of commonly encountered neurological syndromes. Conventional imaging technologies as well as new areas are discussed briefly.

Keywords

Seizures • Perfusion imaging • Disconnection syndrome • Spectroscopy • Positron emission

Introduction

Diffuse low-grade gliomas (DLGG) are relatively uncommon tumors that comprise approximately 15 % of primary brain tumors in adults. The most common DLGG included in the WHO II classification are diffuse astrocytomas, oligodendrogliomas, or oligoastrocytomas [1]. These lesions grow slowly and most commonly (80 %) reach clinical attention following the new onset of seizures [1–3]. DLGG infiltrate into the brain and exert mass effect on the surrounding tissue [4]. When seizures are not present, progressive tumor expansion will result in neurological

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dysfunction (motor/sensory deficits, psychological changes, memory problems) or obstruction of CSF flow (obstructive hydrocephalus). When DLGG become large, they can produce elevated intracranial pressure (ICP), neurological sequelae, and headache. Some studies suggest that the tumor cells themselves are neurotoxic (through cytokine and neurotransmitter release) and can elicit seizures by disruption of neuronal pathways or through local glutamate concentration/metabolism changes [5]. This may explain why seizure is among the most common presenting signs of a DLGG. Incidental discovery of DLGG is not uncommon and with the increased use of and access to MRI. Approximately 10 % are considered incidental, when discovered following head trauma or as a result of a work-up for other unrelated neurological symptoms [3].

DLGG are associated with neurofibromatosis type I [6]. For example, astrocytomas occur with increased frequency in these patients. A differential expression of neuronal-related genes and increased mTOR activation are found in DLGG with NF1 and may characterize phenotypic variations in NF1-associated low-grade astrocytomas.

DLGG are the beginning of a fatal disease for the majority of patients. While the biological behavior of these lesions can be unpredictable, the majority will transform into high-grade tumors. This is the most common cause of mortality. Clinical reviews emphasize the importance of young age, high performance status, and preserved neurological function as predictors of a more favorable prognosis [7]. In addition, tumor size also influences survival with larger tumors having a worse prognosis. Since the transition from a relatively slow-growing DLGG into a rapidly fatal high-grade glioma is the major cause of mortality in this population, efforts to prevent or delay this biological change are the focus of investigation. Malignant transformation results from the accumulation of additional genetic mutations; therefore, laboratory studies are directed toward identifying those genetic events that cause tumor transformation.

Because of the slow growth of some of these lesions, they may appear indolent on sequential imaging, resulting in modest changes in tumor dimensions over time. Some longitudinal studies indicate that the majority of these tumors show continued growth, and while observation and surveillance imaging may be recommended, ultimately most clinically silent lesions will evolve into symptomatic aggressive tumors. The rate of tumor growth also correlates with high-grade transformation. An increase in the rate of volume expansion identifies a DLGG that will become an anaplastic glioma in the near future [8, 9].

Only around 30 % of DLGG are amenable to radiographic complete removal [3]. Consequently, for the majority of patients, only a subtotal resection or biopsy may be possible. Optimal management of these patients requires a comprehensive team approach to treatment including collaboration with neurosurgery, neurology, neuropathology, radiation oncology, and diagnostic radiology.

Typical Case Report

A 32-year-old man with no past medical history was found by his wife on the floor of the family room having a generalized tonic-clonic seizure. In questioning the patient later, he stated that he had been having “weird tastes” intermittently for more than 6 months prior to the first generalized seizure onset. He described the episodes as unpleasant and similar to a “chemical taste, like acid.” These episodes occurred infrequently and last approximately 5–15 s. He stated that “I never thought much about them because the first time it happened I was walking by a bathroom and just assumed it was cleaning solutions.” His wife reported that 2 months prior to the generalized seizure, he was reprimanded at work for poor job performance which was attributed to increasing forgetfulness. The patient admitted that he had been having worsening difficulties with concentration and short-term memory. He also reported that “sometimes I get confused and my thoughts start racing.” This has led to him getting lost while driving to and from work several times.

After the generalized seizure, he was transported to a local hospital where CT and MRI of the brain demonstrated a left temporal lobe

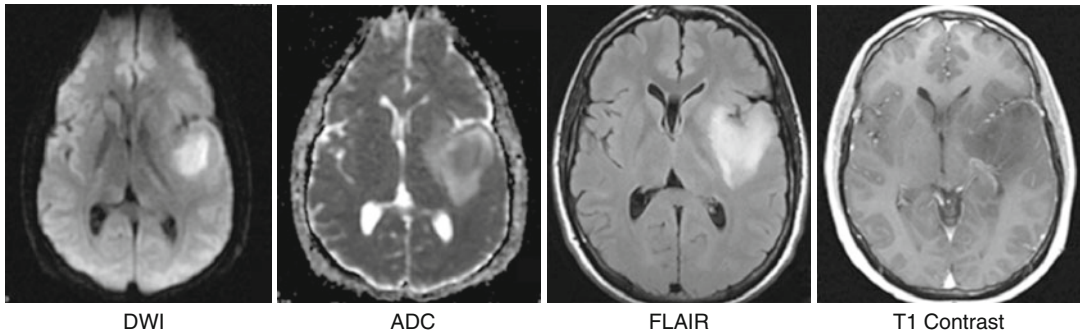

Fig. 12.1 Presurgical magnetic resonance imaging. Magnetic resonance imaging obtained at presentation demonstrates a non-enhancing mass in the left temporal lobe. The mass effect and DWI-ADC abnormality suggest that the abnormality is cellular. *DWI* diffusion-weighted imaging, *ADC* apparent diffusion coefficient map, *FLAIR* fluid-attenuated inversion recovery

non-enhancing mass (Fig. 12.1). He subsequently underwent an awake left temporal craniotomy with subtotal tumor resection. The final histopathology was consistent with an oligodendroglioma, WHO grade II.

This case illustrates a typical history for DLGG. Slow tumor progression can result in subtle findings, and often the patient is either unaware of the impairment or attributes the problem to other causes. Consequently, a detailed history and neurological examination are important to help identify and localize the areas of dysfunction and potential neurological risks of surgery.

Clinical Presentation According to Tumor Location

Frontal Lobe

The most common location for a DLGG is the frontal lobes. Within the prefrontal cortex, DLGG can lead to a "frontal lobe syndrome" due to dysfunction of executive function. Patients may have a noticeable change in personality, motivation, organizational skills, problem solving, insight, and judgment. Alternatively, patients may experience a disinhibition syndrome consisting of inappropriate, often reckless, and promiscuous behavior. Broca's or nonfluent aphasias result from tumors in the posterior inferior frontal region of the dominant hemisphere adjacent to the perisylvian fissure. These patients will have a paucity of spontaneous speech and are unable to repeat, name, read, or write. They may have preserved auditory comprehension and know what he/she wants to say but cannot say it. This is similar to a transcortical motor aphasia except that those patients are able to repeat spoken word.

Temporal Lobe

Tumors involving the superior temporal gyrus of the dominant hemisphere can produce a Wernicke's or fluent aphasia wherein the patient has impairment in their ability to understand spoken speech. These patients are also unable to repeat, name, read, or write, and the speech they produce has frequent paraphasic errors and neologisms. The patient is often unaware of their deficit.

Uncinate fits are seizures produced by tumors of the medial temporal lobe in the vicinity of the uncus. These tumors can produce complex partial seizures consisting of olfactory, gustatory, visual, or auditory hallucinations. There are often automatisms consisting of lip smacking and repetitive behaviors (e.g., flipping pages in a book, repeatedly picking up a phone, and dialing). A distinct feature of temporal lobe seizures is the sense of déjà vu (a misperception that a situation or place being experience for the first time is actually familiar or has been experienced before) or jamais vu (a misperception that a familiar experience is new). In addition, feelings of depersonalization,

anger, rage, anxiety, euphoria, religiosity, and fear can often be associated with complex partial seizures.

Parietal Lobe

DLGG of the parietal lobe can lead to a constellation of sensory deficits. Disorders of tactile gnosis such as astereognosis or the inability to identify an object by touch and feel alone and agraphesthesia or the inability to detect a number written on the patients hand are all cortical parietal functions.

Hemispatial neglect or neglect syndrome is a particularly disabling deficit wherein the patient is unaware or inattentive to one side of their body in the absence of visual loss. This often occurs with tumors of the nondominant parietal lobe and typically involves the contralateral side; however, it can also arise from dominant lesions.

Disconnection Syndromes

Disconnection syndromes occur when the major fiber tracts that connect the primary cortical areas are disrupted. This results in a defect in the flow of inter- and intrahemispheric communication. For example, a dominant lobe parieto-occipital DLGG that infiltrate the splenium of the corpus callosum can lead to a syndrome of alexia without agraphia or pure word blindness. In this case, written word is presented to the unaffected visual cortex, but this information is unable to be transmitted to Wernicke's area due to the involvement of the corpus callosum by tumor. The patient retains the ability to write but is unable to read. Tumors of the dominant temporal-parietal region or angular gyrus can produce syndrome of alexia with agraphia (inability to read or write) or Gerstmann syndrome (dysphasia, dyscalculia, finger agnosia, left-right confusion).

The alien hand syndrome occurs when there is a disconnection between the normal coordinated action of each hand. Tumors involving the genu of the corpus callosum can lead to purposeful but autonomous movements of the nondominant hand. The alien hand is often in conflict ("intermanual conflict") with the hand under control. As such, the alien hand may act to disrupt the normal functioning of the other hand by reaching out to grab a spoon while feeding or pen while writing. An alien hand syndrome can also result from a lesion to the medial aspect of the frontal lobe. In this syndrome, the alien hand is not in conflict with the other hand, but its actions are independent and often involve reflexive movements such as grasping or reaching out for objects.

Radiographic Presentation

The typical presentation of DLGG is a homogeneously isointense to hypointense mass on T1-weighted images and hyperintense on T2-weigthed images. Although many centers have improved image resolution with 3 T MRI, the gold standard continues to be 1.5 T-obtained images with gadolinium contrast administration [10]. However, DLGG are unlike other intracerebral lesions in that there is no "classic" radiographic appearance. Contrast enhancement is uncommon and is seen mostly (25–50 %) in oligodendrogliomas. Calcifications can be present in 20 % of diffuse astrocytomas and 40 % of oligodendrogliomas [11]. Vasogenic edema and mass effect are not usually present as these tumors are slow growing [2].

The presence of contrast in diffuse DLGG (Fig. 12.2) has also been the subject of intense scrutiny as it may predict anaplastic transformation of the tumor and patient survival. Contrast enhancement in a computed tomography study of patients with low-grade gliomas was associated with a shorter survival when compared to patients with a hypodense tumor [12]. A recent retrospective study by Chaichana and colleagues [13] indicated that contrast enhancement was associated with decreased survival and increased recurrence in a population of 189 patients that had no significant difference in treatment paradigms. Furthermore, for those patients that had contrast enhancement and underwent gross total resection, their overall survival and progression-free survival were improved. The volume of

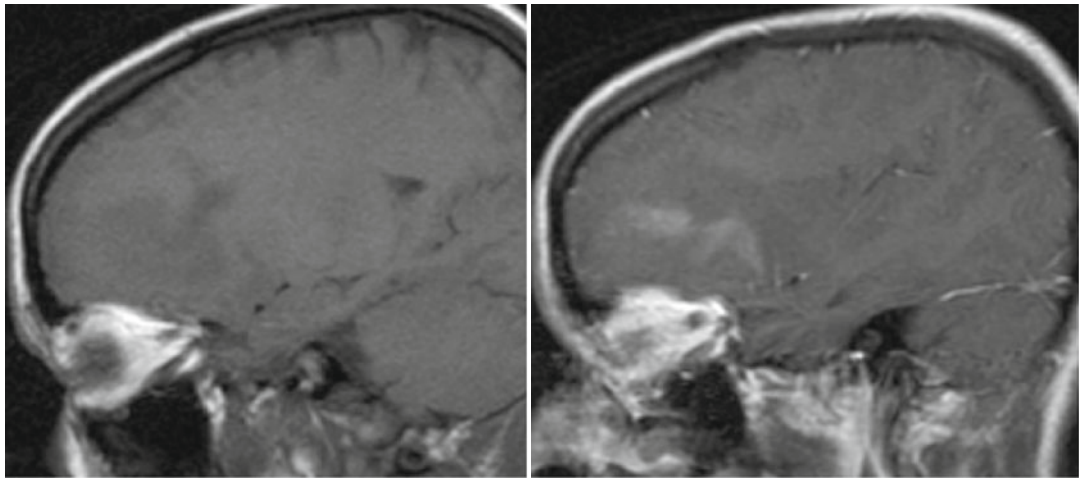

Fig. 12.2 Enhancement in low-grade glioma. T1-weighted non-contrast (*left*) and gadolinium contrast (*right*) MRI showing a diffuse lesion in the frontal lobe

enhancement also carries prognostic information. Patients with more than 4 mL of enhancement have a worse prognosis with only 28 % progression-free at 5 years [14].

Radiographic evaluation of treated lesions is even more difficult as radiation, steroids, and surgical resection can alter the usual appearance of images on MRI. Therefore, a significant amount of effort is directed at evaluating techniques that can improve the accuracy of diagnosis before and after treatment and prevent unnecessary surgical intervention. These techniques will be discussed at length in further chapters. An excellent extensive review of radiographic modalities in DLGG has been previously written by Price [15]. We will provide a brief introduction to some of the most used contemporary imaging modalities and the data supporting their use.

Magnetic Resonance Spectroscopy (MRS)

MRS allows the noninvasive study of metabolism from either single, small regions to multiple regions of a tumor. Figure 12.3 shows an example of a multiregion study in which areas of the tumor in the left temporal lobe show readings most indicative of a higher-grade tumor. All gliomas show a spectrum with increased choline and *N*-acetyl aspartate. Lipid and lactate peaks are rarely elevated in DLGG but increased in higher-grade gliomas [16, 17]. The addition of MRS increases the accuracy of DLGG diagnosis. A study by Möller-Hartmann et al. [17] showed that the spectroscopy data led to a 15 % higher number of correct diagnoses, 6 % fewer incorrect diagnoses, and 16 % fewer equivocal diagnoses.

Spectroscopy has also been shown to be accurate in differentiating high- and low-grade tumors achieving 94 % (long echo time) and 96 % (short echo time) of the area under the curve in receiver operating characteristic analyses [18, 19]. Spectroscopic measurements have been shown to be superior to tumor growth rates and relative cerebral blood volume (rCBV) measurements for predicting anaplastic transformation [20]. However, other studies have suggested that rCBV measurements were better in correctly grading tumors and the addition of metabolic information did not improve diagnostic yield [21].

Perfusion MRI

DLGG lack microvascular proliferation as usually seen in higher-grade gliomas. Perfusion imaging uses rCBV measurements to aid in the

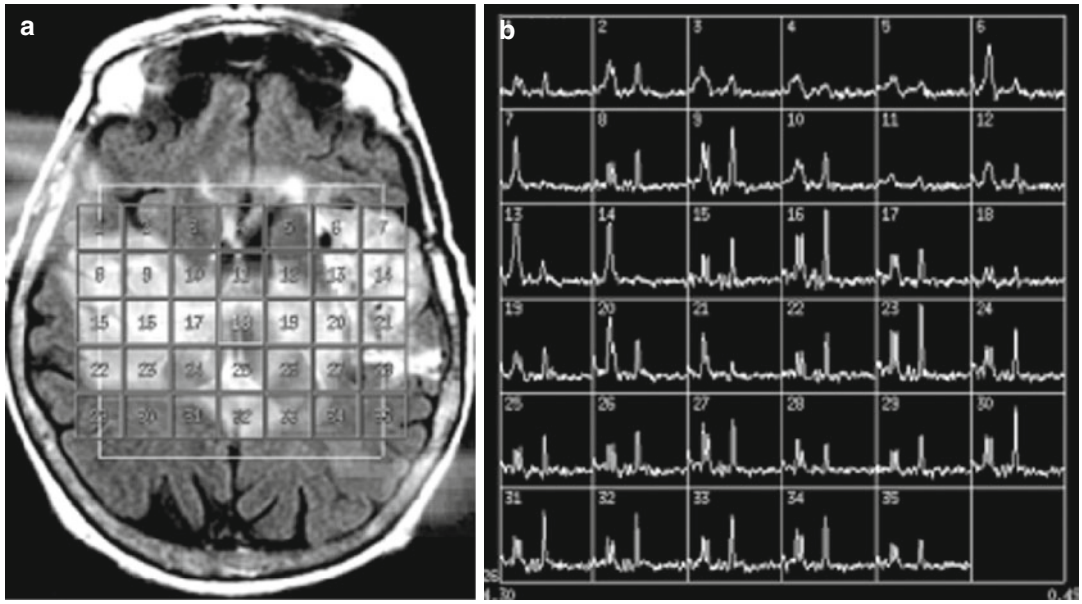

Fig. 12.3 Magnetic resonance spectroscopy in a mixed lesion. Brain axial fluid-attenuated inversion recovery (FLAIR) image showing increased signal throughout the temporal lobes and thalamus. A multivoxel spectroscopy grid was placed over the image. (**b**) Spectroscopic data from the voxels in (**a**). Left temporal readings (voxels 7, 14, and 21) are indicative of progression to high-grade glioma

grading of tumors. rCBV of tumors has been correlated with histological vascularity [22], measures of microvascular density [23], and angiographic vascularity [24].

As with MRS, perfusion imaging has been used to differentiate between high- and low-grade tumors and to predict progression to higher grades. However, there is significant overlap of rCBV values in different tumor grades [25–27]. Furthermore, oligodendrogliomas tend to have higher rCBV values than astrocytic tumors due to their dense network of capillaries in a "chicken wire" pattern [28].

Besides conventional perfusion imaging, there are other methods based on exogenous (with contrast medium) or endogenous tracers (arterial spins) (Fig. 12.4). In arterial spin imaging, arterial blood water is magnetically labeled by applying a pulsed or continuous radiofrequency inversion pulse. The technique provides measurements that are comparable to positron emission tomography using radioactive water [29].

Positron Emission Tomography (PET)

PET imaging has improved our knowledge of cellular metabolism by the development of multiple radiotracers. Currently, these radiotracers allow the evaluation of glycolytic metabolism, protein synthesis, and nucleotide turnover. Given the higher metabolic requirements of tumors in relation to adjacent tissue, it allows for the evaluation of tumor progression and transformation into more malignant phenotypes.

Low-grade gliomas are hypometabolic in PET imaging. High 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG) uptake in a previously known low-grade tumor establishes the diagnosis of anaplastic transformation [30, 31]. However, given the baseline high metabolic activity of brain tissue, high-grade tumors may have uptake that is only similar or slightly above baseline [32]. Delayed FDG imaging at 3–8 h can improve the delineation between tumor and normal grey matter [33] and help overcome the decreased sensitivity due to

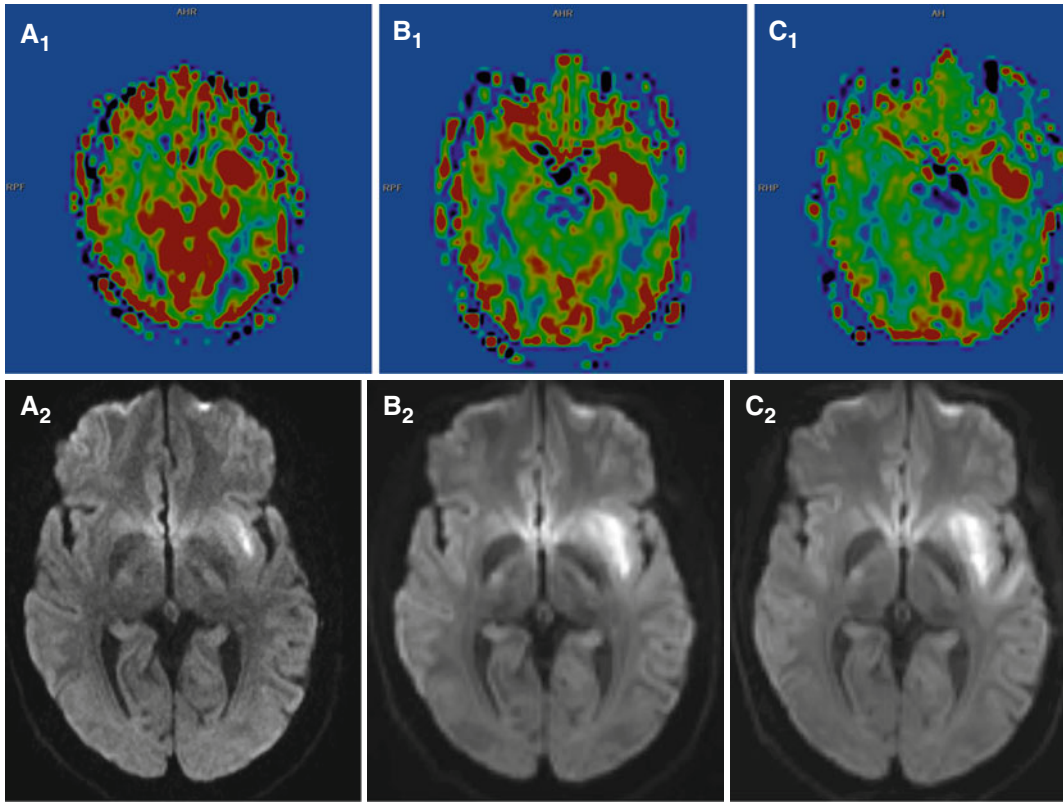

Fig. 12.4 Perfusion- and diffusion-weighted image (DWI) of low-grade glioma. Arterial spin labeling perfusion (A_1–C_1) and DWI (A_2–C_2) follow-up imaging in a patient with a grade 2 oligodendroglioma. The tumor showed initial progression of the lesion in the left temporal/insular regions over a year as demonstrated by an increase in blood flow (A_1 to B_1) and restricted diffusion (A_1 to B_2). Six months later, restricted diffusion was stable (C_2) and was associated with a decrease in perfusion (C_1) to the region

high brain activity. Coregistration or coanalysis of FDG-PET with MRI improves the diagnostic value of PET imaging as it allows examination of the area of interest and determination of lesions in the area [34].

Two areas have attracted attention to overcome the limitations of FDG-PET: imaging of protein synthesis and nucleotide production. Amino acid imaging is based on the increased amino acid transport usually seen during malignant transformation [35, 36]. ^{11}C and ^{18}F isotopes have both been used to track amino acid transport. L-[methyl-^{11}C]-methionine is one of the commonly used amino acids. Methionine PET can detect high-grade gliomas with 97 % sensitivity and DLGG with 61 % sensitivity [37]. Furthermore, it serves as a prognostic marker since WHO grade II and III gliomas have a shorter survival time if they exhibit increased methionine uptake [38].

3′-deoxy-3′-fluorothymidine (FLT) is a thymidine analog that accumulates in proliferating cells [39]. FLT uptake is increased in high-grade gliomas [40, 41]. DLGG have little to no FLT uptake. Its uptake seems to correlate with areas of contrast enhancement and, in general, correlates with the MIB-1 labeling index in higher-grade gliomas [42]. Therefore, this thymidine analog may have increased used in the future to guide biopsies and predict anaplastic transformation.

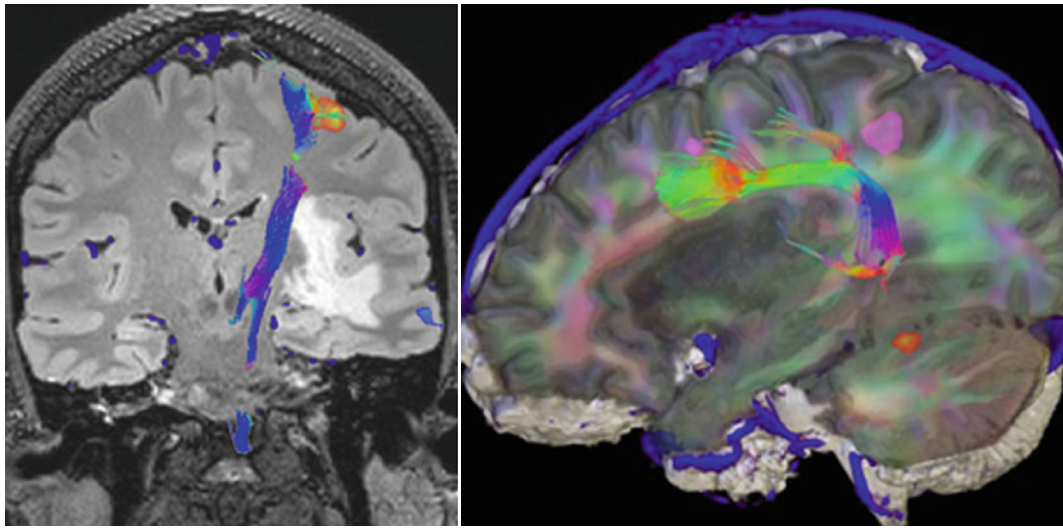

Fig. 12.5 Preoperative functional MRI for resection of left insular low-grade glioma. Functional imaging of the left brain hemisphere demonstrating the course of the corticospinal tract (*left*: *blue*/*violet* fibers) and arcuate fasciculus (*right*: *blue*/*violet*/*green* fibers). In this example, the fiber tracts are medial and superior to the location of the tumor. The MRI provides boundaries for resection to prevent functional impairment

Functional Magnetic Resonance Imaging (fMRI)

Given the infiltrating nature of DLGG, preoperative knowledge of the functional areas involved is crucial for safe resection. Understanding whether functional tracts are involved or deflected can alter the operative approach and dictate the limits of resection (Fig. 12.5). However, fMRI remains too imprecise for complex functions such as language with sensitivity of 81 % and specificity of 53 % [2]. Currently, direct intraoperative stimulation for language mapping remains the gold standard.

The Response Assessment in Neuro-Oncology group (RANO) proposed imaging guidelines in 2011 regarding DLGG to standardize follow-up of tumor growth and clinical trials. A basic MRI was proposed for all centers with and without gadolinium contrast. MRS, perfusion imaging, and diffusion-weighted imaging are restricted to specialized centers. PET imaging, including amino acid imaging, was also recommended. Progression on MRI is defined in two separate domains: development of or an increase in enhancement suggestive of malignant transformation and linear progression of a still non-enhancing lesion. Although PET, MRS, perfusion imaging, and diffusion-weighted imaging can provide insights into anaplastic transformation, their relation to clinical benefit still needs further study and validation [43]. As these technologies become validated, we will likely see an increase in their use and hopefully improved management of DLGG.

Conclusions

DLGG will continue to be interesting but difficult lesions to treat given their slow growth, indolent presentation, and lack of knowledge of the molecular mechanisms that govern their transformation into high-grade gliomas. Advances in imaging and molecular genetics should help bridge this gap and allow the clinician to tailor specific therapies depending on the tumor genotype and predict phenotypic behavior. These advances should advance surgical resections as well as medical treatment, limiting morbidity, improving mortality, and patient's quality of life.

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Epilepsy in Diffuse Low-Grade Gliomas

13

Anja Smits

Abstract

Epileptic seizures occur in the majority of patients with diffuse low-grade glioma (DLGG) as initial symptoms leading to tumor diagnosis. The temporal relationship between seizures and diagnosis in DLGG is, however, not always easily defined; minor focal seizures may go unnoticed for many years, seizures may occur first after radiological diagnosis in previously asymptomatic patients, and, in rare cases, seizures can precede the diagnosis of brain tumor. The risk of developing tumor-related seizures is inversely correlated with the growth rate of the tumor, being highest for patients with slowly growing tumors. The specific location of the tumor and its proximity to the cortex are other important factors affecting seizure risk in patients with DLGG.

About half of all patients with DLGG continue to have seizures before operation, in spite of adequate antiepileptic drug treatment. Poor seizure control has a strong negative impact on the health-related quality of life in this patient group. Improved seizure control in patients with medically refractory epilepsy can be achieved by tumor resection including removal of seizure foci, either as single treatment or in combination with radiotherapy and chemotherapy. When studying the relationship between seizure control and clinical outcome of DLGG, the natural evolution of these tumors has to be considered. The presence of seizures as initial and exclusive symptom at diagnosis is a strong favorable prognostic factor. There is some evidence that preoperative seizure freedom in DLGG is an additional prognostic factor for longer survival. Recurrent seizures after an initial seizure-free period or late-onset seizures are frequently associated with malignant progression, but the exact temporal relationship between seizures and tumor progression in DLGG remains unclear.

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Epilepsy • Seizure risk • Seizure control • Tumor control • Survival • Natural course

Introduction

Epileptic seizures are among the most common symptoms in patients with gliomas. In patients with diffuse low-grade glioma (DLGG), seizures occur most frequently as initial symptoms but are common symptoms of chronic disease as well. The burden of chronic epilepsy imposes a substantial morbidity to this patient group. It is well known that poor seizure control has sustained negative effects on daily functioning and quality of life [30]. Medically refractory seizures often lead to multiple drug strategies, at the price of serious side effects on cognitive and psychological function [29]. Impairment of cognitive function induced by polypharmacy will further deteriorate the possibility to maintain former social and professional activities of daily life [56]. Elderly patients or patients with prior cognitive impairment are particularly prone to disabling sedative and cognitive side effects of antiepileptic drugs (AEDs). Achieving optimal seizure control is therefore an essential part of the clinical management of patients with DLGG. Furthermore, seizures are not always benign. There is a significantly increased mortality in the epilepsy population compared to the general population, especially in patients with a high number of tonic-clonic seizures [50].

From a clinical point of view, it is important to realize that, also in patients with glioma, new-onset seizures can be acute symptomatic seizures and do not necessarily imply epilepsy. Acute symptomatic seizures are seizures provoked by acute medical or neurological insults, for example, during the immediate postoperative period, and do not require long-term AED treatment unless followed by subsequent unprovoked seizures [4].

When discussing seizure activity in patients with DLGG, one needs to discriminate between seizure risk and seizure control. Although obviously related, these two concepts are not identical; high seizure risk in individual patients does not necessarily imply poor seizure control and vice versa.

Seizure risk in patients with DLGG, that is, the risk of developing seizures over time, is affected by a number of tumor-related factors of which the tumor growth rate and the specific location of the tumor are the most important. Seizures occur more frequently in DLGGs than in fast-growing tumors like high-grade gliomas. Within the group of DLGG, patients with oligodendrogliomas carry the highest seizure risk [11]. The specific lobe affected and the proximity of the tumor to the adjacent cortex are additional factors determining seizure risk [47]. Tumors in the vicinity of the primary motor cortex and with limbic and perilimbic cortical location are associated with high seizure risk, whereas occipital tumors are less likely to manifest with seizures.

Seizure control is defined as the individual response of a patient to anticonvulsive treatment, which as a rule consists of pharmacological treatment with AEDs. Improved seizure control in DLGG can also be obtained by surgery, radiotherapy, and chemotherapy. In clinical trials or observational studies, seizure control is usually quantified as the number of seizures during a specific time period prior to the time point of investigation, taking into account the number, type, and serum levels of AEDs during this period. The Engel classification system, originally described in 1987, is the most widely used scale for classifying seizure outcome [18]. This system, based on a four-grade scale (free of disabling seizures, rare disabling seizures, worthwhile improvement, no worthwhile improvement), is easy to use but contains some subjective

assessments. The International League Against Epilepsy (ILAE) proposed a new classification scheme for seizure outcome after surgery, with the aim to provide a more objective measure of the number of seizures [61].

Seizure control, like seizure risk, varies between gliomas of different histological type, but while seizure risk is lower in high-grade gliomas than in DLGGs, seizures may be more difficult to control in high-grade gliomas.

Epidemiology of Seizures

Epileptic seizures as initial symptoms leading to brain tumor diagnosis occur in 70–90 % of all patients with DLGG [52]. However, seizures may go unnoticed for long time periods, and it may not be until seizures become secondary generalized tonic-clonic seizures that patients seek medical ward. The difficulties in recognizing initial nonconvulsive seizures as first symptoms are illustrated by King and colleagues, who investigated 300 consecutive adults and children presenting with new-onset unexplained seizures [28]. The authors showed that about one third of all patients presenting with initial tonic-clonic seizures had previously experienced focal epileptic symptoms, such as temporal lobe auras. This study confirms that patients may be unaware of the importance of focal epileptic symptoms and illustrates the consequential difficulties in defining the exact time point of first clinical symptoms in these patients.

In older patients (≥ 60 year), constituting less than 10 % of all patients with newly diagnosed DLGG, seizures occur less frequently as initial symptoms, that is, at the time point of radiological diagnosis. This discrepancy has been explained by a generally more aggressive tumor growth in the elderly population compared to younger patients, favoring neurological and cognitive deficits over seizures. In older patients, cognitive impairment, language disorders, and sensorimotor deficits were more often present at diagnosis than in younger patients [26]. In addition, tumors were generally larger, and prognosis was worse in the elderly compared to the younger population. Shorter survival could not be explained by an overrepresentation of histological tumor subtypes with unfavorable prognosis, since astrocytic and oligodendroglial tumor subtypes were equally divided irrespective of age [26].

Sometimes DLGGs are discovered incidentally in asymptomatic patients, when brain imaging is performed after trauma or for other reasons. Little has been known about the biology of these tumors, but recent data suggest that they represent an earlier stage in the natural history of DLGGs. Incidental DLGGs were smaller compared to their symptomatic counterparts, were more often located in the frontal lobe or in noneloquent brain regions, and were associated with a female predominance [41]. Importantly, the estimated radiological growth rate of incidental DLGGs was within similar range as the growth rate of symptomatic DLGGs [41]. When not treated, incidental DLGGs became symptomatic at a median interval of 4 years, and patients experienced seizures. Apart from arguing for a more aggressive therapeutic approach in incidental DLGGs than previously thought, this study illustrates that epileptogenesis in DLGGs is a time-dependent process that may stretch over several years.

Intriguingly, the temporal relationship between seizures and brain tumors is more complex. There is evidence to suggest that seizures may sometimes precede the detection of brain tumors. A recent retrospective cohort study of two large English registries for patients admitted for new-onset epilepsy showed that tumors as the underlying cause for epilepsy may not be apparent for several years after seizure onset [27]. A significantly increased risk for developing brain tumor was found during the complete duration of follow-up in both datasets, especially in young patients with new-onset seizures [27]. These findings are of clinical importance and suggest that there is a need for continued surveillance in adults presenting with new-onset seizures.

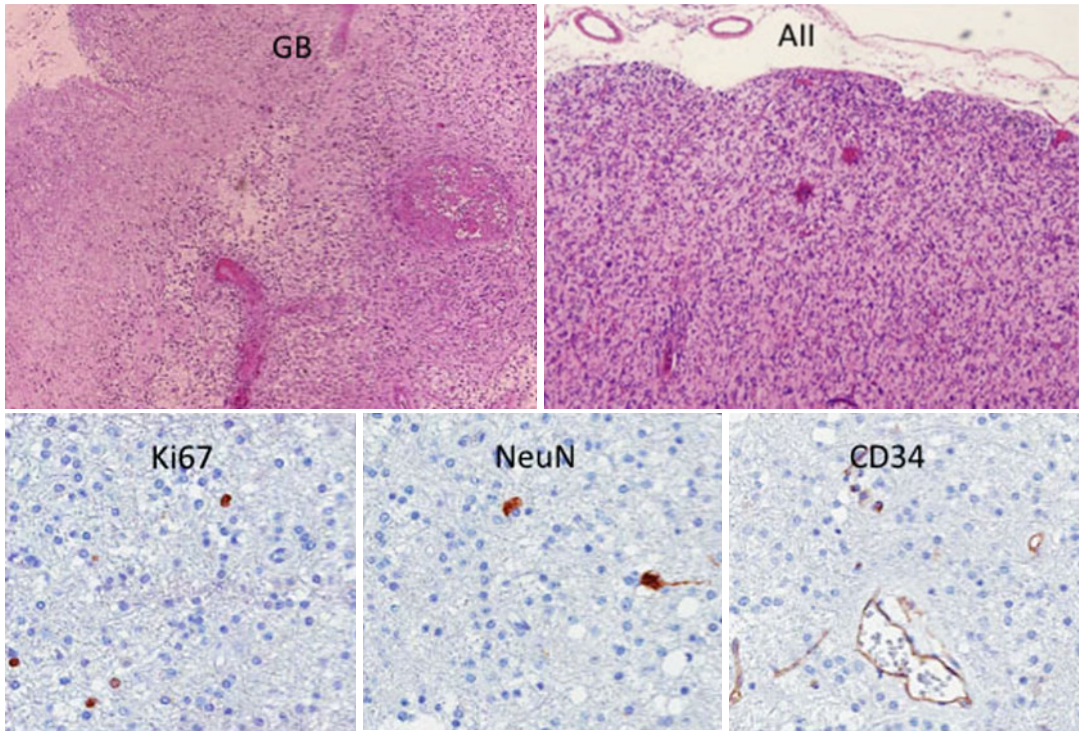

Fig. 13.1 *Upper panels*: Microphotographs showing hematoxylin-eosin stainings of two glioma samples, illustrating the different histological characteristics between high-grade and low-grade gliomas. The glioblastoma (*GB*) shows areas with necrosis and endothelial cell proliferation, while the diffuse astrocytoma WHO grade II (*AII*) shows a highly differentiated tumor invading cortical structures (Photographs kindly provided by Dr. E Aronica, University of Amsterdam, The Netherlands). *Lower panels*: Microphotographs showing immunohistochemical staining of an oligodendroglioma WHO grade II. The antibodies used for immunostaining of specific cell types are, respectively: *Ki67*, identifying a low number of proliferating tumor cells; *NeuN*, neuronal nuclear antigen, identifying some entrapped neurons in the tumor; *CD34*, identifying cells of hematopoietic origin in the tumor

Epileptogenesis

A growing intracranial tumor can structurally and functionally alter the surrounding brain by edema, vascular proliferation, inflammatory reactions, and metabolic changes and thereby promote seizure activity. Seizure development in brain tumors depends on multiple factors such as tumor histology, the peritumoral region, and the integrity of the blood–brain barrier. Thus, the mechanisms underlying seizure development are considered to be basically different for low-grade gliomas and high-grade gliomas. High-grade gliomas are highly invasive tumors that show destructive growth in the surrounding brain parenchyma. Histologically, high-grade gliomas are characterized by a high degree of cell polymorphism and proliferation, necrotic areas, and disturbed microvascularization (Fig. 13.1). Seizure activity in these tumors is most likely related to the immediate physical effects of tissue damage due to mass effect, peritumoral ischemia, and micro bleedings.

In contrast, a partial deafferentation of cortical brain regions causing denervation hypersensitivity is considered to contribute to the epileptogenic milieu in slowly growing low-grade gliomas. DLGGs are infiltrative tumors in nature, but, unlike malignant gliomas, they are characterized by only mild hypercellularity and rare mitotic figures (Fig. 13.1). The typical growth pattern of a DLGG is not that of a solid tumor mass but of

isolated tumor cells infiltrating the brain parenchyma. In oligodendrogliomas, tumor cells have a characteristic propensity to satellite around preexisting structures such as neurons and blood vessels [44] (Fig. 13.1).

Seizure-Related Structural Changes

As discussed, seizure development in DLGG has been related to chronic deafferentation and disconnection of functionally isolated cortical areas and not to a direct effect of tissue damage [3, 47]. Seizures consist of synchronous electrical activity reverberating through complex neuronal networks and must ultimately always include abnormalities on the circuit level. Thus, in parallel with abnormalities at the cellular level, distortions of neuronal networks will contribute to epileptogenesis by upsetting the physiological balance between excitation and inhibition in the brain. The characteristically slow growth of DLGGs is believed to modify existing neuronal circuitries through axonal sprouting, synaptogenesis, and neurogenesis in the peritumoral region [8]. The excessive and random synchronizations of neurons may persist during critical time periods and, after a certain time interval, cause epilepsy.

The almost 100 % seizure risk in patients with glioneuronal tumors such as DNET and gangliogliomas, together with the high frequency of medically refractory seizures in these patients, suggests that these tumor types contain intrinsic epileptogenic properties [3]. Gangliogliomas typically occur in childhood or young adults. These tumors are composed of dysplastic neuronal components and proliferating glial cells and are predominantly located in the temporal lobe. Evidence from microdissection studies suggests that aberrant gene expression in the neuronal tumor component contributes to the development of dysplastic neurons and the aberrant neuronal network, thus predisposing for generating intrinsic epileptic activity [19]. Some proposed mechanisms by which gangliogliomas can actively participate in the generation of seizures are by aberrant release of neuromodulators into the adjacent brain and through electrical activity of their intrinsic neuronal networks [2].

Sometimes patients with chronic tumor-related seizures develop secondary epileptic foci, that is, distant, actively discharging epileptogenic foci that do not correspond to the tumor or to the peritumoral area [57]. This phenomenon is more frequently found in patients with a long seizure history and with temporal lobe tumors. Consistent with the existence of separate epileptic foci, patients with secondary epileptic foci tend to have two different types of focal seizures [13]. Unless complete resection of the epileptogenic regions is performed in addition to tumor resection, these patients will continue to have postoperative seizures.

Peritumoral Cortex

Unlike gangliogliomas, the tumor tissue of DLGGs is electrically inert. Seizure development in DLGG is considered to take place at the interplay between tumor and its surrounding tissue. Consequently, the epileptic focus, that is, the location corresponding with the start of the seizure, is not always contiguous with the tumor. Alterations in ultrastructure of peritumoral brain regions, as well as changes in metabolic activity and in pH, have been demonstrated that may induce an increased excitability [48].

Recent support for the importance of the peritumoral region in tumor-associated epileptogenesis comes from a study by Conti and coworkers, who reported decreased GABAergic inhibition underlying the electric hyperexcitability of this area [14]. GABA (γ-aminobutyric acid) is the main inhibitory neurotransmitter in the brain, and alterations of GABAergic transmission have been postulated as mechanisms underlying epileptogenesis and seizure generation. A major facet of GABAergic transmission is the intimate link between $GABA_A$ receptor function and ion homeostasis [20]. The $GABA_A$ receptor consists of a ligand-gated pentameric chloride channel that is permeable to chloride anions. Two major pumps acting in opposite fashion, NKCC1 and KCC2, determine the chloride gradient.

An abnormal expression of NKCC1 and KCC2 was found in membranes of human peritumoral epileptic tissue transplanted into oocytes, resulting in altered GABA-evoked currents and a subsequent reduction of GABAergic inhibition [14]. Interestingly, immunohistochemistry localized the increase of NKCC1 proteins mainly to neuronal cells and not to glial cells in the peritumoral cortex.

Clinical Seizure Manifestations

Tumor-related seizures in patients with DLGG include all types of focal seizures with or without secondary generalization. While all tumor-related seizures are focal by nature, the duration of the focal seizure component can be extremely short, and new-onset seizures are therefore sometimes erroneously classified as primary generalized tonic-clonic seizures. Secondary generalization is more common in early seizures that occur at diagnosis and before AED treatment is started. Once AED therapy has been initiated, seizures tend to become focal only [22]. Seizure semiology reflects specific tumor localization and the somatotopic organization of cortical brain functions.

Seizure Types

Focal seizures due to brain tumors are divided in simple focal seizures and complex focal seizures. In simple focal seizures, there is no impairment of consciousness. In contrast, patients with complex focal seizures experience varying levels of clouded consciousness. Simple focal seizures are most common in patients with high-grade gliomas, whereas complex focal seizures occur more frequently in DLGG. Apart from tumor histology, the specific location of the tumor is a strong predictor for whether seizures will manifest as simple or complex focal seizures. Tumors that are located in the temporal lobe or insula region are strongly associated with complex focal seizures. In principal, complex focal seizures consist of an initial aura phase that precedes clouding of consciousness and behavioral automatisms. Epileptic symptoms may, however, largely vary from one patient to the other, and the separate seizure components cannot always be distinguished. Patients can also experience isolated auras without other signs of epileptic manifestations. During the aura phase, consciousness is still unclouded, and they will therefore be able to recall and report these initial experiences.

Focal epileptic syndromes can further be classified according to the site of the lesion or epileptic origin. There is often a good correlation between tumor location and seizure type, although distinguishing specific tumor location on clinical grounds solely can be difficult. In particular, temporal lobe seizures can be difficult to differentiate from frontal lobe seizures [36]. The widespread use of modern neuroimaging has devalued clinical capacities of precisely localizing seizure activity to specific brain regions. Nevertheless, it is useful for the neuro-oncologist to be able to recognize the main focal epileptic syndromes.

Seizures caused by temporal lobe lesions are characterized by behavioral automatisms and experiential symptoms. Patients with focal complex seizures due to temporal lobe tumors typically report characteristic auras consisting of memory disturbance such as déjà vu phenomena, visceral sensations such as epigastric rising, or gustatory and olfactory auras. Auditory hallucinations and language or memory disturbances may all be part of complex focal temporal lobe seizures.

Asymmetric tonic seizures, characterized by tonic arm extension and elevation followed by forced head deviation to the side of the extended arm (often but not always contralateral to the tumor), are strongly associated with frontal tumor locations, especially with seizure origin in the supplementary motor area [25]. Focal Jacksonian motor or somatosensory seizures, which are simple focal seizures, occur with tumor location in perirolandic areas. Other typical manifestations of frontal lobe epilepsy are speech arrest or motor agitation, the latter characteristically occurring at night or upon awakening in early morning and in patients with deeply seated frontal lobe tumors. This seizure type is sometimes associated with bizarre behavioral automatisms such as bicycle

pedaling motions and pelvic thrusting and may be mistaken for non-epileptic psychogenic seizures. Such diagnostic problems are for obvious reasons not as common in patients with tumor-related seizures as in patients with cryptogenic epilepsy.

In patients with occipital lobe tumors, focal seizures typically consist of positive visual symptoms such as hallucinations (flickering or blinking lights) and other visual disturbances (micropsy and macropsy), but may also include visual field defects or blurred vision. The differential diagnosis of migraine in patients with known tumors in the occipital lobe is sometimes difficult to exclude.

Status Epilepticus

Status epilepticus is a neurological emergency situation that has been well documented in the general epilepsy population [38]. Status epilepticus is defined as a single epileptic seizure lasting longer than 30 min or, alternatively, a series of seizures during which function is not regained between ictal events for longer than 30 min [23]. Brain tumors are not a common cause of status epilepticus, and in most studies, they represent only 2–5 % of all status epilepticus cases in the general epilepsy population. The associated mortality is typically estimated in the range of 0–20 % [38]. One report has been published on status epilepticus in patients with intracranial tumors exclusively [10]. The authors showed that status epilepticus in these patients occurred most often at the time point of diagnosis and at tumor progression [10]. They also reported a relatively high incidence of status epilepticus in patients with low-grade gliomas and with an oligodendroglial tumor component [10]. A recent study on the efficacy of different AEDs in the treatment of status epilepticus in tumor-related seizures suggests that the response to newer drugs like pregabalin and levetiracetam is as good as that to conventional drug therapy with phosphenytoin [54]. Patients with tumor-related seizures may also have nonconvulsive status, characterized by a sudden onset of symptoms like confusion, disorientation, and aphasia. This condition is not as life threatening as convulsive status epilepticus, but can be difficult to recognize [9].

Anticonvulsive Treatment

There are limited data available on the optimal treatment of brain tumor-related seizures by anticonvulsive drugs. Large controlled trials comparing the efficacy of old and new AEDs have all been performed in patient populations with non-tumor-related focal seizures [46]. A few studies have been published on AED treatment in neuro-oncological patients, all containing a mixture of different glioma types [31, 60]. Thus, more studies on the efficiency and the possible side effects of AEDs in patients with DLGG are urgently needed.

Antiepileptic Drug Treatment

In choosing the optimal AED therapy for patients with DLGGs, the efficacy of the drug, its pharmacodynamic profile, and its potential side effects have to be taken into account [53]. AEDs that induce components of the hepatic cytochrome P450 enzyme system, which are mainly the older drugs like carbamazepine, phenytoin, primidone, and phenobarbital, are not recommended for first-line therapy of tumor-related seizures. Enzymatic induction by these AEDs results in enhanced metabolism of many commonly administered chemotherapeutic agents as well as corticosteroids. Furthermore, highly protein-bound AEDs and chemotherapeutics may interact with each other by replacement, altering the free and bound levels of both compounds. Carbamazepine, in spite of being an effective and well-tolerated drug for focal epilepsy, is therefore not the first drug of choice for patients with DLGG who receive concomitant chemotherapy. The other drawback of carbamazepine for these patients is the potential risk of bone marrow depression.

The prodrug phosphenytoin is a useful and potent drug when administered intravenously in

emergency situations of status epilepticus. Phenytoin itself is not used on a regular basis anymore because of its serious side effects on cognitive function and its complex pharmacokinetic profile with high risk of toxicity.

Valproate is effective for treating focal as well as primary generalized seizures. In contrast to the enzyme-inducing effect of carbamazepine and phenytoin, valproate is an enzyme-inhibiting drug and may thus raise plasma levels of a second drug by reducing its metabolism. This interaction has to be taken into consideration, especially when combining valproate with chemotherapeutic agents that depend on hepatic metabolism. Its inherent antitumor properties, through inhibition of histone deacetylase activity, are of potential interest when treating patients with tumor-related seizures. Anticonvulsive treatment with valproate has been associated with prolonged survival of patients with newly diagnosed glioblastomas who received concomitant temozolomide chemotherapy. It remains unsolved, however, whether the prolonged survival of these patients is due to increased bioavailability of temozolomide or to the intrinsic antitumor activity of valproate [24, 59]. Apart from rare but potentially fatal liver damage, valproate has relatively mild side effects. Valproate may prolong bleeding time and is therefore avoided as a preoperative AED by some neurosurgeons.

Several of the second-generation AEDs that have been introduced for focal epilepsies in the last 10–15 years have established a role in the treatment of tumor-associated seizures, as monotherapy or as add-on drug [46]. In general, these second-generation AEDs do not have the strong enzyme-influencing effects of some of the older AEDs, reducing the risk of drug interactions, and are well tolerated. It seems reasonable therefore to consider these AEDs as first-line anticonvulsive therapy for patients with DLGG. Alternatively, they can be used as add-on treatment to obtain seizure reduction in patients who do not become seizure-free with one of the older AEDs [46, 57]. Examples of new AEDs are levetiracetam, gabapentin, lamotrigine, pregabalin, and zonisamide. Levetiracetam as monotherapy has been shown as effective in the treatment of newly diagnosed focal epilepsy as carbamazepine and is associated with fewer side effects [57]. The psychotropic side effects of levetiracetam that have occasionally been reported may hamper its utility in some patients, especially in the elderly. Lamotrigine is an effective drug for treating tumor-related seizures, but has the disadvantage of a protracted dosage schedule taking several weeks to achieve therapeutic doses [31]. In general, routine prophylactic use of AEDs for patients with DLGG who have not experienced any seizures is not recommended [21, 53].

Medically Refractory Seizures

As discussed earlier, DLGGs are frequently situated in eloquent brain areas that are strongly associated with medically refractory seizures [17]. Thus, while approximately half of all patients with DLGGs presenting with seizures at diagnosis become seizure-free on AED therapy, the other half continues to have seizures in spite of optimal drug strategy [11, 52]. A substantial proportion of these patients will have medically refractory seizures, defined as seizures so frequent or sincere that they limit daily life, despite the use of at least two appropriately described AEDs in adequate serum concentrations [16]. The threshold for seizure control failure is based on studies suggesting that the likelihood of complete seizure freedom diminishes significantly beyond two AED failures [32].

The relatively poor anticonvulsive effect of AEDs in DLGGs suggests that the pharmacological mechanisms of the AEDs do not interfere with critical epileptogenic pathways of these lesions. There are several possible mechanisms that may underlie pharmacoresistance of tumor-related seizures. One active defense mechanism is proposed to occur by multidrug resistance proteins that actively transport AEDs out of the brain, resulting in insufficient concentrations of the active drug metabolite in the tumor or in the blood surrounding the tumor [57]. This concept is supported by expression of multidrug resistance proteins in tumor types that are strongly associated with refractory seizures [1]. Other pharmacological mechanisms of drug failure

include restricted penetration of lipophilic substances into the brain and loss of receptor sensitivity for AEDs in tumor cells [57].

In addition, molecular-genetic factors may be involved in the pathogenesis of tumor-related seizures. A recent report has described an association between postoperative seizure control and the molecular tumor profile of DLGGs [62]. Apart from molecular aberrations in the tumor itself, host-related factors such as the genetic susceptibility of individual patients to seizures are likely to affect both seizure risk and seizure control, although the evidence for this is still poor [5].

Surgery

When AED treatment fails, alternative treatment options should be considered to improve seizure control in DLGG. The effectiveness of surgery on seizure control for patients with DLGG has been demonstrated in a number of studies [11, 52]. For the majority of patients with occasional seizures, radical tumor resection, that is, complete removal of the tumor as defined by imaging criteria, will be a valuable strategy to improve seizure control [31]. In these cases, the epileptic zone typically consists of the tumor and a variable amount of surrounding tissue [31]. In patients with medically refractory seizures, however, the epileptic zone may include significant extratumoral cortical areas, and in these cases, "epilepsy surgery," that is, resection of epileptic foci, needs to be performed for optimal effect on seizure control. The use of intraoperative electrocorticography monitoring allows for the identification of irritative cortical areas with interictal electrographic spikes. This method has therefore improved the possibilities of achieving good seizure control in patients with pharmacoresistant seizures [8]. An alternative approach is performed by the placement of subdural or intraparenchymal grid electrodes followed by extraoperative mapping. This method is mainly used in specialized centers for patients admitted for epilepsy surgery and is performed to record interictal activity and identify seizure start (Fig. 13.2). The method is valuable for selected patients with DLGG who present with complex seizure semiology, including secondary epileptic foci [33]. For patients with paroxysmal symptoms of possible non-neurological causes (e.g., syncope, cardiac arrhythmias, psychogenic seizures), preoperative video-electroencephalographic (EEG) monitoring is used (Fig. 13.3).

Radiotherapy and Chemotherapy

Radiotherapy, either as single nonsurgical treatment or in combination with surgery, has shown beneficial effects in terms of seizure reduction in patients with DLGG. In the EORTC (European Organization for Research and Treatment of Cancer) 22845 trial, designed to compare survival in adult DLGG treated by early versus delayed radiotherapy, seizure outcome by radiotherapy was included as secondary endpoint in patients still progression-free 2 years from randomization [58]. Twenty-five percent of all irradiated patients had seizures compared to 41 % of those not irradiated. Since there was no difference in seizure control at baseline between the two groups, it was concluded that seizures were better controlled in the group receiving radiotherapy. Consistent findings have been found in small retrospective reviews, showing a significant seizure reduction in irradiated patients.

A number of observational studies have reported a favorable effect of chemotherapy for tumor-related seizures [46]. Chemotherapy with temozolomide was effective in improving seizure control at least in a subpopulation of DLGG [7, 49]. Interestingly, significantly better seizure control by temozolomide was found in non-enhancing tumors compared to enhancing tumors in a study on progressive DLGGs [39]. Non-alkylating chemotherapeutic agents may also have positive effects on seizure control, either as single nonsurgical treatment or in combination with radiotherapy, shown in a study of paralimbic DLGGs [55]. An alternative way to improve seizure control in inoperable tumors may be achieved by neoadjuvant chemotherapy followed by surgical resection. This combination induced significant tumor shrinkage in a small series of DLGGs, allowing subtotal or total resection of initially inoperable tumors [6]. Postoperatively, the majority of these patients experienced improved seizure control [6].

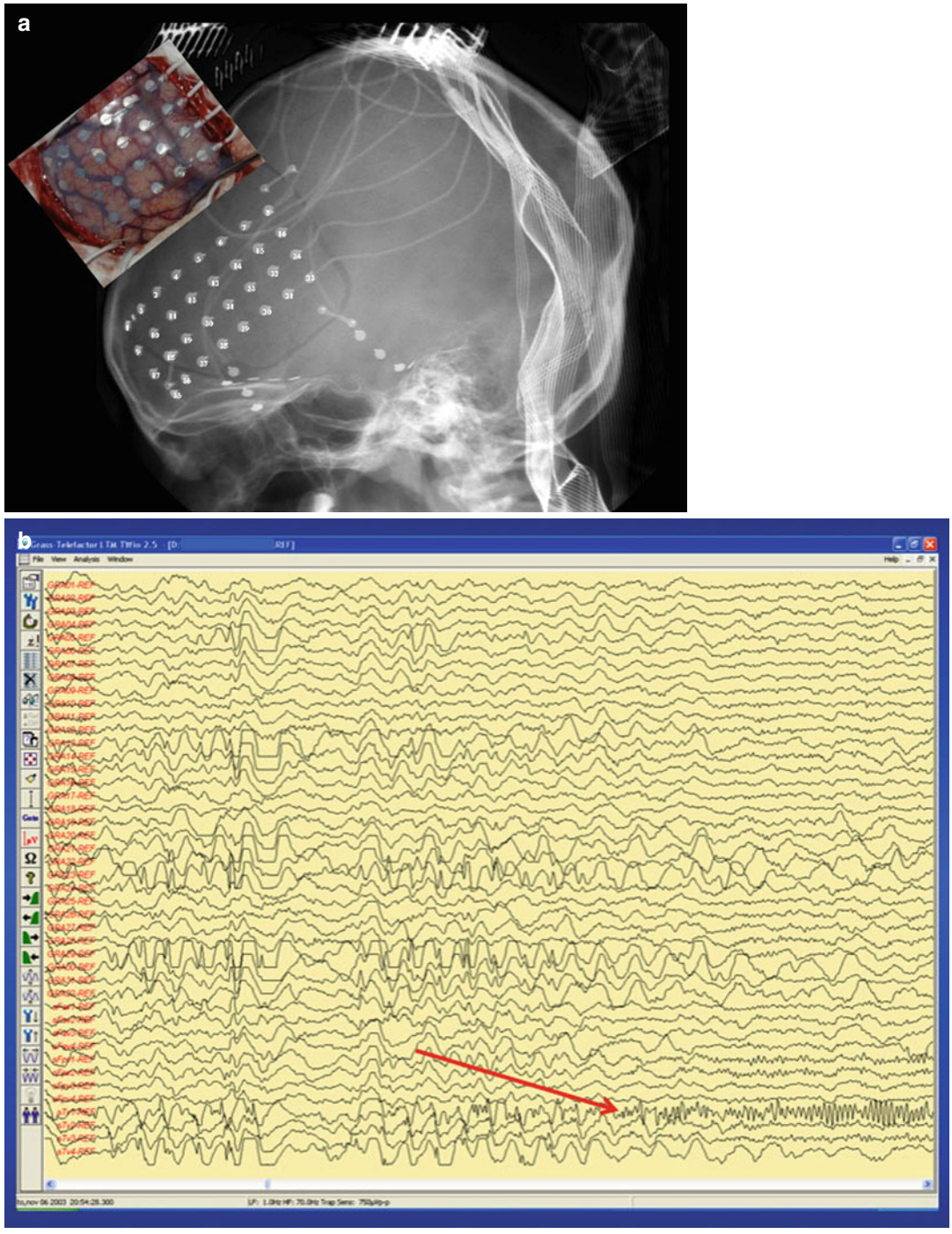

Fig. 13.2 (**a**) Photograph showing a patient with a frontally located grid electrode (4×8 electrodes) combined with strip electrodes subfrontally and subtemporally, allowing for extraoperative mapping of extratumoral seizure foci. (**b**) Photograph showing the EEG (same patient as in (**a**)), showing interictal activity and seizure onset (*red arrow*) (Photographs kindly provided by Dr. R Flink, Uppsala University Hospital, Sweden)

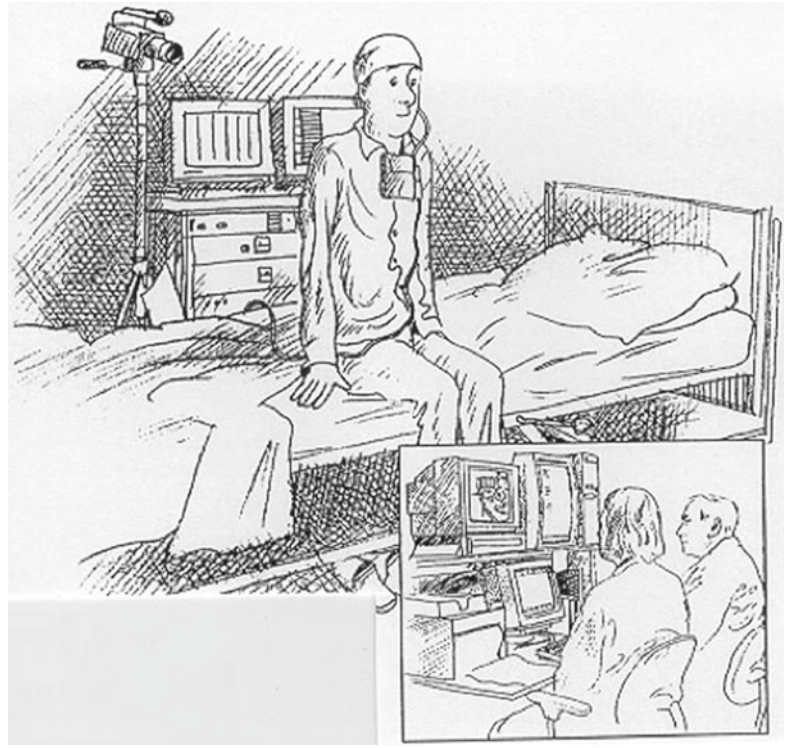

Fig. 13.3 An illustration of the principles for continuous preoperative video-electroencephalographic (video-EEG) monitoring that can be used to differentiate seizure activity from other paroxysmal symptoms such as cardiac arrhythmia or non-epileptic psychogenic seizures. Ongoing EEG activity is recorded in parallel with monitoring clinical symptoms (Kindly provided by Dr. R Flink, Uppsala University Hospital, Sweden)

Seizures in Relation to Tumor Evolution

The frequent cortical involvement of DLGGs explains why seizures are the most common presenting symptoms in these patients. Focal neurological deficits are uncommon at early disease, which is probably related to the typical slow growth of DLGGs allowing functional compensation through brain plasticity mechanisms. Seizures may also bring patients to diagnosis earlier, compared to symptoms of cognitive impairment or neurological deficits with gradual onset and slow progression over time. As such, the presence of seizures at diagnosis is a strong favorable prognostic factor for survival in DLGG [52]. Patients with central tumor location rarely present with seizures as initial symptoms, and these patients are known to have poor outcome [43]. It should be realized though that seizures as initial symptoms are associated with longer survival only in the absence of other symptoms. Once cognitive or neurological dysfunction occurs, prognosis is worse [43]. In agreement, elderly patients with DLGGs presented more often with cognitive impairment in the absence of seizures and showed a more aggressive natural course of disease [26].

In addition to seizures as presenting symptoms, the preoperative seizure control that is obtained by AEDs in individual patients has been proposed as a prognostic factor in DLGG. Thus, longer overall survival was found in patients who presented with single seizures and became seizure freedom before operation [15, 37]. It seems therefore that patients who present with single seizures and become seizure-free on AEDs during early disease constitute a favorable prognostic subgroup of DLGGs. It is possible that tumors

located in non-eloquent areas are overrepresented among these long survivors, thus explaining longer survival by a more extensive tumor resection in this subgroup [51]. Alternatively, other factors related to the natural course of DLGG may explain how preoperative seizure control and survival in DLGG are connected. Patients with persisting seizures before operation were found to have larger mean tumor diameter compared to patients who became seizure-free, suggesting that these tumors represented DLGGs at further advanced steps in their evolution over time [15].

When studying the relationship between seizures and survival in patients with DLGG, the natural evolution of these tumors has to be taken into account. Tumors are diagnosed at various steps along the continuum of their natural course, which has been proposed to occur as a three-step process: an initial silent period, followed by a symptomatic period, and a final period of malignant progression [40]. Longitudinal studies using sequential MRI over time have demonstrated a linear expansion of the mean tumor volume diameter during the entire time course before malignant progression occurs [12, 34]. While surgical resection does not seem to affect individual tumor growth rates, oncological treatment can induce a temporary slowing down of tumor expansion that is usually accompanied by an improvement of seizure control [35, 45].

Although it is well known that recurrent seizures after an initial seizure-free period or late-onset seizures frequently occur during the step of malignant progression, the implications of recurrent seizures for clinical outcome have not been systematically studied. Also, the exact temporal relationship between seizures and tumor progression in DLGG remains unclear. Breakthrough seizures can sometimes precede radiological evidence of tumor recurrence, and repeated imaging over time should be performed in case of sudden deterioration of seizure control. In a series of 234 consecutive patients with primary brain tumors treated with ambulant chemotherapy, half of all patients with late-onset seizures were diagnosed with tumor recurrence or progressive tumor growth [22].

Finally, seizure control in individual patients is probably not exclusively dictated by tumor-related and treatment-related factors. External factors may affect tumor growth rate and thereby indirectly seizure control. The nature of such external factors is still largely unknown, but the hormonal changes that occur during pregnancy have recently been put forward as a possible candidate. An accelerated tumor growth was shown during pregnancy in females with DLGG that was associated with a 40 % increase in seizure frequency [42].

Conclusions

Tumor-associated epilepsy is an important clinical problem for patients with DLGG. Seizures occur in 70–90 % of all patients at diagnosis, and about half of these patients will have breakthrough seizures before tumor resection, despite optimal anticonvulsive drug treatment. A proportion of patients suffer from medically refractory seizures, that is, seizures so frequent or sincere that they limit daily life. The potential benefits of anticonvulsive drug therapy on seizure control in individual patients must be balanced against drug-related side effects on cognition and quality of life. In addition, possible interactions of anticonvulsive drugs with concomitant medication need to be anticipated, especially for patients who receive chemotherapeutics and cortisone.

When drug treatment fails, other treatment modalities including surgery, radiotherapy, and chemotherapy must be considered for optimizing seizure control. To achieve optimal effect of surgery in patients with medically refractory seizures, early surgical resection with resection of the epileptic foci is recommended. Modern tools for mapping extratumoral epileptic foci, such as preoperative video-EEG monitoring and extraoperative electrocorticography monitoring, have largely improved the possibilities of obtaining favorable postoperative seizure control in DLGG. A close collaboration with a qualified multidisciplinary team involved in epilepsy surgery is recommended for these patients.

Future clinical trials are warranted to assess optimal anticonvulsive drug treatment strategies in patients with DLGG and to define the exact role of surgery, radiotherapy, and chemotherapy for seizure control in patients with medically refractory seizures. The etiology of seizure development in patients with brain tumors is still poorly understood. A more comprehensive understanding of the mechanisms involved in tumor-related seizures may explain the variety in seizure control between patients with similar tumor location and histological tumor type and provide a ground for effective and individually based treatment.

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Quality of Life in Low-Grade Gliomas

14

Martin J.B. Taphoorn

Abstract

Outcome measures for brain tumor patients have traditionally been confined to survival and radiological response to treatment. The recognition that duration of survival may not be the only goal of treatment has resulted in health-related quality of life (HRQOL) to become an important (secondary) outcome. It is generally acknowledged that the benefits of longer survival due to tumor treatment should be weighed against side effects of treatment which may have a negative impact on the patient's functioning in physical, psychological, and social aspects.

For low-grade glioma (LGG) patients, outcome measures should include neurocognitive functioning and severity of epilepsy apart from patient-reported measures such as HRQOL. Since these patients have a relatively extended survival compared to high-grade glioma, and may therefore run the risk of long-term complications due to the tumor and treatment, these outcomes are now considered to be of major importance in clinical trials.

From clinical studies we have learned that cognitive deficit and severity of epilepsy in LGG patients have a negative impact on HRQOL.

Keywords

Low-grade glioma • Health-related quality of life • Cognition • Epilepsy • Outcome measures

Introduction

Patients with diffuse low-grade gliomas (LGG) have brain cancer and cannot be cured so far from their disease. Although the disease is expected to have a protracted course over years, and thus LGG patients may enjoy a long period with relatively few symptoms, the tumor will ultimately develop into a high-grade glioma with progressive

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neurological deficit. The far majority of patients with diffuse LGG will therefore die in the end from the disease. This grim outlook for relatively young patients with these tumors, typically affected in their third and fourth decade of life, will be a heavy burden for both the patients and their families.

Like other brain tumor patients, LGG patients not only suffer from cancer but also from a progressive brain disease with specific neurological symptomatology, distinct from other patients with a malignancy. Slowly progressive neurological and cognitive symptoms and signs prevail in LGG patients and influence the patient's functioning and quality of life. Epileptic seizures, often the presenting symptom in these patients, and cognitive disturbances are most prominent in LGG patients, whereas motor deficit and signs of increased intracranial pressure are initially rare. Also, fatigue and mood disturbances are frequently met in LGG patients. Not only the tumor but also its treatment may contribute to a disabling morbidity [1].

Compared to traditional outcome measures such as progression-free survival, overall survival, and radiological response, evaluation of health-related quality of life (HRQOL) and cognitive functioning in brain tumor patients may be time-consuming and burdensome for both the patient and the doctor. Besides, given the relatively low incidence of brain tumors and the ultimately fatal outcome of the disease, the interest in HRQOL and cognitive functions emerged relatively late in these patients [2]. Also, the notion that the disease itself may affect the patient's ability to judge his or her own functioning may hinder the use of patient-reported outcomes (PRO) such as HRQOL.

Cognitive functioning and HRQOL, however, are not only useful as outcome measures in clinical trials for brain tumor patients. They may also serve as an early indicator of disease progression and have prognostic significance, thereby helping in clinical decision-making for the individual patient in daily practice.

With a median survival for diffuse LGG patients between 5 and 15 years, and a high percentage of long-term survivors, preservation and improvement of the patient's neurological and cognitive functioning, as well as the HRQOL, are major goals of treatment [3]. Despite the relatively long-term survival of LGG patients, they are underrepresented in the present literature on HRQOL of cancer survivors [4].

Table 14.1 Outcome measures in low-grade glioma

Traditional outcome measures
Overall survival
Progression-free survival
Radiological response
Patient-oriented outcome measures
Objective measures
Impairment scale (e.g., Edinburgh Functional Impairment Test)
Disability scale (e.g., Karnofsky performance scale)
Handicap scale (e.g., Rankin handicap scale)
Cognitive testing
Seizure severity scale
Subjective measures (patient-reported outcomes)
Symptom scales (e.g., M.D. Anderson Symptom Inventory)
Cognitive functioning scale
HRQOL questionnaires

Outcome Measures in Low-Grade Glioma (Table 14.1)

To measure how a brain tumor patient is functioning is more complex than measuring (progression-free) survival or tumor response to treatment on imaging, which are considered to be *hard* and traditional outcome measures. These traditional outcome measures in trials on LGG are problematic, since the moment of progressive disease in these patients, compared to high-grade glioma, may not be easy to determine. Besides, radiological changes in LGG may be subtle, reflected by small, incremental, and asymptomatic increases in size on T2-weighted and FLAIR MRIs. Not until malignant transformation with emergence of new contrast enhancement is observed, determination of radiological progression in LGG is a challenge. Vice versa, the Macdonald criteria for response evaluation in LGG are not optimal at all, since they are focused on changes in contrast

enhancement, which is atypical for LGG in contrast to high-grade tumors [3].

The World Health Organization's International Classification of Functioning, Disability and Health (ICF 2002) definition on how to describe a patient's functioning discriminates on a basic level the patient's *impairment* (i.e., a hemiparesis or a dysphasia), which can be measured by neurological examination or impairment tests like the Edinburgh Functional Impairment Test (developed for brain tumor patients) [5]. On a higher level, the patient's *activity limitations* reflect the consequences of the impairment in daily life (i.e., the patient with the hemiparesis is unable to climb the stairs, the dysphatic patient is unable to make telephone calls), to be measured with disability or performance scales, such as the Barthel Index for stroke patients and the Karnofsky performance scale [6], which was developed for cancer patients. Finally, how the disability affects the patient's well-being and his social interactions may be reflected in the patient's *participation restrictions* (i.e., the patient who cannot climb stairs will be forced to move to another home/level, the dysphatic patient becomes socially isolated), which can be measured with handicap scales, like the modified Rankin handicap scale in stroke patients.

Although these validated scales provide essential information, not only in clinical trials but as a clinical parameter in the course of disease as well, the existing disability and handicap scales are insufficient for brain tumor patients [7]. Cognitive (dys)functioning, which has a major impact in brain tumor patients but is less visible than a hemiparesis, is hardly measured by these widely used scales, of which the Karnofsky performance scale is the most prominent example.

Measures of impairment, activity limitations, and participation restrictions are generally of less importance in LGG than in high-grade glioma, since the neurological deficit in LGG is limited. Measures of cognitive functions and seizure activity are, however, crucial in LGG patients [1].

For *cognitive functioning*, a highly important outcome measure in LGG, objective cognitive testing is the gold standard. Patient self-report, reflecting the patient's subjective cognitive complaints, is not sufficient, since it is often associated with symptoms of fatigue and depressed mood [8]. Objective, validated, and performance-based testing is mandatory. Formerly, the MMSE has been used in clinical brain tumor trials as a measure of cognitive function. Although MMSE was developed for screening patients for dementia, it has been shown to be a prognostic factor for overall survival in glioma [9]. MMSE, however, is insufficient to measure cognitive functions in brain tumor patients. A comprehensive cognitive test battery (20–30 min is feasible for repeated testing in the LGG patient), covering the different cognitive domains of the brain, that can be administered by health-care providers without neuropsychological expertise is crucial [3].

Since epileptic seizures are prominent in LGG patients, and often the only presenting symptom, measures for *seizure activity* in these patients are equally important. Moreover, epileptic seizures and the use of antiepileptic medication may both have a negative impact on cognitive functioning and HRQOL [10, 11]. Successful treatment of LGG may also result in reduction of epileptic seizure activity and use of medication. Grading systems for seizure activity are mainly derived from studies on outcome after epilepsy surgery but are so far not commonly used in LGG trials [12].

Health-Related Quality of Life: Development of Questionnaires (Table 14.2)

Apart from the objective instruments to measure a brain tumor patient's neurological and cognitive functioning, as well as his disability and handicap, subjective assessment instruments emerged. Examples are symptom scales for fatigue or pain, when one cannot do without patient-reported measures, and HRQOL instruments which are inherently subjective in nature. HRQOL is defined as a multidimensional concept consisting of at least physical, psychological, and social phenomena as perceived by the patient [13]. This is a far more complex outcome measure than performance or handicap scales, demanding a multidimensional instrument. Such

Table 14.2 Health-related quality of life questionnaires used in brain tumor patients

HRQOL instrument	Function	Subscales and number of items for the computation of each scale
EORTC QLQ-C30 (quality of life core questionnaire)	Generic instrument for cancer patients	Physical functioning (5 items) Social functioning (2 items) Emotional functioning (4 items) Cognitive functioning (2 items) Role functioning (2 items) Fatigue (3 items) Nausea (2 items) Pain (2 items) Individual items (1 item each): dyspnea, insomnia, appetite loss, constipation, diarrhea, financial difficulties Global HRQOL (2 items)
EORTC-BN20 (brain cancer module)	Brain tumor-specific instrument	Future uncertainty (4 items) Visual disorder (3 items) Motor dysfunction (3 items) Communication disorder (3 items) Individual items (1 item each): headaches, seizures, drowsiness, hair loss, itchy skin, weakness of legs, bladder control
FACT-G (Functional Assessment of Cancer Therapy – general)	Generic instrument for cancer patients	Physical well-being (PWB) (7 items) Social well-being (SWB) (7 items) Emotional well-being (EWB) (6 items) Functional well-being (FWB) (7 items) FACT-G (27 items) (FACT-G = PWB + EWB + SWB + FWB)
FACT-BrS (Functional Assessment of Cancer Therapy – brain subscale)	Brain tumor-specific instrument	FACT-Br subscale (BrS) computed on 23 items: *I am able to concentrate* *I have had seizures* (*convulsions*) *I can remember new things* *I get frustrated that I cannot do things I used to* *I am afraid of having a seizure* (*convulsion*) *I have trouble with my eyesight* *I feel independent* *I have trouble hearing* *I am able to find the right word*(*s*) *to say what I mean* *I have difficulty expressing my thoughts* *I am bothered by the change in my personality* *I am able to make decisions and take responsibility* *I am bothered by the drop in my contribution to the family* *I am able to put my thoughts together* *I need help in caring for myself* (*bathing*, *dressing*, *eating*, etc.) *I am able to put my thoughts into action* *I am able to read like I used to* *I am able to write like I used to* *I am able to drive a vehicle* (*my car*, *truck*, etc.) *I have trouble feeling sensations in my arms*, *hands*, *or legs* *I have weakness in my arms or legs* *I have trouble with coordination* *I get headaches*
FACT-Br (= FACT-G + FACT-BrS)	Combined instrument	FACT-Br (50 items) (27 FACT-G, 23 FACT-BrS)

Table 14.2 (continued)

HRQOL instrument	Function	Subscales and number of items for the computation of each scale
FACT/NCCN Brain Symptom Index	Brain tumor-specific symptoms	Brain Symptom Index (FBrSI) computed on 15 items: *I get headaches* *I have had seizures* (*convulsions*) *I have weakness in my arms or legs* *I need help in caring for myself* (*bathing*, *dressing*, *eating*, *etc.*) *I have lack of energy* *I have difficulty expressing my thoughts* *I have trouble with coordination* *I get frustrated that I cannot do things I used to* *I have nausea* *I am able to find the right word*(*s*) *to say what I mean* *I am losing hope in the fight against my illness* *Because of my physical condition*, *I have trouble meeting the needs of my family* *I worry that my condition will get worse* *I am afraid of having a seizure* (*convulsion*) *I am able to enjoy life*

patient perspectives are increasingly used as outcomes in care and clinical trials because the importance of assessing the effect of both the disease and treatment on the patient is more and more recognized. HRQOL should be assessed by the patient using a self-reported questionnaire, reflecting the patient's perceived level of functioning. As an alternative, a (semi-)structured interview could be undertaken with the patient.

Both generic and disease-specific questionnaires have been developed and validated to assess HRQOL. Early HRQOL instruments, such as Spitzer Quality of Life Index, are scales to be completed by a doctor or nurse and resemble disability or handicap scales. As the science of HRQOL research developed and broadened, multidimensional, patient-derived HRQOL questionnaires emerged, often via rigorous development and psychometric testing. Examples are the COOP charts, the Medical Outcomes Scale (MOS) Short-Form Health Survey, Nottingham Health Profile, Rotterdam Symptom Checklist, and the Sickness Impact Profile [14]. Cancer-specific tools soon emerged including the Functional Living Index-Cancer (FLIC), Functional Assessment of Cancer Therapy (FACT), and European Organization for Treatment and Research of Cancer (EORTC) tools [13–15]. Currently, common HRQOL tools in use were developed by the EORTC Quality of Life Group: the EORTC QLQ-C30, a 30-item core measure heavily symptom based and designed to be supplemented by disease treatment or symptom-specific modules (questionnaires) [13]. The EORTC QLQ-BN20 module (consisting of 20 items) was developed and validated with brain cancer patients [16, 17] and focused on key symptoms. Both instruments are available in numerous languages and have robust psychometric properties resulting from rigorous international testing and development over time [18].

The EORTC QLQ-C30 measure comprises five functioning scales (*physical*, *role*, *emotional*, *cognitive*, and *social*), three symptom scales (*fatigue*, *nausea/vomiting*, and *pain*), six single-item scales (*dyspnea*, *sleep disturbance*, *appetite loss*, *constipation*, *diarrhea*, and *financial impact*), and the *overall health/global* QOL scale.

The EORTC QLQ-BN20, designed for use with patients undergoing chemotherapy or radiotherapy, includes 20 items assessing *visual disorders*, *motor dysfunction*, *communication deficit*, various disease symptoms (e.g., *headaches* and *seizures*), treatment toxicities (e.g., *hair loss*), and *future uncertainty*.

The items on both measures are scaled and scored using the recommended EORTC procedures [19]. Raw scores are transformed to a linear scale ranging from 0 to 100, with a higher score representing a higher level of functioning or higher

level of symptoms. Differences of at least 10 points (on a 0–100 scale) are classified as the minimum clinically meaningful change in a HRQOL parameter [20]. For example, an increase by more than 10 points on a functional scale would mean a moderate improvement, whereas a decrease by more than 10 points would be interpreted as moderate worsening. Likewise, a rise in a symptom score indicates deterioration, whereas a reduced score means improvement of the specific symptom. Changes of <10 effect points are considered as no change, or to be clinically irrelevant. Changes of >20 points are classed as large effects.

Another widely used brain tumor-specific HRQOL questionnaire is the FACT-Br, to be combined with the generic FACT module (FACT-G).

Apart from HRQOL instruments for cancer patients, symptom scales are alternative PROs.

The MD Anderson Symptom Inventory Brain Tumor module (MDASI-BT) is a symptom scale which has been validated for both primary brain tumor patients and patients with brain metastases [21]. Given that this questionnaire addresses symptoms, it has similarities with the EORTC-BN20. The MDASI-BT module is particularly useful to describe symptom occurrence throughout the disease trajectory and may be used to evaluate interventions designed for symptom management.

Attempts have been made to quantify HRQOL using the Quality Adjusted Live Years (QALY) measure, or comparable scales for use in brain tumor trials. Alternatively, an independent living score (ILS) for use in glioblastoma patients was evaluated [22].

Given the importance of cognitive functions and epileptic seizure activity specifically in LGG patients, these issues are not always well represented in existing HRQOL questionnaires. Regarding self-reported cognitive complaints, the six-item Cognitive Functioning Scale, developed for use in the Medical Outcomes Study, is useful as a PRO [23].

Health-Related Quality of Life: Methodological Issues and Limitations

The ability of patients to cope with limitations and handicap may largely impact the patient's perception of health and satisfaction with life. Due to the exclusively subjective nature of HRQOL questionnaires, it is susceptible to *response shift* when patients are asked to repeatedly report on their HRQOL over time. The patient's perception of his HRQOL may therefore gradually become different from the partner's and health workers' perception.

Because it may become too burdensome, one may also expect that patients with more severe clinical symptomatology and quality of life difficulties are less likely to complete questionnaires over time. These patients (noncompliers), excluded from any analysis, may lead to an overestimation of the actual quality of life [24]. Indeed, the interpretation of serial measurements of HRQOL is affected by *missing data*. Apart from the selection bias due to the clinical condition, in both patient and observer compliance, the filling out of questionnaires decreases over time. However, the main cause of missing data is *administrative failure* arising, for example, when questionnaires are not distributed by a doctor or nurse, distributed at the wrong moment, or handed out without instructions. *Discontinuing HRQOL assessment* at the time of tumor progression in clinical trials will leave the impact of progressive disease on HRQOL unrevealed, which seriously hampers the relevance of these additional outcome measures in trials. Therefore, HRQOL assessment should not stop once tumor progression is declared.

Also, *timing of HRQOL assessment* is of paramount importance. The minimally negative impact of combined procarbazine, CCNU, and vincristine (PCV) chemotherapy on HRQOL in anaplastic oligodendroglioma patients is partly due to a too liberal timing of HRQOL measurement [25].

Methodological problems may arise due to the study design, for example, using HRQOL instruments unknown to the clinicians. Other patient-related factors outside the clinical situation encompass lack of motivation on the part of the patient, misunderstanding instructions, and/or filling out questionnaires incorrectly.

Several approaches can be undertaken to minimize avoidable loss of data on the quality of life. Research staff and patients understanding the relevance of these data to be collected is of critical importance. When writing a research protocol,

HRQOL assessment as a trial endpoint must be explicitly defined, the way of data collection clearly specified, and the analysis of HRQOL parameters should be described in order to prevent problems related to understanding the data and analysis discussion that may or may not be appropriate. Administrative problems can be addressed by training staff responsible for data collection to check for completeness of assessments at submission, document reasons for missing data, and structurally contact patients who miss appointments. To reduce patient-related missing data, it is important to motivate patients. At trial entry, patients should be fully informed regarding the importance of HRQOL assessments and how and when they will be done. Multiple questionnaires addressing similar issues in a different format and/or a high frequency of assessments will result in a low overall compliance.

Touch-screen devices have been developed to replace filling out of paper questionnaires, which may also help to increase compliance, both in trials and in clinical practice. An example is the Computer-based Health Evaluation System (CHES), developed by Holzner and colleagues [26].

When patients are unable to self-report, for example, due to cognitive disturbances, one might consider using proxies or health-care professionals to rate patient HRQOL. In the past, this method was regarded far from optimal. There is often a considerable variability reported in the results between a doctor and cancer patient assessment of patient HRQOL. The same can hold true for HRQOL scores between the patient and partner/proxy [27]. However, a review found moderate to good agreement in various studies evaluating the concordance between patient and proxy measures [28]. Mixed results have been reported for patients and health-care providers. Proxies and health-care providers tend to report more HRQOL problems than do patients themselves, and proxy ratings tend to be more in agreement with patient physical HRQOL domains compared to the psychological domains. Also, specific agreement between brain tumor patients and proxy HRQOL reports was evaluated. The EORTC QLQ-C30, EORTC-BN20, and FACT-Br showed moderate agreement between patient and proxy HRQOL assessment, provided cognitive functioning was not severely affected [29]. A recent study also demonstrated fairly good agreement between patient and proxy data [30]. Still, the use of a non-patient-based report should only be used when patients are incapable of self-report and is considered to be of less value in determining patient HRQOL. Specifically in brain tumor patients with increasing cognitive deficits in the course of disease, a divergence, between the perceived HRQOL by patients on the one hand and proxies or health-care providers on the other hand, may be anticipated.

Health-Related Quality of Life in Low-Grade Glioma Patients

Early HRQOL studies on glioma patients described functional disability and cognitive dysfunction mainly in long-term survivors [31, 32]. The disturbances found are largely ascribed to adverse effects of radiation and chemotherapy and clearly have a negative impact on patient functioning and HRQOL. Especially radiotherapy has long been considered to be the major reason of cognitive dysfunction in both children and adults [33]. Several studies, however, have convincingly shown that the (recurrent) tumor itself, tumor-related epilepsy, and antiepileptic drugs are important causes of cognitive decline as well [34–37].

Other researchers used KPS as HRQOL measures in retrospective analyses for malignant gliomas [38]. These studies indicate that the level of functioning after diagnosis and treatment is sustained or improved for a period of time until patient condition deteriorates due to progressive disease. Death usually occurs within 2–3 months after the decline commences, as confirmed in a large analysis of various brain tumors by Sachsenheimer et al. [39].

Regarding HRQOL in LGG, the studies conducted to date suggest that many survivors of LGG suffer from cognitive deficits, assessed both objectively and subjectively, and a compromised HRQOL with increased fatigue and/or depression [40–42].

According to the results of a large cross-sectional study in the Netherlands in 195 LGG survivors, in the recurrence-free phase of disease,

a decreased HRQOL in long-term LGG survivors is related to the amount of cognitive deficit and the severity of epilepsy [10, 11]. Approximately one quarter of LGG patients reported serious problems with cognitive functioning, particularly memory and concentration. Of note, the overall and generic HRQOL in LGG survivors is not different from that in patients with hematological malignancies without involvement of the central nervous system [11]. The specific diagnosis and treatment history may therefore be of less importance in the determination of generic HRQOL outcomes than is often assumed. Having been diagnosed and treated for a malignancy, in itself, has a negative impact on generic HRQOL. Both patient groups in the Dutch study have a significantly worse HRQOL compared to healthy controls, matched on characteristics such as age, sex, and education. Apart from reporting that their overall health was poorer than that of healthy controls, both patient groups reported poorer role and social functioning, as well as mental health and energy levels. Surprisingly, only a minority of patients reported strong feelings of uncertainty regarding the future. Since patients were in stable condition, without disease progression their responses may have reflected their relatively optimistic outlook at that time point.

Next to LGG patients themselves, also their partners may suffer from a compromised HRQOL [43].

A recent prospective study on HRQOL in 43 adult LGG patients demonstrated an improved HRQOL over time, provided no tumor recurrence was met. This study also confirmed that use of antiepileptic drugs has a negative impact on cognitive functions [44].

Patients with high-grade glioma experience worse quality of life than patients who have an LGG [45]. Next to grade, tumor size and location correlate with HRQOL. Large tumors, tumors in the nondominant hemisphere, and tumors located anteriorly in the brain are associated with poorer HRQOL scores. The unexpected finding as to the nondominant hemisphere tumors having worse HRQOL compared to the dominant hemisphere may be due to more cognitive deficits in dominant hemisphere tumors; the patient's ability to judge his quality of life is reduced with increasing cognitive deficit.

Disease-specific signs and symptoms have a major impact on quality of life. Neurological signs and symptoms as seizure frequency, motor deficits, and functional status have proven to diminish HRQOL [10, 11, 46, 47]. As to nonspecific signs and symptoms in patients with systemic cancers, fatigue and depression are identified as the leading factors diminishing HRQOL [48]. Also in LGG patients, fatigue is one of the most common symptoms and, therefore, one of the leading symptoms of decreasing quality of life [42]. Clinically, significant symptoms of depression have shown to be present in a significant portion of glioma patients and are probably higher than the prevalence in the general cancer population [49]. Thus, depressive symptoms are a serious clinical issue negatively affecting HRQOL in these patients and are related to shorter survival in LGG patients [50].

Disease recurrence has a significantly deleterious impact on patient's life. Patients carry a significant symptom burden, and neurological deficits are more severe at the time of recurrence compared to the initial presentation [47]. Not surprisingly, HRQOL of patients with tumor recurrence is more comprised compared to patients without recurrence at the same time from diagnosis [51].

The number of randomized studies in glioma patients with HRQOL as outcome measure is small. A systematic review from 2002 described only five trials [2], of which only one was performed in LGG patients [52].

Effect of Surgery on Health-Related Quality of Life

Reduction of tumor mass may alleviate neurological symptoms and cognitive deficits thereby improving HRQOL. Also, surgery may reduce the burden of epilepsy in LGG [53]. On the other hand, surgery and perioperative injuries may cause neurological and focal cognitive deficits as a result of damage to normal surrounding tissue. Although these deficits are often transient, they may result in a temporarily lower perceived quality of life. Studies on the effects of surgery in LGG patients have mainly focused on cognitive functioning,

mainly language. Despite extensive surgery, with intensive monitoring and awake surgery especially for tumors in the dominant hemisphere, dysphasia following surgery is relatively mild and in most cases transient [54, 55]. HRQOL is not negatively affected by a wait-and-scan policy in patients with suspected LGG, compared to patients who have a histologically proven LGG [40, 56].

Effect of Radiotherapy on Health-Related Quality of Life

Radiotherapy for LGG has been demonstrated to prolong progression-free survival but not overall survival [57]. It could be hypothesized that by postponing progressive tumor growth, patient functioning and thereby HRQOL would be preserved by radiotherapy. Since cognition and HRQOL were not included as outcome parameters in that particular study, the results from a recently completed EORTC/RTOG clinical trial in LGG addressing these issues are highly relevant. Radiotherapy may result in a decrease of epileptic seizures, with a positive impact on HRQOL [57].

Apart from beneficial effects, radiotherapy may also have a negative impact on HRQOL in LGG patients. The only randomized trial with HRQOL as secondary outcome in LGG compared high-dose radiation (59.4 Gy in 6.5 weeks) with low-dose radiation (45 Gy in 5 weeks) [52]. As to survival, there was no difference between treatment arms.

A specifically composed HRQOL questionnaire consisted of 47 items assessing a range of physical, psychological, social, and symptom domains was used in the trial to measure the impact of treatment over time. Patients receiving high-dose radiotherapy had a more compromised HRQOL reporting lower levels of functioning and more symptom burden following completion of treatment. Statistically significant differences were observed for fatigue/malaise and insomnia immediately after radiotherapy and in leisure time and emotional functioning at 7–15 months after treatment.

In LGG patients in the recurrence-free phase of disease, no negative impact of former radiotherapy on HRQOL was observed [11].

Despite the absence of a negative impact of radiotherapy on long-term HRQOL, most feared is a decrease in cognitive functioning which may be partly due to irreversible radiation encephalopathy in long-term LGG survivors [34, 37].

Effect of Chemotherapy on Health-Related Quality of Life

Successful chemotherapy regimens in glioma patients are PCV chemotherapy (combination of procarbazine, CCNU or lomustine, and vincristine) and temozolomide. Compared to PCV, temozolomide has the advantage of oral administration and less bone marrow as well as subjective toxicity. In LGG patients, temozolomide chemotherapy is not only successful in terms of survival duration but has been proven to maintain or even improve HRQOL while patients are on treatment [58]. Because of the chance of long-term toxicity in LGG survivors treated with radiotherapy, temozolomide is now being compared to radiotherapy in terms of both efficacy and cognition and HRQOL in an EORTC-RTOG trial [59].

Effect of Supportive Treatment on Health-Related Quality of Life

Symptomatic medications prescribed for glioma patients include antiepileptic drugs and steroids (dexamethasone). Because the occurrence of seizures can diminish HRQOL, it can be assumed that treatment with antiepileptic drugs would improve quality of life. Conversely, an adverse effect of antiepileptic drugs on cognition has been demonstrated in the Dutch cross-sectional study in 195 LGG survivors. The impact of seizures and antiepileptic drugs on cognition and quality of life showed both cognitive functions and HRQOL to deteriorate in LGG patients [10, 11]. The cognitive deficits could primarily be ascribed to the use of antiepileptic drugs, whereas the low HRQOL scores were mainly related to poor seizure control [10].

HRQOL in Clinical Practice

In daily practice, prognostic factors such as age and functional status are used to select brain tumor patients who will probably benefit from aggressive treatment and patients who will probably not. HRQOL parameters have shown to be independent prognostic factors in various types of cancers. At present, the prognostic value of baseline HRQOL data in predicting survival of glioma patients is, however, questionable. There are no data specifically regarding LGG patients, but studies performed in high-grade glioma determined the prognostic significance of FACT scores [60]. HRQOL was closely related to functional status, and after correction for this in a multivariate analysis, no prognostic significance of HRQOL scores remained. Two EORTC brain tumor studies in high-grade glioma with respect to this issue were analyzed by Mauer et al. [61, 62]. Classical analysis of EORTC QLQ-C30 subscores, controlled for major prognostic factors as age and performance status, identified cognitive functioning, global health status, and social functioning as statistically significant prognostic factors for survival in glioblastoma patients. In patients with anaplastic oligodendrogliomas, emotional functioning, communication deficit, future uncertainty, and weakness of legs were found to be significant prognostic factors. In a bootstrap analysis, HRQOL scales were added to other predictive factors in a prognostic model and revealed that the HRQOL scales did not improve the prognostic value of known clinical factors. More importantly, fewer parameters are required in the prognostic model using clinical factors compared to the model using HRQOL data. From these analyses it can be concluded that, although various HRQOL scales have prognostic value, they have no additional value over already known clinical factors.

However, in another respect, HRQOL data may have value in daily clinical practice. Routine HRQOL measurements of oncology patients visiting the outpatient department, with information provided to physicians, have shown to have a positive effect on physician-patient communication. In some patients, these measurements improved HRQOL and emotional functioning. However, measurement of HRQOL, symptoms, and functioning is still far from being implemented in daily practice. In the future a core set of standard and disease-specific questions repeated at key points of the disease trajectory (beginning of treatment, mid-treatment, during follow-up, at relapse) should be implemented to allow comparison over time. A small set of focused HRQOL questions could be used at each visit (e.g., during treatment the focus could be on side effects). Furthermore, clear interpretation of scores is important, and decision guidelines should be provided to the clinicians [63]. The use of touch-screen devices in the outpatient clinic will be helpful in this respect [26, 64].

Conclusions and Future Outlook

It is now generally acknowledged that the benefits of longer survival due to tumor treatment in cancer patients should be weighed against side effects of treatment which may have a negative impact on the patient's functioning in physical, psychological, and social aspects. The small number of brain tumor patients compared to other cancers, such as lung and breast cancer, and the dismal prognosis for the far majority of brain cancer patients have for long added to a lack of interest for PROs like HRQOL in these patients. The finding that new (combination) treatments are of benefit for specific subgroups of primary brain tumors and that long-term LGG survivors may run the risk of late treatment complications has elicited an increased interest for outcomes such as cognitive functions and HRQOL.

For LGG patients, the most important outcome measures are cognitive functioning, seizure activity, symptom scales, and HRQOL. These outcomes will more and more be used in both clinical trials and daily clinical care for LGG patients. For cognitive functioning we dispose of standardized test batteries, covering the key cognitive domains in an objective way. Cognitive testing has proven to be an extremely important outcome measure in LGG patients with a relatively long survival, where the immediate effects of extensive surgery and the long-term effects

of radiation and other medical treatments are key issues.

Performance scales, such as the Karnofsky performance scale developed for cancer patients, are frequently used in glioma patients in lack of a brain cancer-specific tool. Since the Karnofsky performance scale does neither reflect neurological impairment such as hemiparesis nor cognitive deficit, its use for brain tumor patients to determine performance status is limited. Possibly specific activity of daily living (ADL) scales, like an instrumental ADL scale developed for dementia, should be developed for brain tumor patients [65].

Regarding the further development of HRQOL instruments, computerized adaptive testing (CAT) is an important new development to improve existing HRQOL measures. The basic idea of CAT is to tailor the questionnaire to the individual patient. Based on the responses to the preceding items, it is estimated which item should be asked next to get the maximal information. For LGG patients, items on cognition, epilepsy, fatigue, and mood deserve special attention [66].

Clearly, HRQOL has become an important outcome measure in brain tumor patients, notwithstanding its limitations. A further development of both patient-reported and other patient-oriented outcomes for brain tumor patients in general and LGG in particular, is needed for both doctors and for the patients and their families.

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Metabolic-Oncological MR Imaging of Diffuse Low-Grade Glioma: A Dynamic Approach

15

Rémy Guillevin

Abstract

Today, magnetic resonance imaging is the "gold standard" of brain tumor imaging, but remains widely used only under its conventional aspect. Recent advances on MRI sequences development and use provided a new conceptual approach of diagnosis and follow-up of WHO II glioma based on multiparametrical and dynamic study of their metabolism allowed by spectroscopy (even multinuclear) and perfusion-weighted imaging, namely, oncological biometabolic imaging. We discuss in this chapter the different aspects and methodological issues and address some practical consequences on MRI clinical practice.

Keywords

MRI • Spectroscopy • Perfusion • Metabolic imaging • Metabolism • Mathematical modeling

Introduction

Until now, conventional magnetic resonance imaging is the method of choice for the noninvasive assessment of suspected intracranial tumors, but has limited sensitivity and specificity [1, 2]. Based on WHO classification, grade II gliomas are deemed low grade, but exhibit nuclear atypia and may progress inexorably, with various timescales from one case to another. In the past 10 years, there have been great advances in diagnostic imaging procedures with increasing accuracy of preoperative assessment of glioma. Multinuclear—phosphorus and proton—magnetic resonance spectroscopic imaging, diffusion-weighted imaging, and perfusion-weighted imaging all have contributed to an improvement of sensitivity and specificity in the diagnosis and monitoring of gliomas [29, 38, 43, 80, 92]. Yet, tumor volume, related cerebral blood volume, and CNI had arisen as prognostic factor of outcome or therapeutic response, as their kinetic profile can

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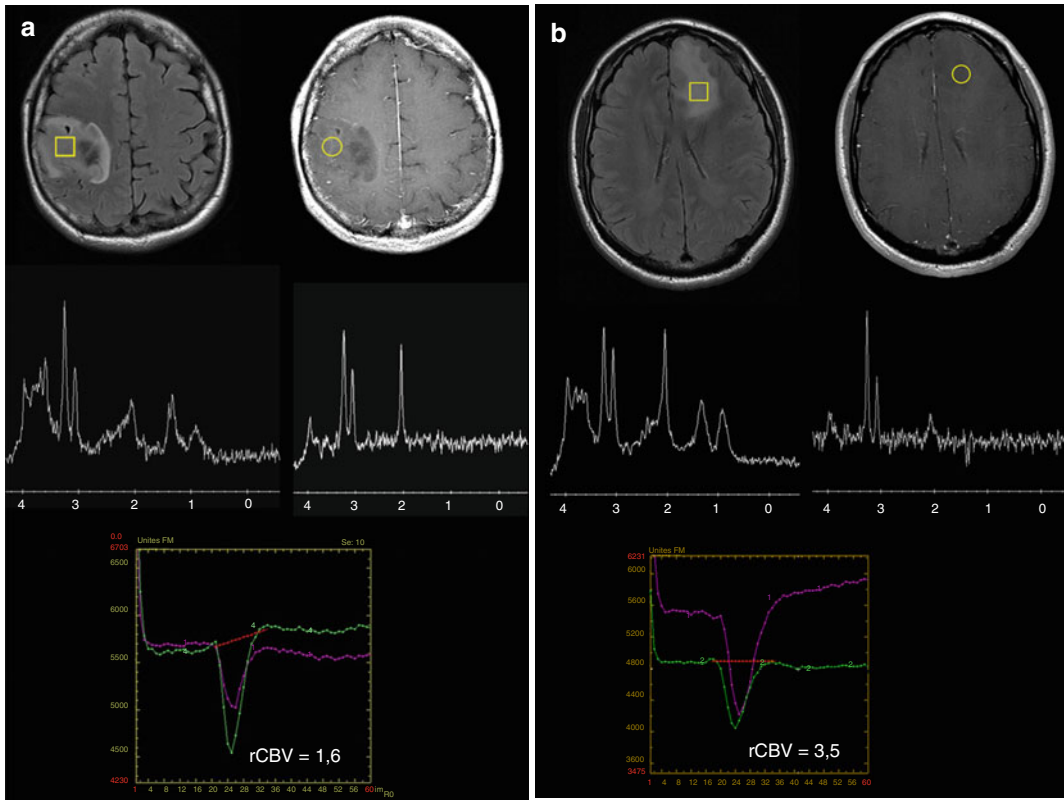


Fig. 15.1 (**a**) Right Rolandic lesion demonstrates heterogeneous aspects on both T2-FLAIR- and T1-weighted images, with slight increased signal on T1 post-contrast images, while (**b**) left frontal lesion appears homogeneous and without contrast enhancement. However, metabolic analysis (*squares*) shows a dramatic increase of CNI and resonance of lactates and free lipids, associated with a rCBVmax (*circle*) value of 3.5, suggesting that the lesion (**b**) may be of higher grade than the (**a**) one (pathologically confirmed)

be properly assessed. In addition, recent advances in molecular biology have been shown to be correlated to specific features on MRI.

Refining the Diagnosis: Which Aggressiveness Within the Grade?

From Diagnosis to Prognosis

Gliomas are extremely heterogeneous in terms of imaging appearance. They are infiltrative lesions with margins often poorly defined on both T1- and T2-weighted images [3]. Low-grade gliomas are identified as a region of hyperintensity on T2-weighted images, with various degrees of heterogeneity, and hypointensity on T1-weighted images (Fig. 15.1). They are typically non-enhancing after injection of gadolinium, but contrast enhancement has been reported at various frequencies [4, 5], ranging from less than 10 % to more than 50 %, and the prognostic significance remains controversial [6, 7]. When present, contrast enhancement has been described as heterogeneous, patchy, and faint. In a retrospective study, Pallud et al. [8] noticed that the presence of contrast enhancement alone, regardless of its pattern, had no prognostic value and that only the presence of a nodular-like contrast enhancement pattern or of a progressive contrast enhancement over time, both suggesting malignant transformation, was associated with a worsened prognosis. However, it must be underlined that there are interobserver discrepancies in contrast enhancement assessment

due to the fact that comparisons are subjective and difficult because qualitative evaluations are performed at various institutions using different imaging parameters. Especially, it must be noted that spin echo sequences must be preferred to gradient spoiled echo on T1-weighted images for contrast enhancement assessment [9, 10].

Diffusion-weighted imaging (DWI) is sensitive to molecular motion of water within brain tissue and provides information about compositional, structural, and organizational features of biological tissues [11]. Such information can be obtained by addition of diffusion gradients to widely available echo planar imaging techniques. Quantitative parameters are the apparent diffusion correlation (ADC) and the fractional anisotropy (FA).

Recent studies of data have suggested that variations in ADC within the anatomic lesion are able to distinguish between different histological subtypes for grade II glioma [12–14] as well as differentiating between upgraded and non upgraded recurrent low-grade gliomas.

Recently, Brown et al. [15] proposed a quantitative method of T2-weighted MR images texture analysis (using S-transform) allowing discrimination between oligodendrogliomas with or without codeletion 1p-19q, with a sensitivity of 0.67 and a specificity of 0.75.

Metabolic Imaging Using In Vivo Spectroscopy: Back to Histological Considerations?

Until now, the major issue of magnetic resonance spectroscopy (MR) was to improve the accuracy of distinction between low- and high-grade gliomas (Fig. 15.1) [16, 17] and to assess their infiltration of the brain using multivoxel and/or monovoxel spectroscopy [18–20]. It has been demonstrated that prognosis may be greatly different between LGGs with similar features on both pathological examination and conventional MRI, without contrast enhancement [21]. The major point here is to establish by noninvasive imaging the progressive transformation from low-grade 2 to "intermediate" and then to grade 3 gliomas of the WHO classification.

Pathological, immunohistological, and molecular biological criteria, while still controversial, are widely used as they could reflect a more or less aggressive potential and could be predictive of further anaplastic transformation [22, 23]. The presence of prominent nuclear or cellular atypias (anisokaryosis with complex nuclear morphology, anisocytosis, dystrophic cells), as well as elevated Ki-67 indices, is suggestive of a more aggressive behavior of a grade 2 glioma, in the absence of true cytological signs of anaplasia as hypercellularity, increased mitotic count, microvascularization, and necrosis.

Some studies have already demonstrated correlations between Cho level and Ki-67 level for gliomas of various grades, but not specifically regarding the degree of aggressiveness within a homogeneous group of WHO grade 2 gliomas [24, 25]. In a recent study [26], authors compared spectral profiles and histological and immunohistochemical data of WHO grade 2 gliomas, using specific methodology for sampling the tumor as complete as possible. Strong correlations were established between specific spectral patterns and three ranges of Ki-67 indices, using not only the choline index but also other metabolites such as Naa, lactates, and lipids (Fig. 15.2).

The rise of lactate resonance suggests anaerobic metabolism within proliferating cells (tumoral anabolism), with progressive increase of CNI, and precedes the increase of microvascular density [27]. This point is in concordance with the fact that an increase of cellular density induces vascular development, since the normal brain parenchymous vascular network becomes insufficient, as reported in previous histopathological studies [28–31]. Moreover, the resonance of lactate appeared as an independent predictive factor of a Ki-67 between 4 and 8 % [26].

At a further stage, corresponding to the third Ki-67 range, membrane breakdown may happen within actively growing tumors, without cell necrosis [30, 32] as reflected by the appearance of a resonance of free lipids, and before any

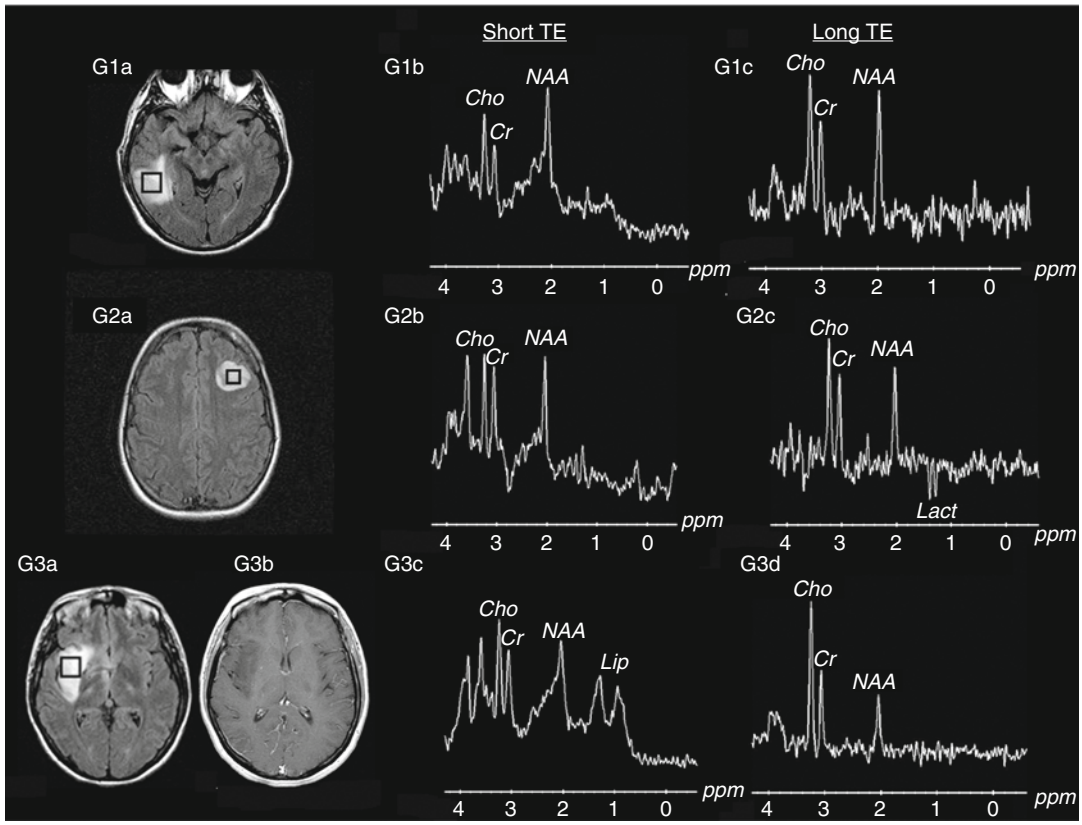


Fig. 15.2 Right temporal lesion (*G1a*). MRS: slight increased CNI at 1.1, without significant resonance of free lipids or lactates (*G1b;c*). Ki-67 value is 3. Left frontoparietal mass (*G2a*). MRS: increased CNI at marked resonance of lactates (*G2c*), without associated free lipids resonance (*G2b*). Ki-67 value is 5 %. Right insular WHO 2 glioma (*G3a*), without post-contrast enhancement on T1-weighted images (*G3b*). Increased CNI at 2.5, with deeply decreased Naa/Cr (*G3d*) and clear resonance of free lipids (*G3c*). No evidence of lactates (*G3d*) [26]

visible modification on conventional MR images [33, 34]. Indeed, resonance of free lipids has been established as an independent predictive factor of a Ki-67 index of more than 8 % (which is agreed to be an abnormally high value for "benign" lesions) [26]. The correlation between free lipids resonance and cellular atypia further supports this assumption, also noted by Nafe et al. [35]. Then, from a spectro-MR point of view (on spectral grounds), a "critical point", which is a change in the glioma aggressiveness reflected by a higher proliferative capacity, could be located between the first and the second Ki-67 ranges.

The finding that 2-hydroxyglutarate (2HG) is present at high levels in IDH-mutated gliomas has raised the possibility that this metabolite could be detected noninvasively by MRS using a widely available ^{1}HMRS sequence and difference editing [36, 37]. As the presence of an IDH 1 or 2 mutation makes the diagnosis of glioma when evaluating a brain mass, the ability to detect 2HG by MRS will be a valuable diagnostic tool and important prognostic marker as well.

Multinuclear spectroscopy may provide additional information on the metabolic behavior of LGG. ^{31}P MR spectroscopy allows quantification of phosphorylated metabolites as phosphoesters (membranous turnover), phosphocreatine (high energy), ATP, and inorganic phosphates (intracellular pH). In a recent study [38], for those of LGG which demonstrated a Ki-67 ≥ 8 %, an increase of the ratio PME/PDE due to an increase of PME more than the increase of PDE, an increase of ratio Cho/Cr, a resonance of free lipids, associated to a resonance of lactate, and an increase of pHi (Fig. 15.3) were found. These results, especially

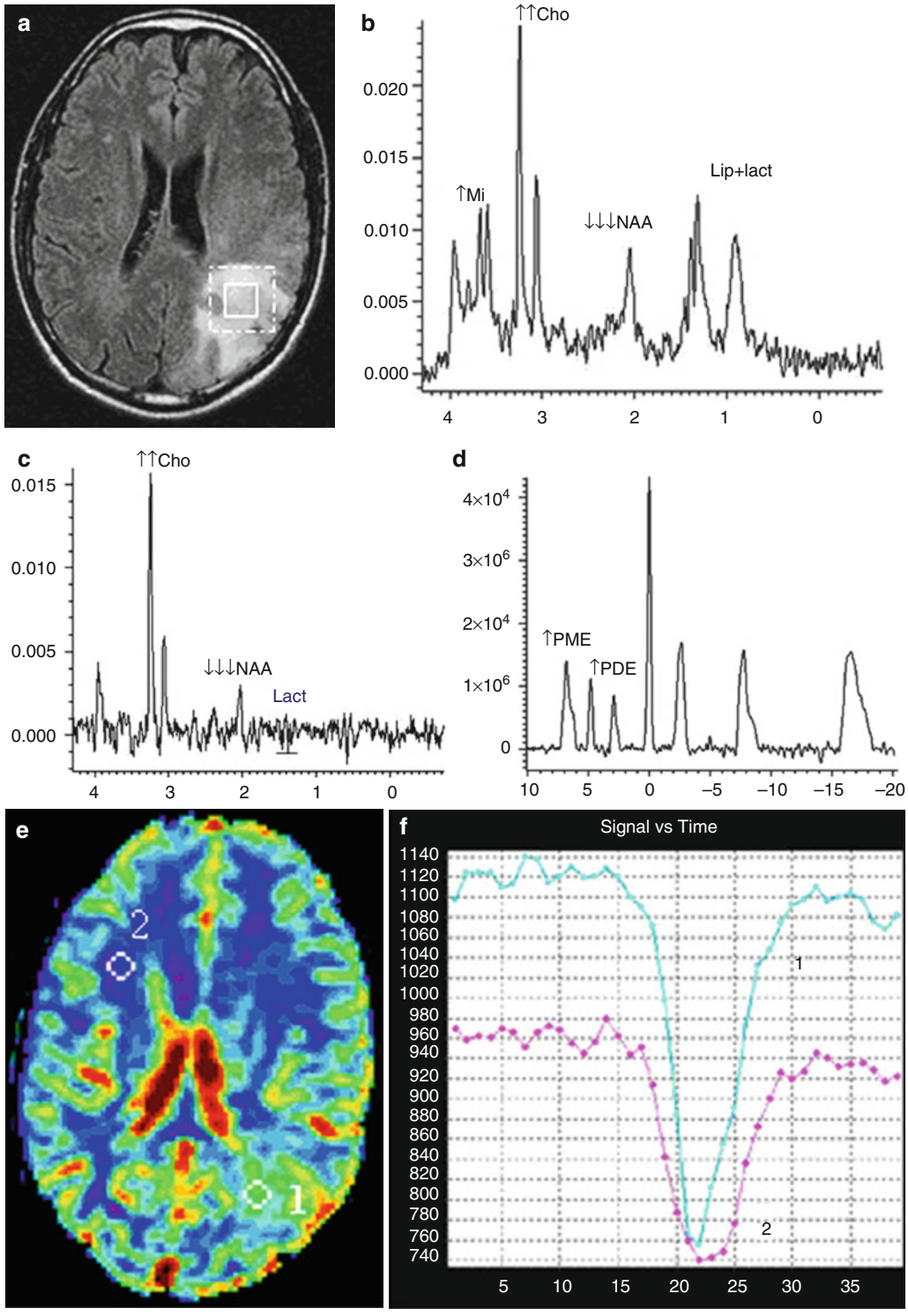


Fig. 15.3 Multinuclear spectroscopic imaging of low-grade glioma. (**a**) Location of proton voxel and phosphorus voxel on the left frontal lesion, T2-FLAIR sequence. (**b**) 1H MRS short TE showing an increase of choline, a dramatic decrease of NAA, and large presence of lipid/lactate. (**c**) 1H MRS long TE, presence of lactate. (**d**) 31P MRS, an increase of PMEs, deltaPi = 4.92, pHi = 7.08. (**e**) Perfusion MRI, a mapping of cerebral blood volume (rCBV), and (**f**) perfusion MRI, first pass curves: hyperperfusion with rCBV = 2.23 [38]

the intracellular alkalinization, were consistent with experimental data [39], suggesting that this phenomenon may constitute an anaplastic transformation factor. While available only in few centers, this tool may be very useful during the next years, especially for monitoring LGG, as it is noninvasive.

The Other Aspect of the Metabolic Imaging: The Perfusion

From Metabolic Modifications to Increased Perfusion: Which Link? [40]

Many studies have demonstrated the utility of perfusion imaging (Fig. 15.1) in the study of brain tumors [22, 29, 30, 41–44], and the rCBV has been shown to be particularly relevant to the prediction of clinical outcome in these patients [22, 27, 43, 45–47]. In particular, Danchaivijtr et al. suggested that DSC imaging may show significant increase in rCBV up to 12 months before contrast enhancement is apparent on T1-weighted images. Similarly, Caseiras et al. [48] demonstrated that rCBV value at study entry was significantly associated with an adverse event and also that rCBV was significantly negatively associated with survival. However, the ability of PWI to discriminate between the histological subtypes remains controversial. Some authors did not find significant differences in the rCBVmax values between the different subtypes of glioma. In addition, although mean rCBV values were higher for oligodendroglioma than for astrocytoma for some authors [40, 46, 49, 50], others did not find such difference [51, 52]. This reflects contradictory findings specific to oligodendrogliomas and highlights a potential weakness of perfusion imaging when used alone. Several studies have shown that the regional cerebral blood volume correlated with the histological grade and the mitotic activity of gliomas. Lev and Rosen [44] reported that the elevated rCBV was a stronger predictor of survival than the degree of contrast enhancement in low-grade gliomas. Law et al. demonstrated that LGGs with high rCBVmax are associated with an 18-fold more rapid progression and a higher propensity for malignant transformation compared with the LGGs with low rCBVmax [46]. While microvascular permeability has already been assessed using T2*-weighted MR technique, but retrospectively and mostly for glioma grading, without controlling therapeutic factors [53], Dhermain et al. [54] found a microvascular leakage (MVL) in 14 patients (WHO II glioma) with low perfusion, suggesting that this feature could reflect an early process in the evolution of unfavorable LGG. More, Law et al. [42] suggested that even with conventional unfavorable features, patients without MVL could represent favorable subgroup with LGG at an early stage.

The rCBVmax value has been positively associated with the Cho variation, which increases with the progression of low-grade glioma. rCBV has also been correlated with glucose uptake and tumor angiogenesis in human gliomas [55, 56]. Finally, in the work performed by our team [40], the Cho/NAA, Lac/Cr, and Lip/Cr ratios were determined to be independent predictive factors of rCBVmax. Moreover, the cutoff value found in this study for the Cho/NAA and Lac/Cr ratios that enabled the discrimination between the two rCBVmax subgroups (<1.7 versus ≥ 1.7) provided additional information related to the previous Law et al. study, which provided data for the prediction of progression-free survival [47]. Thus, the Lac/Cr ratio of 1.54 may be important in clinical decision making, as it has a sensitivity of 75 % and specificity higher than 95 % for predicting an rCBVmax ≥ 1.7. In the same work, it was demonstrated that the resonance of lipids (Lip/Cr) was also predictive of rCBVmax (Figs. 15.4 and 15.5).

The relationship prediction tested in prospective studies by multivariate analysis using probabilistic models of multiple linear regression and binary logistic regression has been shown to

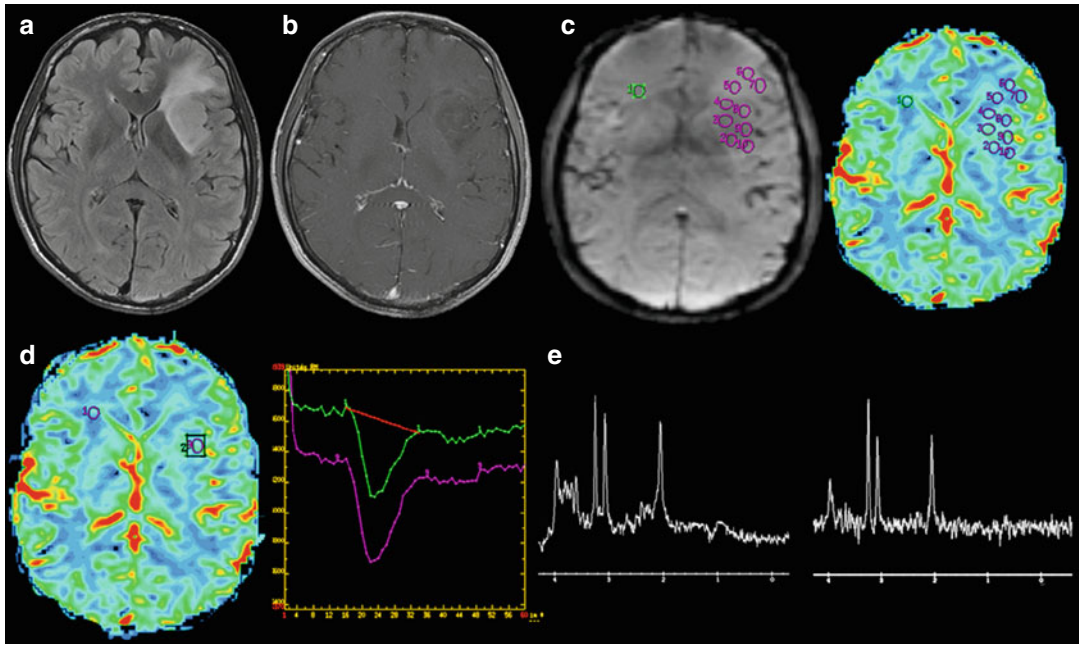


Fig. 15.4 Pathologically proved WHO grade II oligodendroglioma in 32-year-old man. (**a**) Transverse T2-FLAIR-weighted MR image shows increased signal intensity involving left frontal lobe. (**b**) Transverse T1-weighted contrast-enhanced MR image shows subtle contrast enhancement. (**c**) Transverse gradient-echo dynamic susceptibility-weighted contrast-enhanced MR image with relative CBV color overlay map with sampling ROIs for calculation of the maximal value of CBV. (**d**) CBV color overlay map with ROI of rCBVmax (1.6, gamma curve) coregistered with the spectroscopic voxel. (**e**) Proton MRS shows increased Cho/Cr ratio, Cho/Naa ratio, and no free lipids and lactate resonances [40]

support the transitivity of probabilistic functions [57–59]. Thus, we can safely assume that the spectroscopy data (Cho/NAA, Lac/Cr, and lip/Cr ratios), as independent predictive factors, probabilistically support rCBV and that rCBV in turn, as an independent predictive factor, probabilistically supports adverse events (progression or death) [27, 60]. Therefore, based on these assumptions, the Cho/NAA, Lac/Cr, and lip/Cr ratios probabilistically support adverse events. Consequently, ^{1}HMRS adds prognostic information to the clinical outcome as a predictor of adverse events (progression or death). Moreover, ^{1}HMRS may be relevant in cases of misinterpretation of rCBV values between the subtypes of gliomas. These points highlight the importance of early ^{1}HMRS testing and close follow-up of LGGs after they are detected. In addition, it may provide a new parameter for clinical decision making that may help better anticipate and more effectively plan therapeutic management.

Caseiras et al. [48] compared prospectively rCBV, ADC, and tumor volume changes over 6 months. They demonstrated that tumor growth was the best of these parameters in predicting outcome.

Therapeutic Follow-Up

As detailed in another chapters, volumetric evaluation may provide predictive arguments for patient outcome, as demonstrated by some authors [61, 62]. They performed a large study of the dynamic course of LGGs under TMZ

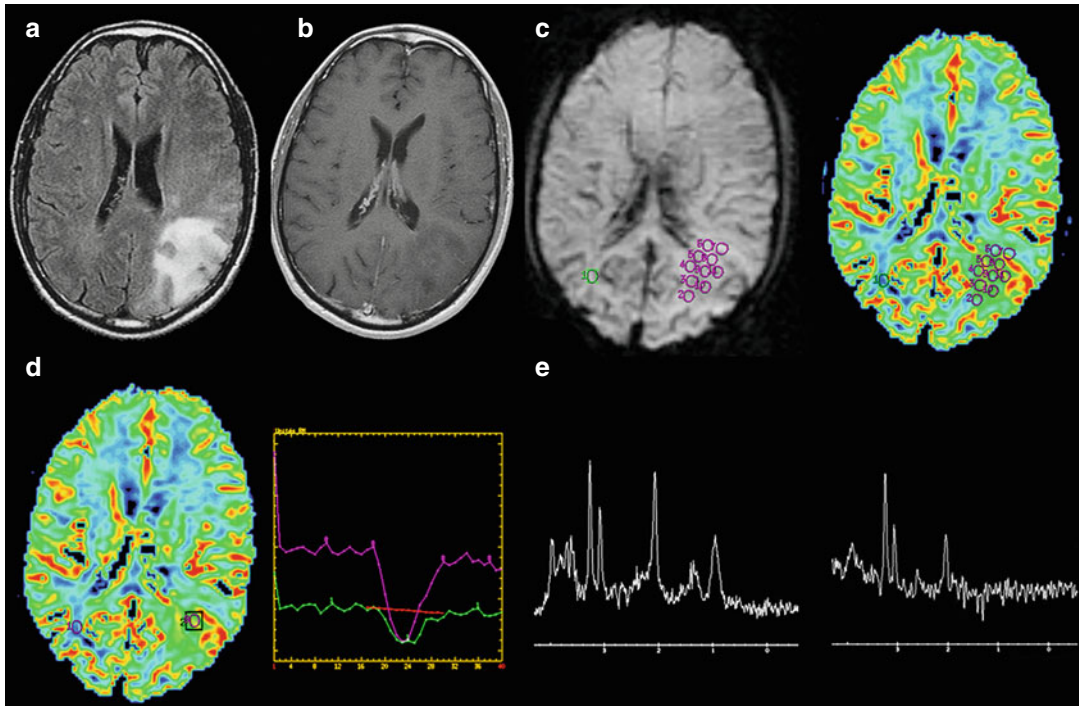


Fig. 15.5 Pathologically proved WHO grade II astrocytoma in 28-year-old man. (**a**) Transverse T2-FLAIR-weighted MR image shows increased signal intensity involving left parieto-occipital lobe. (**b**) Transverse T1-weighted contrast-enhanced MR image shows no contrast enhancement. (**c**) Transverse gradient-echo dynamic susceptibility-weighted contrast-enhanced MR image with relative CBV color overlay map with sampling ROIs for calculation of the maximal value of CBV. (**d**) CBV color overlay map with ROI of rCBVmax (2.18, gamma curve) coregistered with the spectroscopic voxel. (**e**) Proton MRS shows increased Cho/Cr ratio and Cho/Naa ratio with free lipids and lactate resonance [40]

treatment using mean tumor diameter (MTD) growth with a linear mixed model [61, 62] that was extrapolated from serial T2-weighted images. Nevertheless, as the authors stated in their work, the MTD remains a morphological parameter that requires from months to a year to provide reliable measure assessment. Some studies [61, 63] have suggested that chemotherapy may affect tumor burden without causing overt morphological modifications that can be visualized by MRI, possibly by affecting the sole infiltrative part of the glioma. The apparent response to TMZ, however, may appear to be delayed for several months if MRI-measured tumor volume is used as the principal parameter. Consequently, the biological behavior of the glioma (e.g., whether it is stable or progressive) appears to be affected by the TMZ treatment (even at short delays). Metabolic data obtained from proton magnetic resonance spectroscopy (^{1}H-MRS) has proved valuable in therapy evaluation [64] and monitoring [65].

Therapeutic monitoring using ^{1}HMRS may be of interest by providing additional information to volumetric assessment. This point is consistent with the statement that metabolic changes precede and determine morphological changes and tumor growth [66]. In a study on LGG under temozolomide [67], the authors reported that immediately after the first month of treatment, a dramatic change in the metabolite ratios, when compared with the tumor volume, is observed. The Cho/Cr and Cho/Naa ratios fall dramatically to the same extent, whereas the tumor volume decreased much more slowly (Fig. 15.6). The decrease in choline levels is then most probably a direct consequence of the therapeutic effect of

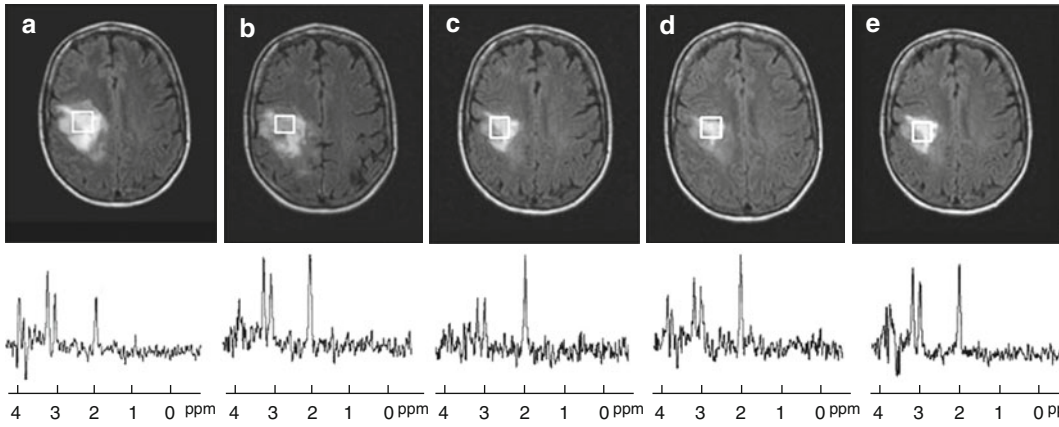


Fig. 15.6 A series of FLAIR images from the patient from Fig. 15.2, together with the spectrum at long TE. The data demonstrate a reduction in tumor volume during the response phase. The images displayed are (**a**) 1 month, (**b**) 3 months, (**c**) 6 months, (**d**) 8 months after the first dose, corresponding to metabolite turnover prior to tumor regrowth, and (**e**) at 12 months [67]

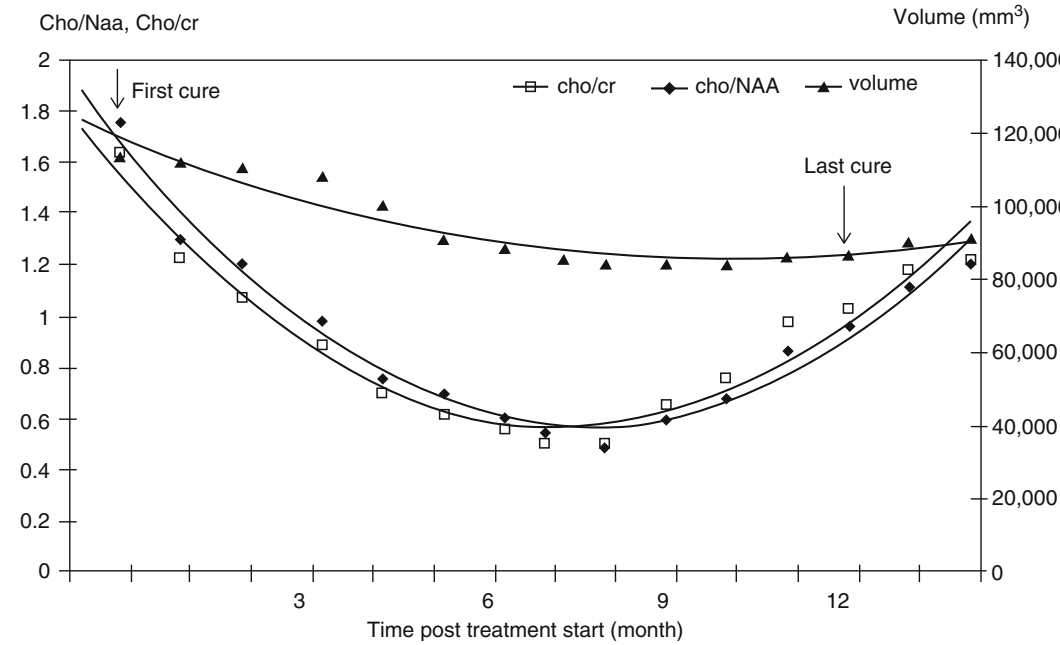


Fig. 15.7 Changes over time in metabolite ratios and tumor volume for a "response-relapse" patient. Note that data spectroscopy is consistent with volume evaluation in both the response phase and the relapse phase and that metabolite ratio changes quicker than the tumor volume in both phases. The metabolite ratios in the response phase decrease until 8 months and begin to rise before the tumor volume does [67]

temozolomide and the subsequent neoplastic cell death [68]. Moreover, the inflection point in the metabolite curve appears from 1 to 2 months before the same event on the tumor volume curve for the patients who displayed tumor recurrence (Fig. 15.7). This observation suggests that MRS may be an earlier MR marker of tumor recurrence than the volumetric data documented in this study as in the study of Tedeschi et al. [20]. More, minimal change of tumor volume, as registered

using dedicated volumetric software, may be difficult to assess by other methods, as MTD one, then leading to delay its assessment. This feature may allow additional time for optimizing adjuvant therapy. After the inflection point in the metabolite ratio curve, a dramatic increase in both Cho/NAA and Cho/Cr ratios is observed; the range of variation of metabolite changes is much more intense than the variation in tumor volume for this part of the curve and in the response phase (Fig. 15.7). In addition, multivariate analysis on spectroscopic data reported in this study provides predictive factors of LGG response under temozolomide treatment. Indeed, the mean relative decrease of Cho/Cr ratio slope at 3 months after the beginning of chemotherapy by temozolomide is predictive of the tumor response over 14 months of follow-up. This point emphasizes the importance of Cho/Cr as a tumor anabolism marker, whatever the degree of neuro-axonal tissue impairment. Yet, Cho/NAA did not appear as a predictive factor of response. This feature is consistent with the results of a previous study performed by Hlaihel et al. [69]. Also, the mean relative decrease between Cho/Cr ratio slope and Cho/NAA ratio slope at 4 months after the beginning of the treatment is predictive of the relapse over 14 months of follow-up. Their relative evolution immediately after the beginning of the treatment may predict the relapse over the follow-up period (Table 15.1). This point gives a reliable index of relapse risk that may be useful in clinical decision making. Last, observation of both lactates and free lipids resonances may provide additional information on LGG metabolic behavior, as stated previously [70].

Few authors have reported variability in the response to temozolomide treatment [71, 72]. Some patients respond in the first month, but others react a long time after the first dose (e.g., only after several months), as it was the case for three of our patients in the "nonresponder" group. Likewise, the relapse phase may occur over a variable time frame. Hence, it is important to monitor patients over a long period, even if predictive factors may be obtained early in the follow-up.

Postradiation Changes or Glioma Progression?

Standard MRI aspects of postradiation changes and progression may be confounding due to overlap findings. Especially, "soap bubble" and "Swiss cheese" patterns may be related to both causes and thus are not reliable [73]. More advanced MR techniques, including DWI, PWI, and MRS, provide specific physiological information, thus leading to increase the specificity of MR examination. Due to increased cellularity, recurrent tumor demonstrates significant lower ADC values compared with radiation changes [74–77]. Normalized rCBV increases over 2.6 (significantly) in case of tumor progression [78]. A combination of thresholds for Cho/Cr and Cho/Naa may identify tumor progression with 83–84.5 specificity and 89–90 % sensitivity [79, 80]. However, each sequence may have its limitations. ADC and DSC

Table 15.1 Mean (±SD) of metabolic ratios and response to treatment [67]

Mean	$\frac{\Delta V_n}{V_o}$	$\frac{Cho}{Cr}$	$\frac{Cho}{NAA}$	$\frac{\Delta\frac{Cho}{Cr}}{\left(\frac{Cho}{Cr}\right)_o}$	$\frac{\Delta\frac{Cho}{NAA}}{\left(\frac{Cho}{NAA}\right)_o}$	$\left(\frac{\frac{Cho}{NAA}-\frac{Cho}{Cr}}{\frac{Cho}{NAA}}\right)_n$
No response	0.005 (±0.003)	1.525 (±0.159)	1.669 (±0.156)	0.016 (±0.007)	0.014 (±0.008)	0.097 (±0.016)
Response/no relapse	0.159 (±0.096)	1.256 (±0.444)	1.555 (±0.487)	0.446 (±0.200)	0.416 (±0.205)	0.018 (±0.061)
Response/relapse	0.116 (±0.057)	1.253 (±0.465)	1.266 (±0.450)	0.255 (±0.220)	0.323 (±0.204)	0.004 (±0.070)

values may be both affected by intercurrent changes due to radiation effects, as necrosis within growing tumor, edema (DWI), aneurysmal formation, telangiectasia, and proliferation of endothelial cells (DSC). Matsusue et al. demonstrated that a multiparametric score threshold of 2 improves diagnostic accuracy to 93.3 %. Amide Proton Transfer (APT) MRI expands the range of molecular MRI techniques by allowing indirect detection of the amide proton signals in the backbone of endogenous proteins and peptides. By its cellularity, glioma may show hypersignal on APT images, while hyposignal is observed in necrotic area (postradiation). This sequence may be useful during the next years [81].

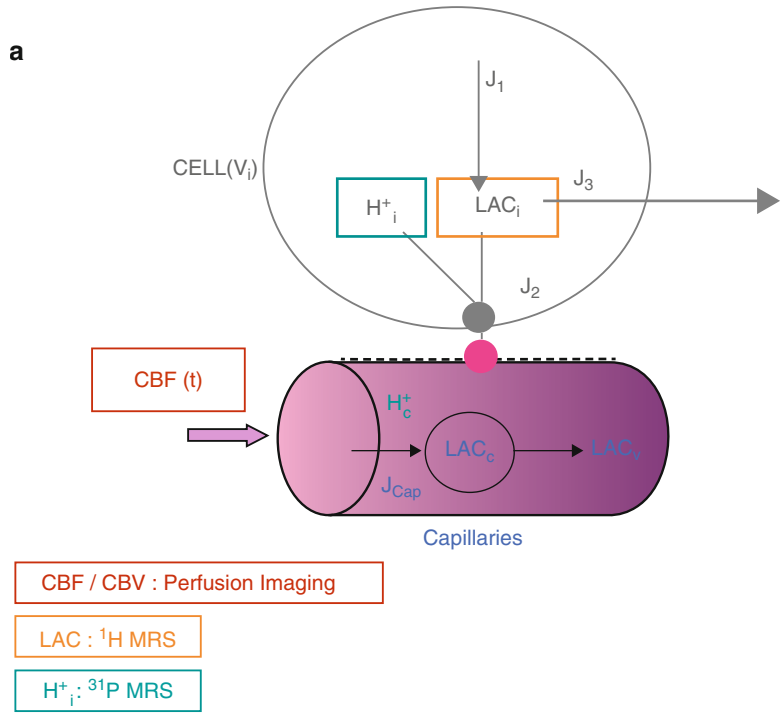
New Frontiers: Mathematical Modeling of Tumor Energy Metabolism

From practical to theoretical approach: how to carry out all the parameters? As stated above, the correlation between the Lac/Cr ratio and increased rCBV is consistent with previous experimental results. This suggests that the metabolism of WHO grade II gliomas may adapt by modifying lactate transporters [70] and increasing anaerobic glycolysis to survive the hypoxic conditions [16, 17]. Furthermore, this linear correlation is consistent with the mathematical model of brain lactate kinetics in the study of the compartmentalization of brain energy metabolism between glia and neurons, which was described by Aubert et al. [82]. This model suggests that lactate clearance from brain tissue via the bloodstream plays only a minor role; these findings are also consistent with the Kuhr and Korf study [83].

The WHO grade II gliomas exhibit characteristics affecting their therapeutic management [84]. Their delay of change in tumor grade is highly variable from one subject to another [85]. To date, only clinical events or continuous observations of the growth of the tumor volume on repeated examinations can evoke a change in tumor grade. It is therefore necessary to repeat the MRI at regular intervals to obtain a predictive parameter, namely, the growth of average diameter [86]. However, in addition to requiring regular inspections, this approach is purely morphological and has the disadvantage of revealing the consequences of changes of tumor bio-metabolism, which occur upstream of tumor growth and of malignant transformation of the lesion. Indeed, switching the metabolism of grade II glioma to a much closer metabolism to that of high-grade gliomas occurs very early during the natural history of gliomas [70]. It is therefore obvious that volume growth and metabolic changes are not synchronous and that metabolic changes precede the growth of tumor volume. Therefore, it seemed important to attempt an investigation of these changes in tumor metabolism, using a biomathematical model with simulations from metabolic data, collected in vivo by magnetic resonance. Yet, we have proposed a model combining the parameters of ^{1}H MRS, ^{31}P MRS, and the MRI perfusion.

Based on different previous definition of "metabolic" subclasses of WHO grade II gliomas, the main question asked is the mechanism involved in the decrease in lactate. Factors that may play a major role are particularly the regional blood flow, the values of pH, and the lactate transport which runs via the MCT, carriers lactate-H^+ [87]. To better understand the pathophysiological mechanisms involved, one study [38] constructed a mathematical model consisting of a system of nonlinear ordinary differential equations (Fig. 15.8a, b). The authors implemented this model using in vivo data from multinuclear MRS (^{1}H/^{31}P) and perfusion MRI. This model is based on existing physiological models [88, 89], and its variables or parameters are mostly measured by MRS and MRI perfusion. They tried to explain the great variability of regional blood flow, lactate, and pHi, which were difficult to explain coherently.

The implementation of the model with the data from this study allows identification of different subgroups based on lactate, pHi, and data from MRS perfusion. Thus, gliomas-expressing lactate and an increase in CBF, also show an increase in pHi. These results are consistent with experimental study of Hubesh et al. [90] which showed that despite a potential increase



b

$$V_i \frac{dLAC_i}{dt} = J_1 - J_2 - J_3 \overset{def}{=} J - J_2$$

$$V_c \frac{dLAC_c}{dt} = J_{cap} + J_2$$

$$\frac{dLAC_i}{dt} = \frac{1}{V_i}\left[J(t) - T\left(\frac{LAC_i H_i^+}{K_H + LAC_i H_i^+} - \frac{LAC_c H_c^+}{K_H + LAC_c H_c^+} \right) \right]$$

$$\frac{dLAC_i}{dt} = \frac{1}{V_c}\left[2\,CBF(t).(LAC_a - LAC_c) + T\left(\frac{LAC_i H_i^+}{K_H + LAC_i H_i^+} - \frac{LAC_c H_c^+}{K_H + LAC_c H_c^+} \right) \right]$$

Fig. 15.8 (**a**) Description of the mathematical model with parameters collected from MR sequences. (**b**) Differentiel equations system of the mathematical model [38]

in glycolysis, the pHi of tumor cells is likely to increase due to alterations of membrane transporters proton/bicarbonate.

On the other hand, it seemed paradoxical that lactate, for which the authors have shown that it would be a very early marker of changes of tumor metabolism [26], has very different values, and sometimes a non-monotonic evolution. Taking into account intracellular pH and rCBF by the model of lactate allows to have a mathematic simulation of the high dispersion of results obtained from MRS and perfusion MRI without recourse neither to the hypothesis of capillary recruiting highly unlikely in WHO grade II gliomas nor to the capillary proliferation excluded at this stage. However, these results strongly suggest changes in the transport of lactate across the blood–brain barrier and the membranes of tumor cells, that is, modifications of density and/or kinetic properties of MCT. This hypothesis can be in relation with the resonance of free lipids observed in lesions showing no detectable lactate resonance. It is consistent with recent data from the literature which suggest also

qualitative or quantitative changes of MCTs in glial tumors [91]. This prompted us to further study the properties of the model: whatever the parameters values, the model has a unique stationary point, which is asymptotically stable. Numerical computations using MATLAB software confirm this point [92]. In addition, this finding is consistent with a clinically observed fact that, within a short time scale from minutes to days, metabolites concentrations within the tumor appear nearly constant. Moreover, explicit and sufficient conditions are derived, ensuring that a stationary point is in a viability domain in the first quadrant.

Conclusion

On a practical "imaging" point of view, glioma should be considered as a dynamic multiparametrical system. Baseline oncological examination should be complete, including standard MRI, DWI, PWI-DCE/DSC, and ^{1}HMRS. It is critical that all the examinations must be performed on the same magnet (at least of the same field strength) for a same patient, using the same parameters from one session to another. In particular, T2-FLAIR-weighted images must be acquired using identical specific parameters and read on a same workstation using stable windowing and leveling, thus allowing proper assessment of tumor boundaries and size. In the same way, ^{1}HMRS, after initial oversampling of the tumor, should be performed using the same voxel size within the same location from one exam to the next. In addition, specific spacing of examinations must be established thoroughly for the patients, regarding the strategic therapeutic plan retained for him. As stated above, it should be recommended a close monitoring by imaging using both metabolic and volumetric assessment of the tumor behavior at the beginning of a new therapeutic modality, for example, radiotherapy or chemotherapy. Once obtained a "kinetic" profile, MR controls could be spaced. During the next few years, improvement of both descriptive and prognostic models should allow optimization of imaging follow-up.

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Positron Emission Tomography in Diffuse Low-Grade Gliomas

16

Karl-Josef Langen, Frank Willi Floeth, Michael Sabel, and Norbert Galldiks

Abstract

MRI is currently the method of choice for the diagnosis of diffuse low-grade gliomas and provides an excellent depiction of structural changes in the brain. Nevertheless, the delineation of the tumor from normal brain tissue and nonspecific abnormalities on MRI such as edema- or treatment-related changes can be difficult. Positron emission tomography (PET) provides additional information on tumor metabolism and is helpful in many clinical situations. In particular, PET using radiolabeled amino acids has a wide range of applications and helps to solve a number of clinical issues. At initial diagnosis, amino acid PET may be helpful to estimate the prognosis of a low-grade glioma and to decide on the therapeutic strategy. The method improves targeting of biopsy and provides additional information of tumor extent which is helpful for planning neurosurgery and radiotherapy. In the further course of the disease, amino acid PET allows a sensitive monitoring of treatment response, the early detection of tumor recurrence, and an improved differentiation of tumor recurrence from treatment-related changes of the brain tissue. In the past, the method had only limited availability due to the low number of PET scanners and the use of radiopharmaceuticals with a short half-life. In recent years, however, the number of PET scanners in hospitals has increased considerably. Furthermore, novel amino acid tracers labeled with positron emitters with a longer half-life have been developed and clinically validated which allow a more efficient and cost-effective application. These developments and the well-documented diagnostic performance of PET using radiolabeled amino acids suggest that its

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application continues to spread and that the method may be available as a routine diagnostic technique for certain indications in the near future.

Keywords
Low grade glioma • PET • Radiolabeled amino acids • C-11-methionine • F18-fluoroethyltyrosine (FET)

Introduction

MRI with its excellent soft tissue contrast, high spatial resolution, and its multiplanar reconstruction capabilities is currently the method of first choice for the diagnosis of cerebral gliomas. Despite these unsurpassed properties of MRI, many problems in the diagnostic assessment of low-grade gliomas remain and a number of pivotal questions concerning the management of low-grade gliomas cannot be answered satisfactorily. Thus, at initial diagnosis, diffuse low-grade gliomas may exhibit only minimal changes in the brain tissue which can hardly be distinguished from benign lesions. In larger tumors, the differentiation of glioma tissue from surrounding edema may be difficult, particularly when the tumor is not sharply demarcated from normal brain tissue due to diffuse tumor cell infiltration. Although commonly assigned as low-grade gliomas, the course is clinically diverse and for some patients, the disease has a benign course, whereas others experience rapid progression [1]. The decision of early surgical intervention or a "watch and wait" policy is crucial on the basis of both clinical (e.g., age, histology subtype, and the presence of neurological deficits) and MRI criteria (e.g., tumor size) [2]. In the further course of the disease, the tumors may exhibit regional malignant transformation, which is difficult to detect, especially when the blood–brain barrier (BBB) remains intact. In these patients with a heterogeneous tumor and intact BBB, biopsy guidance may be difficult especially. Monitoring of treatment response is another important factor to optimize individual treatment strategy which is only a late sign based on volumetric measures in MRI. After treatment, postoperative or radiogenic changes in peritumoral brain tissue may result in contrast enhancement on MRI that cannot be reliably distinguished from vital tumor tissue of recurrent glioma [3].

Therefore, alternative imaging methods reflecting metabolic features of the tumor tissue have attracted the interest of neuro-oncologists for many years in order to facilitate the process of clinical decision-making in this challenging tumor entity. PET is a powerful method in nuclear medicine that has shown great potential for the diagnostic assessment of malignant tumors. The most widely used tracer for PET is ^{18}F-Fluorodeoxyglucose (FDG), which is accumulated in the majority of tumors due to an increased energy demand and consequently an increased glucose metabolism. FDG has been used for the evaluation of brain tumors since the early days of PET, and a relationship of FDG uptake and tumor grade of gliomas and prognosis of cerebral gliomas have been reported in numerous studies [4]. In low-grade gliomas, however, FDG uptake is generally low and there is high FDG uptake in the surrounding normal brain tissue. Therefore, the usefulness of this tracer for low-grade gliomas is limited. In this chapter, the most promising PET tracers for the diagnostic assessment of low-grade gliomas will be reviewed. The chapter deals mainly with the application of radiolabeled amino acids owing to the ability of these substrates to penetrate the intact blood–brain barrier (BBB) and to depict brain tumors with a high tumor-to-background contrast. These tracers are well investigated, allow decisive diagnostic information in cerebral gliomas with respect to many clinical aspects, and are quite close to be established in routine clinical diagnosis (Table 16.1) [14, 35–37].

Radiopharmaceuticals for PET in Low-Grade Gliomas

Today, the most widely used application of PET is the measurement of glucose metabolism with FDG in various types of cancer. In cerebral

Table 16.1 Clinical applications of amino acid PET in low-grade gliomas

	Clinical potential	References
Detection and differential diagnosis	+	[5, 6]
Biopsy guidance	+++	[7–9]
Tumor extent	+++	[10–13]
Grading of gliomas	+	[12, 14–18]
Prognosis	+++	[19–22]
Resection planning	+++	[13, 23, 24]
Radiotherapy planning	++	[25, 26]
Detection of recurrence	+++	[27–29]
Therapy monitoring	+++	[30–34]

+ limited value, ++ helpful in a small fraction of patients, +++ high clinical impact

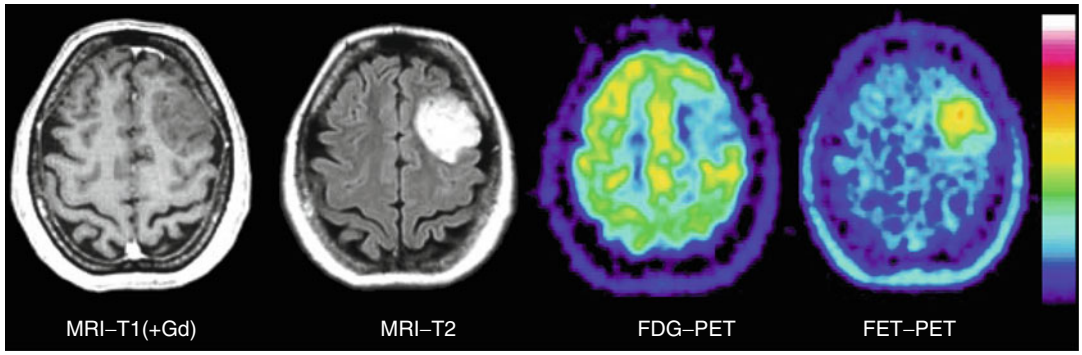

Fig. 16.1 Astrocytoma WHO grade II in the left hemisphere. The T1-weighted MRI after application of Gd-DTPA shows no contrast enhancement indicating an intact BBB, and depiction of the tumor in the T2-weighted MRI is similar. FDG PET shows hypometabolism and is not helpful to guide biopsy. FET PET identifies a hot spot within the tumor and detects an optimal biopsy site

gliomas, FDG uptake is correlated with the degree of malignancy of the tumor (WHO grading) and with the patient's outcome [4, 15]. Due to the high rate of glucose metabolism especially in the gray matter of the brain, however, it is difficult to distinguish glioma tissue from normal brain tissue by FDG PET. While most high-grade gliomas WHO grade III and nearly all grade IV glioblastomas show an increased FDG uptake compared to the white matter, low-grade gliomas WHO grade II usually exhibit an indifferent or even a decreased FDG uptake (see Fig. 16.1). Therefore, FDG PET is not useful to delineate low-grade gliomas from the surrounding brain tissue.

Nevertheless, FDG PET has been shown to be useful to detect malignant transformation in low-grade gliomas and may therefore be useful for follow-up in low-grade gliomas [4]. PET studies of regional blood flow and oxygen metabolism of brain tumors using ^{15}O-labeled tracers showed a high variability and have been found to be of limited value for the diagnostic assessment of cerebral gliomas [38]. The use of proliferation markers such as [^{18}F]3′-deoxy-3′-fluorothymidine (FLT) showed even a better correlation with the grade of malignancy and prognosis of cerebral gliomas than FDG uptake [39]. An image-guided biopsy study demonstrated that FLT is a useful marker of cellular proliferation that correlates with regional variation in cellular proliferation, but was unable to identify the margin of gliomas [40]. This is caused by the fact that FLT is not able to penetrate the intact BBB and accumulates normally only in areas with contrast enhancement on MRI [16, 39, 41]. Therefore, portions of the tumor with an intact BBB which is the predominant finding in low-grade gliomas cannot be detected with FLT PET. Also, ^{11}C-Choline has been used as a marker of cell membrane phospholipids in brain tumors and shows a significant correlation of uptake with

the degree of malignancy in gliomas [42]. Tracer uptake, however, is limited as with FLT to areas with BBB disruption, and therefore, these tracers offer limited additional information compared to a contrast-enhanced MRI.

Another interesting approach is to investigate the presence of intratumoral hypoxia using ^{18}F-Fluoromisonidazole [43]. Hypoxia in tumors is a pathophysiological consequence of structurally and functionally disturbed angiogenesis along with deterioration in the inability of oxygen to diffuse through tissues. A PET study in patients with cerebral gliomas demonstrated areas of hypoxia in glioblastomas, but all investigated low-grade gliomas showed low uptake of ^{18}F-Fluoromisonidazole[44]. This is not unexpected since tumor growth and angiogenesis in low-grade gliomas are still in a balance so that this approach is particularly attractive for the evaluation of high-grade gliomas.

At present, the most promising PET tracers for the investigation of low-grade gliomas are radiolabeled amino acids of the class of large neutral amino acids such as [Methyl-^{11}C]-L-methionine (MET) or O-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET) [14, 35, 45]. Because the uptake of these amino acids by both the white and gray matter of normal brain tissue is relatively low, cerebral gliomas can be distinguished from the surrounding normal tissue with high contrast. It was long assumed that increased uptake of MET in brain tumors reflects an increased protein synthesis rate. Experiments in mice, however, demonstrated that an inhibition of protein synthesis did not influence the uptake of radiolabeled MET in tumors and brain [46] suggesting that alterations of amino acid transport rather than increased protein synthesis caused increased uptake in tumors. This was also confirmed by kinetic PET studies in humans with L-[2-^{18}F]fluorotyrosine, a tyrosine derivative which in contrast to FET is incorporated into proteins [47]. That study showed an increase of transport-related rate constant K_1 but not of metabolism-related rate constant k_3 in glioma. A number of experimental studies suggest that the increased uptake of MET by human glioma is caused mainly by increased transport via specific amino acid transporters predominantly by the system L amino transporter and its subtype LAT1 [14, 48]. Furthermore, the predominant role of transport phenomena for increased amino acid uptake in gliomas is confirmed by the observation that PET using amino acid derivatives like FET which is not incorporated into protein exhibit nearly identical results concerning brain tumor imaging as MET PET. Thus, a number of studies have shown that imaging of cerebral gliomas with MET and FET is rather similar [49–51]. Similar results in cerebral gliomas have also been observed when comparing PET using MET and 3,4-dihydroxy-6-[^{18}F]fluoro-phenylalanine (FDOPA)[52], but this tracer shows additional uptake in the striatum which may cause problems in the delineation of gliomas in those areas.

Since large neutral amino acids also enter normal brain tissue, a disruption of the BBB, i.e., enhancement of contrast media in CT or MRI scans, is not a prerequisite for intratumoral accumulation of MET or FET (see Fig. 16.1). Consequently, uptake of these tracers has been reported in many low-grade gliomas without BBB leakage [14, 19].

Most PET studies of cerebral gliomas have been performed with the amino acid MET [14], although the short half-life of ^{11}C (20 min) limits the use of this technique to the few centers that are equipped with an in-house cyclotron facility. In contrast to MET, ^{18}F-labeled amino acids (half-life 109 min) such as FET and FDOPA can be transported from a cyclotron unit to multiple external PET centers. This enables a wider application of amino acid PET in clinical diagnosis. One of the best established ^{18}F-labeled amino acids is FET that can be produced in large amounts for clinical purposes like the widely used FDG [48, 53, 54].

Animal experiments have shown that FET, in contrast to MET, exhibits no uptake in inflammatory cells and in inflammatory lymph nodes, but false-positive uptake has been observed for both tracers MET and FET in human brain abscesses, demyelinating processes, near cerebral ischemia, and hematomas. Therefore, increased uptake of the tracers is not specific for cerebral gliomas although increased amino acid uptake has a high positive predictive value for cerebral gliomas [5]. The report is focused on the clinical experiences with MET and FET, which are at present the best validated amino acid tracers for PET.

Clinical Applications of PET in Diffuse Low-Grade Gliomas

Detection of Low-Grade Gliomas and Differential Diagnosis

The diagnostic potential of amino acid PET to detect low-grade gliomas is limited since MET and FET exhibit increased uptake only in a fraction of about 60–80 % of low-grade gliomas [5–7, 19, 35]. The limited sensitivity of MET and FET PET represents no problem in the clinical setting since MRI has a high sensitivity to detect low-grade gliomas and the MRI examination is always ahead of the PET study. The specificity of MET and FET PET for neoplastic lesions is generally affected by possible tracer uptake in the area of benign processes such as hematoma, ischemia, and acute inflammatory processes [14, 55–58]. In the largest study to date evaluating MET PET in a consecutive series of 196 patients with suspected brain tumors, differentiation between gliomas and nonneoplastic lesions was correct in 79 % using a threshold of the tumor/brain ratio of 1.47. Exclusion of high-grade gliomas (99 low-grade gliomas vs 24 nonneoplastic lesions) yielded a sensitivity of 67 % and specificity of 72 % for distinguishing low-grade gliomas from nonneoplastic brain lesions [6, 35]. In an actual study, we have evaluated the diagnostic performance of FET PET in a series of 174 newly diagnosed cerebral lesions with suspicion of glioma which included 72 histologically confirmed diffuse low-grade gliomas. In that study, the sensitivity of FET PET to detect low-grade gliomas was 79 % and the specificity 48 % [92]. These results are similar to the observations in previous publications with smaller groups of patients [5, 7]. Thus, the diagnostic accuracy of amino acid PET for low-grade gliomas is not sufficient in the clinical setting, and the possibility of nonspecific uptake in inflammatory cells or reactive glial tissue in the penumbra of benign lesions must be borne in mind. Therefore, a histological evaluation of suspicious brain lesions by biopsy remains necessary under most circumstances.

Identification of an Optimal Site for Biopsy

An important aspect of the diagnostic assessment of low-grade gliomas is the definition of the areas within the tumor in which the rate of cellular proliferation is highest. Since the tumor biology is dominated by the most aggressive parts of the glioma, representative tissue samples are vitally important for histological tumor diagnosis, prognostication, and treatment planning. The ability of MRI to show the most rapidly proliferating portions of diffuse low-grade gliomas is limited, particularly when the tumor shows no contrast enhancement in MRI which is the predominant finding in low-grade gliomas. FDG and FLT PET are usually negative in low-grade gliomas and provide no information on regional heterogeneity of metabolic activity in these tumors. Radiolabeled amino acids exhibit increased uptake in the majority of diffuse low-grade gliomas and are helpful to optimize the targeting of biopsies and prevent the problem of nondiagnostic biopsies from nonspecifically altered tissue (Figs. 16.1 and 16.2). Biopsy controlled studies have shown that MET and FET uptake correlate with microvessel and cell density in non-contrast-enhancing gliomas [59–61]. Vascular density is a frequently described feature linked to early malignant transformation in gliomas [62]. A number of studies have compared the diagnostic potential of PET using FDG and MET or FET to identify metabolic hot spots in cerebral gliomas to guide biopsy [7–9]. These studies consistently report that regionally increased FDG uptake, if present, is congruent with that of increased MET or FET uptake. For the subgroup of low-grade gliomas, there is only limited experience. In a study with 32 patients that included 10 low-grade gliomas, MET PET allowed the definition of a biopsy target in all low-grade gliomas, while FDG showed increased uptake in only one of these tumors [9]. In a patient series of 22 histologically confirmed low-grade gliomas, FET PET identified a local maximum for biopsy guidance in 16 of the tumors (72 %), while FDG identified a metabolic spot in only 2 (9 %) of the low-grade gliomas [7]. In the actual study of our center mentioned above including 72 histologically

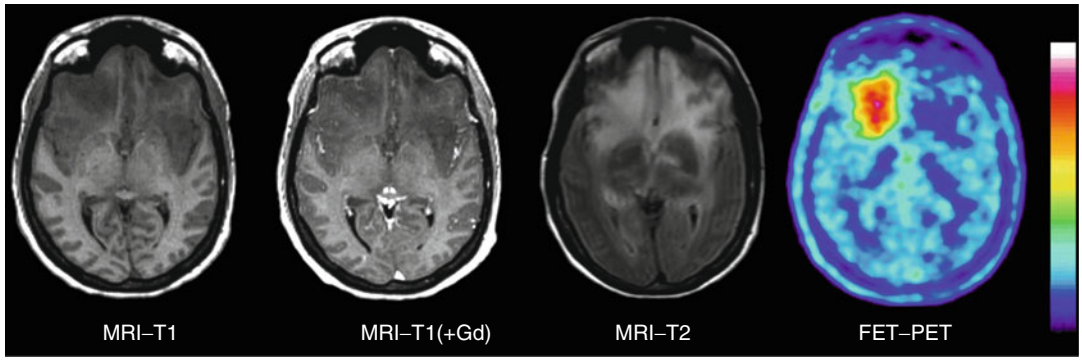

Fig. 16.2 Astrocytoma WHO grade II in the frontal lobe. T1-weighted MRI after application of Gd-DTPA shows no pathological contrast enhancement and a tumor cannot be clearly delineated. T2-weighted MRI shows widespread abnormalities within the complete frontal lobes on both sides and is not helpful to depict the tumor. FET PET identifies a clear tumor with high tracer uptake in the lower right frontal lobe

confirmed diffuse low-grade gliomas, FET PET identified a local maximum in 79 % of the tumors [92]. These data suggest that amino acid PET can be considered as a promising tool for identifying metabolic hot spots in low-grade gliomas to target biopsies. Nevertheless, it is not yet proven beyond doubt that the maximum concentration of amino acid uptake in low-grade gliomas corresponds to the most aggressive part of the tumor and further studies are needed to investigate this aspect.

Delineation of Tumor Extent for Treatment Planning

Multiple studies in which the radiological findings were compared with the histological findings in tissue samples obtained by biopsy or open surgery have provided clear evidence that PET using radiolabeled amino acid detects the solid tumor mass of cerebral glioma tissue more reliably than either CT or MRI [8–12]. This is especially relevant for the non-enhancing parts of gliomas in MRI, which predominantly occurs in low-grade gliomas. In a study exploring the potential of FET PET to image the extent of cerebral gliomas, 52 neuronavigated biopsies were taken from cerebral gliomas of 31 patients. Tumor tissue was found in 94 % of biopsies from areas where FET PET was positive, but only in 53 % of the suspicious areas identified by MRI [12]. In that study, 12 biopsies yielded the histopathological diagnosis of a diffuse low-grade glioma and FET uptake was increased in all but one of the areas from which the biopsies were taken. In contrast, none of these areas showed contrast enhancement on MRI. Another study investigated the role of FET PET as a surrogate marker for accumulation of 5-aminolevulinic acid (5-ALA), which is used as a metabolic marker of malignant glioma cells for fluorescence-guided resection [63]. In that study, patients with 17 low-grade gliomas were included. FET was positive in 7 of the tumors, while 5-ALA was observed in only one of the low-grade gliomas, which showed corresponding contrast enhancement. These data indicate that amino acid uptake in PET is a more sensitive indicator of low-grade glioma than 5-ALA fluorescence.

Since amino acid PET appears to be a valuable instrument to detect the solid tumor mass of cerebral gliomas, this technique has been used for resection planning. In a study evaluating integrated MET PET and MRI-guided resection of 103 brain tumors, a large fraction of low-grade gliomas was included [13]. Resection planning in 59 low-grade gliomas demonstrated that the PET volume did not match the MR volume and improved the tumor volume definition in 88 % of the cases. Similar results were reported in a recent study [23]. These data suggest that resection of low-grade gliomas guided by amino acid PET may increase the amount of cytoreduction and

thus patient's survival. It needs, however, to be considered that MET and FET show increased uptake in only 60–80 % of low-grade gliomas and that the resection of the tumors with low amino acid uptake cannot be improved. On the other hand, there is some evidence that low or even absent amino acid uptake in low-grade glioma is associated with a favorable prognosis and it is questionable whether early resection of these tumors is recommendable [19].

The improved imaging of glioma tissue using amino acid PET has also been applied to improve planning of radiation treatment of brain tumors [25]. A number of centers have started to integrate amino acid imaging into CT- and MRI-based radiotherapy planning, particularly in high-grade gliomas and when high-precision radiotherapy is to be given or in the setting of dose-escalation studies or for the re-irradiation of recurrent tumors [64–68]. Experiences with amino acid PET radiotherapy planning of low-grade gliomas are limited but indicate improved sensitivity in detecting postoperative residual tumor and a benefit for radiotherapy planning in patients with inconclusive MRI findings [26]. Improved outcome of the patients with radiotherapy planning by amino acid imaging compared with conventional therapy planning, however, has not yet been proven.

Glioma Grading and Prognosis

FDG PET is considered as a relative accurate predictor of tumor grade and prognosis of cerebral gliomas, and the detection of foci with increased FDG uptake in low-grade glioma is highly suspicious for malignant transformation [15]. This, however, is often associated with contrast enhancement in MRI so that the additional value of FDG PET in this situation is limited. Most PET studies employing amino acids have shown that gliomas of different WHO grades overlap in their degree of amino acid uptake, so that the tumor grade cannot be reliably predicted with this technique [12, 14, 16, 17]. A high potential to differentiate high-grade and low-grade gliomas has also been claimed for FLT, but FLT uptake goes along with BBB disruption and there is a high fraction of anaplastic astrocytoma without significant contrast enhancement on MRI which consecutively are negative in FLT PET [16].

Using FET PET, a number of studies have demonstrated that the evaluation of tracer kinetics in the tumors may be helpful for tumor grading [17, 18, 69, 70]. High-grade gliomas are characterized by an early peak of the time–activity curve around 10–15 min after tracer injection followed by a decrease of FET uptake. In contrast, time–activity curves slightly and steadily increase in low-grade gliomas of WHO grade II. Using dynamic evaluation of selected regions of the tumor, high-grade and low-grade gliomas could be distinguished with an accuracy > 90 % in primary tumors as well as in recurrent tumors [17, 18, 70]. A study using MET demonstrated that unlike FET PET, the uptake characteristics of MET PET do not allow the classification of low- and high-grade tumors on an individual patient basis [71].

Considering gliomas of all WHO grades, the prognostic significance of amino acid uptake remains a matter of controversy. Some studies seem to show that lower amino acid uptake especially in astrocytic glioma is associated with a better prognosis, but there is generally high uptake in oligodendroglioma in spite of their apparently better prognosis [14, 72]. Obviously, the oligodendrocytic gliomas are a different entity with respect to amino acid transport characteristics.

There appears to be, however, an important clinical role of amino acid PET in prognostication for patients with low-grade gliomas. Some of these patients will enjoy a stable course with an excellent quality of life for many years or a decade even without treatment, while others experience rapid tumor progression with malignant transformation to a high-grade glioma and a poor prognosis. A better identification of individuals with either a poor or a favorable prognosis is highly desirable to optimize treatment. A study with MET PET showed that these patients benefit from a surgical procedure only when increased amino acid uptake can be demonstrated [20]. In a series of 24 patients with low-grade gliomas, patients with a tumor/brain ratio > 2.2 had a significantly shorter survival time than the patients with a tumor/brain ratio < 2.2 [21].

Another study revealed the combined evaluation of FET PET and MR morphology as a statistically significant prognostic predictor for patients with newly diagnosed low-grade gliomas [19]. Within a 7-year period, a group of 33 consecutive patients with previously untreated non-enhancing WHO grade II glioma were included in a prospective study. A baseline, both MRI and FET PET were performed before histology in all patients on tissue samples by biopsy, and a "watch and wait" strategy without further treatment was started. During the follow-up, it turned out that baseline FET uptake and a circumscribed versus a diffuse growth pattern on MRI were highly significant predictors for patients' course and outcome: Those low-grade gliomas that were well delineated on MRI and showed no FET uptake had an excellent prognosis with long progression-free intervals, good clinical condition, and late malignant transformation. In contrast, patients with low-grade gliomas with diffuse tumor margins on T2-weighted MRI and FET uptake had a poor outcome with early progression in combination with malignant transformation to an HGG, rapid clinical deterioration, and die earlier. Thus, combined assessment with FET PET and MRI can identify a subgroup of patients with "stable" low-grade gliomas who may be best "treated" with "a watch and wait" strategy and another subgroup of patients with "instable" low-grade gliomas who should receive early and aggressive treatment to avoid malignant transformation.

The Diagnostic Assessment of Recurrent Tumors

Early detection of recurrent tumor is of particular interest. It is difficult to distinguish recurrent glioma from nonspecific post-therapeutic changes with conventional MRI alone, because pathological contrast enhancement may reflect either regrowth of tumor or tissue necrosis after radio- or chemotherapy [73]. Furthermore, contrast enhancement is usually missing in recurrent low-grade gliomas and MRI cannot differentiate between tumor, edema, and nonspecific postoperative changes, unless a mass effect or distinct bloating of cortex or other gray matter structures are seen [6]. The potential of FDG-PET in differentiating tumor recurrence from radionecrosis is limited because of the higher frequency of nonspecific uptake [74]. Multiple studies have shown that MET PET is highly sensitive to detect recurrent tumor but the specificity for the differentiation of vital tumor tissue from nonneoplastic changes is not optimal and in the range of 70 % [14]. The specificity of FET PET for the differentiation of recurrent tumor from nonneoplastic changes appears to be higher than that of MET PET. In a study involving 46 patients, the sensitivity and specificity of FET PET for the detection of recurrent gliomas were 100 and 93 %, respectively, compared with 93 and 50 %, respectively for MRI [75]. Thus, especially FET PET is considered as a valuable tool in differentiating recurrent tumor from nonneoplastic changes.

Monitoring of Radio- and Chemotherapy

Imaging for radiological response assessment in low-grade gliomas is based on serial measurements of T1- and T2-weighted MRI. Low-grade gliomas usually show no contrast enhancement on MRI due to an intact BBB, and the diffusely infiltrative nature of these tumors makes the assessment of tumor boundaries difficult. Changes in apparent tumor size that are seen in MRI are taken as indicators of the response to therapy but this approach is limited by the difficulty in distinguishing vital tumor tissue and unspecific treatment effects. The feasibility and usefulness of PET for treatment assessment and follow-up in cerebral gliomas of all grades of malignancy after radiotherapy and chemotherapy have been explored in multiple studies, and the diagnostic accuracy compared with conventional MRI is considered to be very efficient.

The current experience concerning treatment monitoring in brain tumors with PET is based mainly on the data obtained in patients with high-grade gliomas. Several studies evaluated the role of amino acid PET using MET and FET in patients with high-grade gliomas to monitor external fractionated radiation therapy [76]; treatment effects during standard chemotherapy regimen, i.e., adjuvant temozolomide [77] or chemotherapy with procarbazine, CCNU, and vincristine (PCV) [78];

dose-intensified chemotherapy with temozolomide [79]; and experimental treatment such as intracavitary radioimmunotherapy [80], convection-enhanced delivery of paclitaxel [81], tyrosine kinase inhibitor treatment [82], and antiangiogenic treatment with bevacizumab [83]. The currently available data suggest that a reduction of the tumor to brain ratio of amino acid uptake and the metabolic volume of high-grade glioma is a sign of a response to treatment.

Besides cytoreductive surgery, local radiotherapy is an important treatment option especially in patients with an astrocytoma WHO grade II. The possibility of late side effects of radiotherapy (e.g., neurotoxicity) in this group of patients with a much longer life expectancy makes it necessary to identify groups that benefit from early radiotherapy compared with those in whom radiotherapy should be delayed until the time of tumor progression. The role of MET PET has been evaluated in a small number of retrospective studies, mostly in comparison to FDG PET. Roelcke and coworkers evaluated the effects of postoperative external fractionated radiotherapy using MET and FDG PET in patients with an astrocytoma WHO grade II [30]. Tracer uptake was assessed by tumor to brain ratios during follow-up and at the time of first tumor progression, and was not significantly different in patients who received external radiotherapy after tumor resection ($n=13$) in comparison to patients treated with surgery alone ($n=17$). Different results could be observed in brachytherapy after implantation of ^{125}I seeds. One year after seed implantation, FDG uptake did not change in patients with low-grade glioma, but a significant decline of MET uptake was detected [31, 32] indicating that MET PET may provide more information on therapeutic effects than FDG following brachytherapy. The different results of these studies may be explained by different follow-up times and radiotherapy modalities used in the study protocols.

In order to assess the response to chemotherapy using amino acid PET in patients with low-grade gliomas, several studies with limited numbers of patients have been performed. In a prospective study, FET PET was used to evaluate the response to an intensified temozolomide regimen in 11 patients with progressive non-enhancing low-grade glioma WHO grade II [33].

After initiation of treatment, the authors compared the reduction of the metabolically active tumor volume as assessed by FET PET with the reduction of the tumor volume delineated by fluid-attenuated inversion recovery-weighted (FLAIR) MR images. In patients who showed a clinical response, a reduction of the metabolically active tumor volume after initiation of treatment could be observed in FET PET earlier than volume reductions on FLAIR MRI sequences (see Fig. 16.3). In a retrospective study, the effect

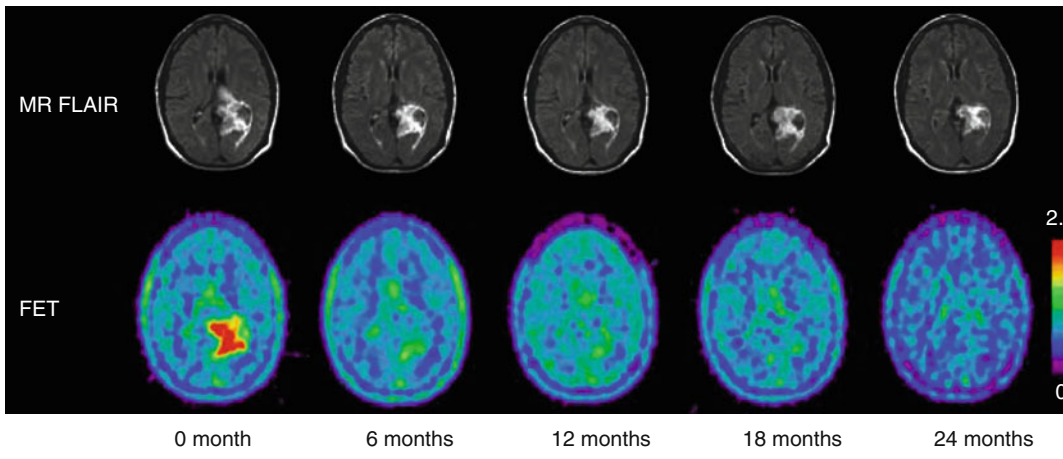

Fig. 16.3 Patient with an oligoastrocytoma WHO grade II during chemotherapy with temozolomide. FET PET identifies response to treatment at an early stage of disease, while T2-weighted MRI remains ambiguous [33] (Reproduced, with permission, from Springer Science + Business)

of PCV chemotherapy was examined using MET PET in seven patients with an oligodendroglioma WHO grade II [34]. Similar to the above-mentioned study, changes of tumor volume in MRI FLAIR sequences and metabolically active tumor volume derived from MET PET were monitored. MRI FLAIR and MET PET provided concordant information on tumor to PCV treatment, but MET PET was found to be more sensitive for the assessment of PCV responsiveness.

The findings indicate the sensitivity of amino acid PET for detecting early treatment response in low-grade gliomas. Furthermore, the early identification of nonresponders may help to minimize negative impact of chemotherapy on quality of life.

Perspectives for PET in the Management of Low-Grade Gliomas

Diagnostic assessment of diffuse low-grade gliomas by PET using radiolabeled amino acids permits a more specific representation of the spatial extent of the tumors than is possible by conventional MRI alone. This has been shown to be advantageous for the planning of biopsies, resections, and radiotherapy. Valuable prognostic information can be obtained at initial diagnosis to optimize individual treatment strategy, and the treatment response can probably be judged early in the course of treatment. Recurrent tumors can be differentiated from post-therapeutic changes with a high degree of specificity. Other imaging methods like proton magnetic resonance spectroscopy (MRS) may also yield metabolic information that is markedly more specific than that obtainable by conventional MRI for the differentiation of tumor tissue from nonspecific changes [84]. A relationship between increased FET uptake and abnormal high choline concentration in gliomas as measured by MRS has been demonstrated [85]. Unlike PET, however, MRS can only be used to analyze selected small volumes or partial areas in single planes, and the quality of the studies is often impaired by susceptibility artifacts. Furthermore, diffusion-weighted imaging (DWI) has been considered, but the clinical relevance of this methods is not yet established [86]. Other techniques like perfusion-weighted MRI are more easily available than PET and may yield information that is correlated with the degree of malignancy of gliomas [84]. The diagnostic accuracy of this technique in comparison with amino acid PET, however, remains to be investigated.

Diffusion tensor imaging (DTI) can contribute valuable diagnostic information on the involvement of white matter structures (e.g., nerve pathways). First studies have demonstrated the potential benefit of integrating fiber tracking by DTI and FET PET [87, 88]. These studies indicated complementary information and more detailed understanding of peritumoral fiber tract alterations in gliomas, which are more complex as described so far.

The scientifically documented utility of amino acid PET of low-grade gliomas seems to justify its introduction as a routine diagnostic technique for certain indications, but it remains to be confirmed that this will improve the overall quality of care. The guidelines of the European and the German Association of Nuclear Medicine for brain tumor imaging using labeled amino acid analogues have been published in recent years [89, 90]. The logistical prerequisites for amino acid PET have become markedly less difficult to achieve in recent years with the introduction of FET PET, and more than 25 hospitals in Germany and Austria have already integrated this approach into the routine diagnostics of patients with brain tumors (Personal inquiry, K.-J. Langen 2012). Applications for approval by National Food and Drug Administration are under way. The benefit of amino acid PET in cerebral gliomas appears to be well justified by its clinical utility since the costs of PET imaging are relatively small in relation to the expenses of local or systemic treatment approaches and, consecutively, the management of possible adverse effects. The information provided by amino acid PET assists to optimize the individual treatment strategy and to minimize negative impact of treatment approaches on quality of life. The future will also be strongly influenced by the integration of PET and MRI in one imaging device [91]. The advent of hybrid

PET-MRI systems offers a multimodal approach for the investigation of brain tumors and improved patient comfort due to a significant reduction in measurement time and improved spatial and temporal co-registration of PET and MRI data.

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Dynamics of DLGG and Clinical Implications

17

Emmanuel Mandonnet

Abstract

Diffuse low-grade glioma is defined by a common morphotype on histological analysis. However, these tumors exhibit a wide heterogeneity in their degree of biological aggressivity, resulting in a large range of survival times. Several factors at diagnosis have been found to identify different subgroups of prognosis, but there is growing evidence that the intrinsic dynamics of the tumor – that can be evaluated over a short initial follow-up – plays a major role in predicting overall prognosis at an individual scale. We will review our current knowledge of DLGG dynamics on molecular, histological, radiological, and clinical scales. In particular, we emphasize the importance of estimating the initial radiological dynamics from two successive morphological MRI. Finally, we describe how the spontaneous dynamics can be modified by the different treatment modalities, including surgery, chemotherapy, and radiotherapy.

Keywords

Diffuse low-grade glioma • Tumor dynamics • Tumor kinetics • Growth rate

The histological definition of diffuse low-grade glioma (DLGG) actually comprises a wide spectrum of tumoral behaviors. This apparent diversity of tumoral biodynamics, coupled to differing levels of sensitivity to treatments, explains the wide heterogeneity of DLGG survival. In this chapter, we propose to review our current understanding of DLGG dynamics, to study how to determine individual dynamics, and to analyze how this information can be integrated in the decision process of personalizing treatments sequences.

Multiscale Multimodality Approach of Pretreatment Longitudinal Follow-Up

The Cellular and Subcellular Scales

Despite considerable advances in the understanding of molecular biology in cancer, very little is known about the *sequence* of cellular and

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molecular events underpinning the tumorigenesis and tumor growth in DLGG. First, the cell of origin, if it exists, remains supputative (Cf chapter 6). Second, the very first mutations leading to the transformation of the cells of origin, and then to the indolent proliferation in the initial low-grade phase, are also not elucidated. It is however believed that IDH mutation [65] is an early event in DLGG, preceding the 1p-19q codeletion in oligodendroglioma and the p53 mutation in astrocytoma, as shown in a study with biopsy samples performed longitudinally [63, 64]. For oligodendroglioma, it has been recently shown that they exhibit a modest and stable somatic mutational load, CIC being the sole gene very frequently (close to 70 %) mutated in tumors with IDH mutation and 1p-19q codeletion [4, 66]. On the contrary, in astrocytoma, tumor cells with p53 mutations acquire a growth advantage, with an increasing percentage of mutated cells on longitudinal samples [25], a phenomena that could contribute to increase the genomic instability over time, possibly through an early methylation of MGMT [18, 39], and later in time of DNMT1 [18]. This difference in genomic instability between 1p-19q-deleted oligodendroglioma and p53-mutated astrocytoma probably contributes to explain the difference of prognosis between these two kinds of tumors. Finally, it should be noted that most studies focused on genetic changes observed at malignant progression; hence, there is a need of more studies exploring the genetic dynamics between two longitudinal biopsies after repeated surgery [35] for a recurrent DLGG, before any malignant transformation.

The Histological Scale

Historically, neuropathologists were among the first to attempt to picture some elements of glioma growth and invasion dynamics. Their analysis entirely relied on the ultimate snapshot of the tumor, that is, the postmortem specimen. This method enabled, for example, to describe the preferential extension of glioma along white matter tracts [36, 57].

Daumas-Duport introduced an histological classification of oligodendroglioma (low-grade astrocytoma do not exist in her classification, because astrocytes are interpreted as being always reactive to the oligodendroglial tumor, see chapter 3), based on the spatial organization of the cells [14]: most low-grade glioma belong to the type III, composed of isolated tumor cells (ITC), while some others do exhibit both solid tumor tissue and ITC (type II). Interestingly, when endothelial hyperplasia and/or a contrast enhancement is observed on MRI with gadolinium [13], the tumor structure is always a mixture of solid tumor tissue and ITC (type II). This mixed architecture thus probably constitutes a more malignant stage of the disease. Of course histological analysis gives only a single snapshot of the dynamics, and one cannot know whether the type II arises later in time from a type III or from a type I (composed only by solid tumor tissue). However, the fact that there exist some foci of higher tumor cell density and minute microangiogenesis in about 15 % of type III oligodendroglioma [14] supports the idea that the transition from a type III toward a type II usually occurs, with a likely continuous transition between these two types. Moreover, it has been shown that ITC can be detected, at a very low density, outside the area of T2 hypersignal on MRI [46] (confirming that there exists a cell density threshold of radiological visibility, see Chap. 28). This structure of low-density radiologically non-visible part of the DLGG could be named type IV. Thus, it can be hypothesized that surrounding areas of type IV progressively evolve toward radiologically visible type III and within the type III zone, anaplastic micro-foci arise,[1] whose further growth and coalescence ultimately leads to a type II.

[1]We do not know whether this is a random process or whether some biological law governs the spatiotemporal dynamics of these events.

The MRI Follow-Up: A Three-Period Story

The Silent Phase

This phase corresponds to the "hidden" tumor life, from its biological birth to its clinical revelation (most often by a seizure). Very little is known about this part of the story. However, with the increasing availability of magnetic resonance imaging, these tumors are now more frequently discovered incidentally. Two series have been reported in the literature [44, 52], establishing that incidental DLGG present a continuous and spontaneous radiological growth during the silent period preceding their clinical revelation. Interestingly, growth rates were found very close to those reported in symptomatic patients, with a median growth rate at 3.9 mm/year in one study and 3 mm/year in the other [44, 48, 52].

These results suggest that the growth rate measured in a symptomatic patient is a good estimate of what it was during the silent phase. In other words, one can extrapolate backward in time the growth curve of the diameter, leading to an estimation of the radiological birthdate of the glioma, through the formula d_0/v, d_0 being the diameter at first MRI and v the estimated velocity of diameter expansion (VDE) during the silent phase. Of course, this procedure assumes that the dynamics properties of the glioma remained unchanged during the whole silent phase, a strong hypothesis which cannot be verified. However, it has been possible for a specific patient to show that the estimated date of the radiological birthdate was not unrealistic: in this case, an old MRI without any lesion was available, and the date of this MRI was indeed anterior to the predicted date of radiological birthdate [15].

The "Low-Grade" Phase

Qualitative Follow-Up

Radiologically, the analysis of the tumor shape can already yield some information regarding the growth pattern of DLGG. Chen et al. [10] proposed a simple classification: tumors originating from the gray matter will remain bulky, without definite involvement of white matter while continuously growing (the so-called expansive growth), whereas tumors originating at the junction of gray and white matters will grow predominantly along the adjacent white matter fiber tracts. While we agree that some tumors look radiologically much more bulky than others, we do not believe that DLGG remain restricted to the gray matter. They always invade the adjacent white matter, along the U-shape fibers, joining two successive gyri. This radiologically bulky phenotype should not be confused with the histological Daumas-Duport type II: indeed, a type III DLGG may appear bulky on MRI (see example in Daumas-Duport et al. [14]). Interestingly, we noted that in the course of the postoperative period, the radiological bulky phenotype might shift toward a more diffuse one (see Fig. 17.1).

Several reports have also illustrated that the shape of DLGG on MRI is imposed by the architecture of white matter fiber tracts. It is indeed well known that projecting or associative pathways constitute a major road of tumor cells invasion for high grade glioma [36, 40, 57]. For DLGG, a similarity between the tumor shape and the anatomical description of fiber tracts has been reported [28, 32]: in particular, para-insular tumors may extend along the uncinate fasciculus, the arcuate fasciculus, or the inferior fronto-occipital fasciculus (IFOF), and the sagittal stratum (corresponding to the merging of IFOF and optic tracts).

Moreover, a longitudinal radiological study demonstrated on a series of 16 patients the preferential extension along these tracts [32], as it has been also confirmed by computational simulations based on a biomathematical model of tumor growth [26]. Of note, if it is clear that white matter pathways can facilitate tumor cells invasion within a tract, one could also imagine that the interface between two orthogonal pathways acts as a barrier against cells invasion. This phenomenon has been less investigated in the literature.

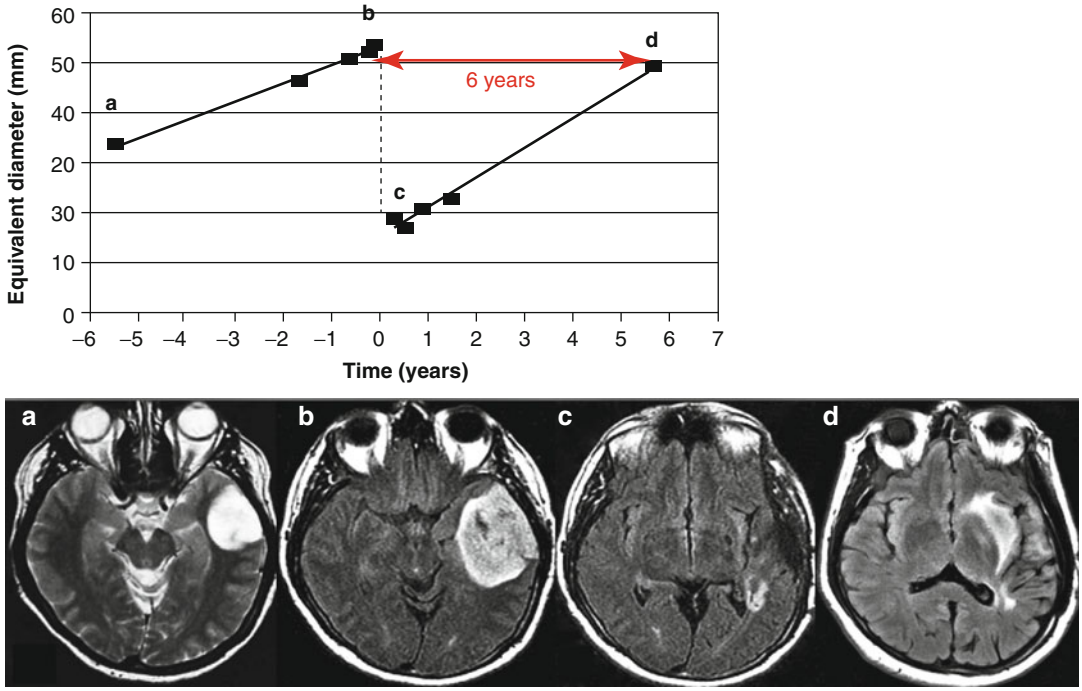

Fig. 17.1 Illustrative case of pre-versus postoperative growth rates. Note that one can estimate the oncological gain of surgery: the re-evolution curve is translated by about 6 years. Note also the evolution of radiological phenotype, from bulky preoperatively to diffuse postoperatively

Quantitative Follow-Up

The old belief that DLGG may alternate indolent and growing periods is still alive, although not supported by any relevant studies. A probable explanation is that a minor increase of tumor diameter is difficult to identify just by "eyeball" qualitative comparison of two consecutive MRI (see Fig. 17.2). Tumor diameter can be measured by different techniques, either with linear measurements of one, two, or three diameters, or it can be deduced from a full 3D volumetric segmentation. It has been clearly evidenced that estimating tumor size by segmenting (manually or semiautomatically) each axial slice on a computer reduces greatly the intra- and inter-reader variability [61]. Indeed, as stated in van den Bent [62], DLGG are often irregular in shape and grow anisotropically, resulting in poor reproducibility of area or volume estimation based on linear measurements. The increasing availability of softwares allowing to perform segmentations on DICOM images (be they on dedicated stations in neuroradiology and radiotherapy department or even on PC – e.g., Osirix and ImageJ) renders any other technique based on one, two, or three diameters measurements old fashioned. Segmented volumes are then converted in diameters, by computing a *volumetry-based diameter* (d_v):

$$d_V = (2 \times V)^{1/3}$$

Finally, the growth curve of this equivalent diameter can be plotted over time, and the growth rate of the radiological diameter d_V can be estimated for each patient from a simple linear regression, giving the velocity of diametric expansion (VDE).

This methodology allowed to prove that DLGG are not radiologically stable. Mandonnet et al. [31] first showed quantitatively the spontaneous radiological growth of DLGG on successive MRIs in a subset of 27 histologically proven DLGG that were followed before oncological treatment. The average VDE was close to 4 mm/year, with a minimum at 2 mm/year. These initial

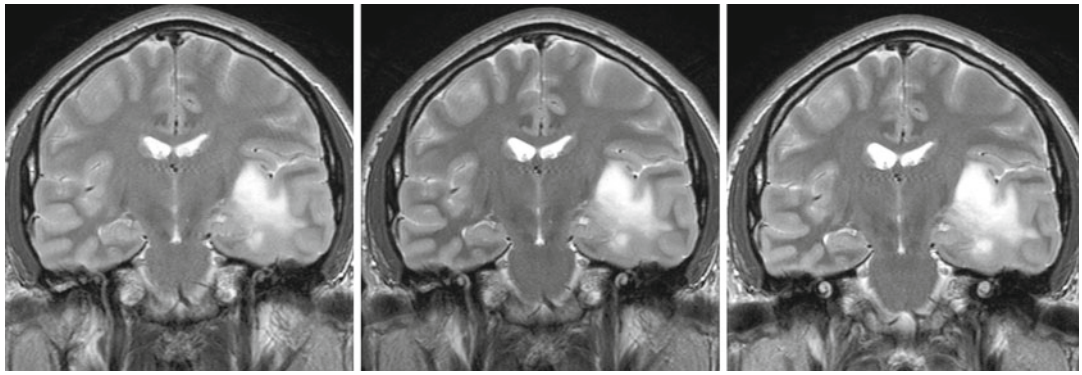

Fig. 17.2 Eyeball versus quantitative estimation of growth. From *left to right*, three successive MRIs of the same patient, each separated by 9 months. Whereas growth is difficult to assess visually on two successive exams, quantitative measurements by full 3D segmentation reveal a 4 mm/year growth rates

results were confirmed by the same group on a larger series of 143 histologically proven DLGG that ranged individual VDE from 1 to 36 mm/year [41], with a median VDE at 4.4 mm/year. In the series of Ricard et al. [55], the 39 patients with an available follow-up before chemotherapy also had a median VDE of 4.4 mm/year. Several independent groups have recently confirmed these results in series of patients harboring histologically proven supratentorial DLGG, as summarized in Table 17.1:

- Brasil Caseiras et al. [6] found that all patients exhibited a volume increase over a 6-month period (minimum of 1.9 mL) in a series of 34 patients. However, this group did not convert their volumic growth rates in terms of VDE, precluding to make any comparison with other series.
- Hlaihel et al. [24] reported a median VDE of 3.5 mm/year in a series of 21 patients.
- Peyre et al. [50] measured a minimal VDE of 2.2 mm/year in a subseries of 13 patients with an available follow-up before chemotherapy, with an average VDE at 5.5 mm/year.
- Pallud et al. [46] found that all of the eight studied DLGG with pretreatment imaging follow-up experienced an increase of their diameter, the minimal VDE being at 1.1 mm/year, with a median value at 3.3 mm/year. The same group reported, on another series of 19 patients, a median VDE of 4.5 mm/year, with a minimal value of 0.6 mm/year [47].

Table 17.1 Distribution of the velocity of diametric expansion in series of patients harboring a LGG, as reported by different groups

Authors	Cases (n)	Median VDE (mm/year)	Range (mm/year)	Median follow-up (years)
Mandonnet et al. 2003 [31]	27	4.1	2–8	4.75
Pallud et al. 2006 [41]	143	4.4	1–36	1.8
Ricard et al. 2007 [55]	39	4.76	–	3.6
Brasil-Caseiras et al. 2009 [6]	34	volumic increase		0.5
Hlaihel et al. 2010 [24]	21	3.65	–	1.9
Peyre et al. 2010 [50]	13	5.5	2.2–21.4	–
Pallud et al. 2010 [46]	8	3.3	1.1–3.7	–
Goze et al. 2012 [19]	64	3.5	0–24.3	0.8
Pallud et al. 2012 [47]	19	4.5	0.6–16.9	0.7

- Goze et al. [19] also reported a median VDE of 3.5 mm/year in a series of 64 patients. In this series, it seems that at least one patient presented a null VDE, but this could be due to the very short follow-up (median of 0.8 years, with a minimum of 0.25 years).

Finally, as already stated, two studies have evidenced a continuous growth of diameter in series of incidentally discovered DLGG:

- Pallud et al. [44] reported an average VDE of 3.9 mm/year in a series of 47 patients.
- Potts et al. measured volumic increase in eight patients with initial follow-up. After conversion to VDE [48], this yielded a value close to 3 mm/year.

Thus, these quantitative studies never reported a case of an indolent untreated DLGG with a stable tumor volume and a null VDE or a case of an untreated DLGG alternating indolent and growing periods. In summary, DLGG present a systematic, spontaneous, and continuous radiological growth (although sometimes as slow as 1 mm/year), before any transformation into a higher grade of malignancy.

The Transition Toward Higher Grade

The transition toward a glioma of higher grade is a somehow unforeseeable event, albeit unavoidable, in the natural history of a DLGG. It has been well proven that the greater the initial tumor volume (or its residue after surgery), the higher the risk of imminent anaplastic transformation. Whereas the reference definition of anaplastic transformation is based on the histological criteria of a grade III or IV glioma, it is now widely admitted that it can be also diagnosed by *the appearance in the longitudinal follow-up* of a new contrast-enhanced nodule on T1-gado MRI.

As it will be shown later, a VDE greater than 8 mm/year on initial follow-up is highly suggestive of an imminent malignant transformation. However, for DLGG with an initial growth rate at 4 mm/year, it has never been shown whether malignant progression is preceded by an increase of the growth rate or not. This point will be further discussed in Chap. 28. Advances in modern imaging methods,[2] including perfusion MRI and spectroscopic MRI, when performed at initial diagnosis, can yield valuable data regarding the anaplastic risk of an individual tumor [54], as detailed in the chapter 15. The aim of this paragraph is to put these results in a dynamic perspective and to emphasize what additional information can be gained from a longitudinal application of these techniques.

Perfusion MRI

Several studies have shown the interest of the value of rCBV max at the initial perfusion-weighted MRI in predicting malignant transformation or even overall survival. All studies evidenced a threshold value in the range 1.7–2.2 [12, 22, 27, 29]. However, on an individual basis, the prognostic value is limited by the fact that it has been established that oligodendroglioma exhibit a greater rCBV max than astrocytoma [5, 7, 30]. One longitudinal study proved that an annual rate of rCBV max increase greater than 2/year is predictive for the appearance of contrast enhancement within the next 6–12 months [12]. Thus, a high value and/or a rapid increase of rCBV max precede by 6–12 months the onset of contrast enhancement.

Metabolic MRI

The advent of spectroscopic MRI enabled to measure noninvasively the concentrations of some molecules of metabolic interest, offering to track metabolic changes sustaining the transition toward malignancy. For example, it has been clearly shown that the choline levels correlate with increased cellular density and proliferation rate [17, 38]. To our knowledge, there are very few studies in the literature analyzing longitudinal datasets of spectroscopic MRIs for DLGG patients. Whereas for treated patients spectroscopic data seem to amplify the volumetric evolution [23, 24], studies in untreated patients provided diverging results regarding the possibility

[2]PET imaging with different molecular markers will not be discussed here considering the limited number of longitudinal studies. We refer the reader to the PET imaging chapter for the interest of these techniques in DLGG.

to predict anaplastic transformation (see Price [54] for a review). This can be explained by methodological limitations, like the variability of the spatial location of the ROI in the mono-voxel technique [2] – a limitation that should be overcome by the multivoxels technique [38]. An alternative explanation will be proposed in Chap. 28.

With the discovery of high frequency of IDH mutations in oligodendroglioma, there has been a regain of interest for the study of glycolysis and oxidative phosphorylation in tumorigenesis. The lactate resonance is supposed to be a surrogate marker of a glycolytic switch, decoupling the glycolysis from oxidative phosphorylation in the tricarboxylic cycle [51]. Not surprisingly, no lactate resonance is observed for DLGG with a proliferation index inferior to 4 %, whereas a lactate peak is detected when proliferation index is comprised between 4 and 8 % [20]. Interestingly, the lactate resonance is no more evidenced for tumors with a proliferative index greater than 8 %. As explained by a mathematical model [21], this could be linked to the combined effect of MCT overexpression (excreting lactate out of the cells) and increased cerebral blood flow, washing out the lactate through the capillaries. An alternative explanation would come from the theory of metabolic symbionts [51]: the lactate produced by glycolysis in hypoxic cells would be "recycled" by normoxic cells, through the transformation in pyruvate (then entering the tricarbonic acid cycle for oxidative phosphorylation). Hence, one should keep in mind a dynamic view of the tumoral metabolism when analyzing the lactate peak on spectroscopic MRI.

The Cognitive Follow-Up

A striking feature of DLGG patients is that they do not have any focal neurological deficit. This means that brain networks plasticity can cope with lesions growing up to 4 mm/year, without major consequence on motor or language function. However, studies with extensive assessment of cognitive status have evidenced that healthy controls scored better than DLGG patients [1]. Hence, it can be suspected that the decline in cognitive status of DLGG patients is a continuous process during the silent and low-grade period, as for the MRI evolution. However, very few studies have focused on the longitudinal cognitive follow-up of "wait-and-see" cohorts. Indeed, only one longitudinal study has been recently published, showing a worsening in non verbal delay recall scores after a 1 year "wait-and-see" follow-up [11]. Of course, at malignant transformation, the brain plasticity capabilities are overwhelmed by the fast growing tumor (VDE greater than 8 mm/year). At that time, focal neurological deficits, together with an increased seizures frequency, are commonly observed. All together, one can assume that the tumoral dynamics (as measured by the VDE) should be correlated with the dynamics of cognitive deterioration: the higher the tumor growth rate, the faster the cognitive deterioration. This hypothesis would deserve more clinical studies.

Factors Influencing DLGG Growth Rates

First of all, the different DLGG histological subtypes (oligodendroglioma, astrocytoma, mixed glioma) do not exhibit significant differences regarding the radiological tumor growth rates, as previously demonstrated in several studies [6, 33, 41, 55].

Two studies have investigated the link between DLGG genetic subtypes. In the first one, it was shown that DLGG with 1p-19q codeletion grew significantly slower than DLGG without (median VDE at 3.4 mm/year) and that DLGG with immunohistochemical overexpression of p53 grew significantly faster than DLGG without (median VDE at 4.2 mm/year). The second study [19] confirmed that growth rates of DLGG are lower when 1p-19 codeletion is present, whereas IDH status does not influence growth rates. This suggests that the favorable outcome of 1p-19q codeleted patients might be in part related to a tumor inherently more indolent, and that on the contrary, the good prognostic value of IDH mutation could result from a

better efficacy of treatments or a lesser risk of malignant progression.

Only one study investigated quantitatively the effects of pregnancy on the radiological growth rates of DLGG [42, 45]. The results showed that DLGG accelerated significantly their radiological growth rates during pregnancy, above levels detected either before pregnancy or after delivery in 75 % of cases. These changes in tumor growth were associated with an increase in seizure frequency in 40 % of cases and radiological and clinical changes during pregnancy motivated further oncological treatment after delivery in 25 % of cases. These results also underline that young women with DLGG should be informed of the oncogenic role of pregnancy.

The Prognostic Value of Pretreatment VDE

The first study focusing on the prognostic significance of spontaneous MRI growth rates on overall survival was conducted on a retrospective series of 143 DLGG with measurements of the evolution of the diameter over time [41]. Overall survival was significantly higher in the low growth rates subgroup (median survival of 15 years for a VDE lower than 8 mm/year) than in the high growth rates subgroup (median survival of 5.6 years for a VDE at 8 mm/year or more).

The prognostic significance of spontaneous MRI growth rates on predicting progression into a higher grade of malignancy was addressed in two recent prospective studies. Brasil Caseiras et al. [6] proved in a series of 34 patients that "tumor growth within 6 months was better than baseline volumes, relative cerebral blood volume, or apparent diffusion coefficient in predicting time to malignant transformation in untreated DLGGs and was independent of other parameters." They found a threshold of 6.21 mL of growth within 6 months, with a mean time of progression of 3.91 years versus 1.82 years. However, the prognostic significance of the evolution of the tumor volume (and not diameter) over time may be a combination of two independent other prognostic factors, the initial tumor volume and the tumor growth rate [43]. Or stated differently, the same amount of volumic increase may correspond to a large tumor with a low growth rate (of diameter) or a small tumor with a high growth rate (of diameter) [43]. Thus, VDE, obtained by the evolution over time of the diameter (deduced from the total segmented volume *V*), appears as a more reliable parameter than the evolution of the tumor volume to assess selectively the prognostic significance of radiological tumor growth rates.

Hlaihel et al. confirmed these results and demonstrated that an elevated VDE higher than 3 mm/year was correlated with a greater risk of progression into a higher grade of malignancy, with an average VDE at 7.87 mm/year in transformers group versus an average VDE at 2.14 mm/year in non transformers group [24]. The VDE threshold at about 8 mm/year is thus as a strong predictor of both malignant progression-free and overall survivals.

Assessing Treatment Efficacy by Volumetry-Based Diameter Measurements: When Patients Can Serve as Their Own Controls

As DLGG are slow-growing progressive tumors, with considerable variability in patient characteristics and therapeutic modalities, the accurate evaluation of the different oncological treatments efficacy constitutes a clinical challenge. Along with clinical response – particularly on seizure frequency – we will show that the quantitative assessment of the individual VDE by diameter evolution over time on consecutive MRI is a useful adjunct in the follow-up armamentarium.

Surgery

Using the VDE methodology, it has been shown in a retrospective study of 54 DLGG patients that radiological tumor growth rates remain unchanged after surgical resection [33] (see Fig. 17.1 for a

typical case). This result reinforces the idea that the survival benefit of surgery is mediated by a cytoreductive effect, as already stated by several studies [3, 37, 53, 56, 59].

However, two patients of the 54 under study exhibited a decrease of their tumor growth rates greater than 3 mm/year whereas in two other patients, surgery failed to stop an ongoing anaplastic transformation, resulting in an increase of 3 mm/year on the tumor postoperative growth rates [33]. Thus, the precise quantitative assessment of VDE obtained pre- and postoperatively by repeated measurements of the diameter would help analyze the effects of surgical resection on an individual basis and should allow guiding the decision making of a postoperative oncological treatment.

Of note, these results highlight the inadequacy of progression-free survival as an endpoint in DLGG clinical trial. Indeed, this notion is ill defined after surgery, as there is no progression-free period in patients exhibiting a residual tumor after an incomplete resection.

Chemotherapy

Similarly, the tumor response to chemotherapy can be demonstrated quantitatively by tracking the diameter evolution. Ricard et al. [55] first quantified the tumor response after temozolomide chemotherapy in 107 DLGG. In addition, they evidenced different patterns of response, depending on the 1p-19q codeletion status:

- Almost all patients exhibited an initial decrease of the diameter after temozolomide onset.
- The median VDE after temozolomide onset was – 9.2 mm/year.
- Tumor relapse occurred more frequently and earlier in tumors without 1p-19q codeletion.

A similar study was recently performed to quantify the tumor response after PCV chemotherapy in 21 DLGG. Peyre et al. [50] demonstrated that:

- Tumor diameter decreased initially after PCV onset in all patients.
- The median diameter decrease after PCV onset was 10.2 mm/year, a value very close to the value reported by Ricard et al. [55] with temozolomide.
- An ongoing diameter decrease in 20 of the 21 patients after PCV discontinuation that was prolonged more than 2 years in 60 % of the DLGG under study.

These results demonstrate the same radiological quantitative response following TMZ and PCV chemotherapies. They challenge the idea that a prolonged duration of chemotherapy is necessary for treating DLGGs and raise the possibility of a chemotherapy monitoring based on the quantitative changes of the tumor diameter over time. As a consequence, VDE changes could be used as a quantitative reproducible parameter for the assessment of the response to chemotherapy for DLGG that is extremely difficult to judge using the McDonald criteria [49].

Radiotherapy

In a very recent study, VDE have been determined after radiation treatment. Pallud et al. studied a consecutive retrospective series of 32 adult supratentorial DLGG treated with first-line radiotherapy with an available imaging follow-up [47]. They demonstrated that:

- Diameter decreases initially after radiotherapy onset in all patients during a mean 49 months.
- The median VDE after radiotherapy onset was 16.7 mm/year, a value close to those reported after chemotherapy.
- The post-radiotherapy VDE carried a prognostic significance on overall survival as the fast post-radiotherapy tumor volume decrease (VDE at −10 mm/year or faster) was associated with a significant shorter OS (median 47.9 months) than the slow post-radiotherapy tumor volume decrease (VDE slower than −10.0 mm/year) (median 120.8 months). One hypothesis would be that fast responders had a tumor with high proliferation rate. Once the radiosensitive cells have been killed, the tumor shrinks according to an equally high apoptosis rate. But the radioresistant clone will recur quickly, in keeping with a high proliferation rate. This

phenomenon should be explored in a near future by biomathematical modeling.

Finally, the actual distinction between tumor tissue and radiation-induced changes appears sometimes difficult to ascertain and could limit the assessment of tumor response after radiotherapy by morphological MRI.

Clinical Applications

The spontaneous growth rate on MRI being a strong prognostic factor, it should be estimated systematically for each patient. This means that patients should get a second MRI before any oncological treatment, thus allowing the measurement of the VDE through the evolution of the diameter over time. This raises the issue of the intra- and interobservers reproducibility in measuring the VDE and how the interval between the first MRI at radiological discovery and the second MRI before oncological treatment could influence the reliability of the estimation. These issues have never been investigated. On one hand, a longer period would allow to enhance measurements reliability, but on the other hand, a too long period could put the patient at risk of progression to a higher grade of malignancy. Of course, in rare patients presenting with increased intracranial pressure and/or neurological deficit, treatment should be prompted, precluding to perform a second MRI after an observational period. On the contrary, for those DLGG incidentally discovered, an every 4 months follow-up has been proposed, with a threshold of VDE at 2 mm/year triggering surgical treatment [58]. Otherwise, we propose the following scheme according to Chang's score [8, 9] (based on four risk factors: presumed eloquent location, age > 50, KPS < 80, diameter > 4 cm):

- For patients presenting a Chang's score 3 or 4, the second MRI should be performed during a 6-week interval after radiological discovery. A diameter increase greater than 1 mm would then correspond to a VDE greater than 8.7 mm/year, suggesting tumor aggressiveness close to those of malignant gliomas. Treatment modalities and follow-up should be selected accordingly.
- For patients presenting with Chang's score 0–2, a 3-month interval is certainly reasonable, with an expected diameter increase of 1 mm corresponding to the average VDE of 4 mm/year. In a very small proportion of cases, a surprisingly higher diameter increase, greater than 2 mm, will be evidenced, leading again to consider the DLGG as of higher aggressiveness and risk of progression to a higher grade of malignancy.

As there is currently no mean to cure the patient, the principle of DLGG management consists in optimizing the sequence of the different treatment options, including surgery, chemotherapy, and radiotherapy, with the aim to delay malignant transformation while preserving quality of life [34]. To guide the physician in the decision-making process, three main parameters have to be included in the analysis: clinical status (and especially seizure frequency in these usually otherwise asymptomatic patients), tumor volume, and VDE. The principle is indeed to reduce as much as possible the tumoral volume (which is recognized as being an important predictive factor of malignant transformation), while preserving an optimal quality of life. Functional neurooncological surgery is thus the standard treatment as first line [16, 60]. Figure 17.3 illustrates how VDE can be integrated in the decision algorithm all along further follow-up. It is thus of utmost importance to assess quantitatively postoperative tumor volumes, with a first reference image at 2–3 months after the day of surgery, and then, a follow-up MRI every 3–6 months.

Conclusion and Perspectives

The better knowledge of DLGG dynamics, thanks to quantitative radiological follow-up, has greatly contributed to shift the treatment paradigm from a conservative one toward a proactive one. In the future, the dynamics on other scales should also be taken into consideration. Once a better picture of molecular, histological, and cognitive dynamics would be gained, an integrated view of DLGG dynamics should be analyzed for each patient. Then, treatment should be adequately selected

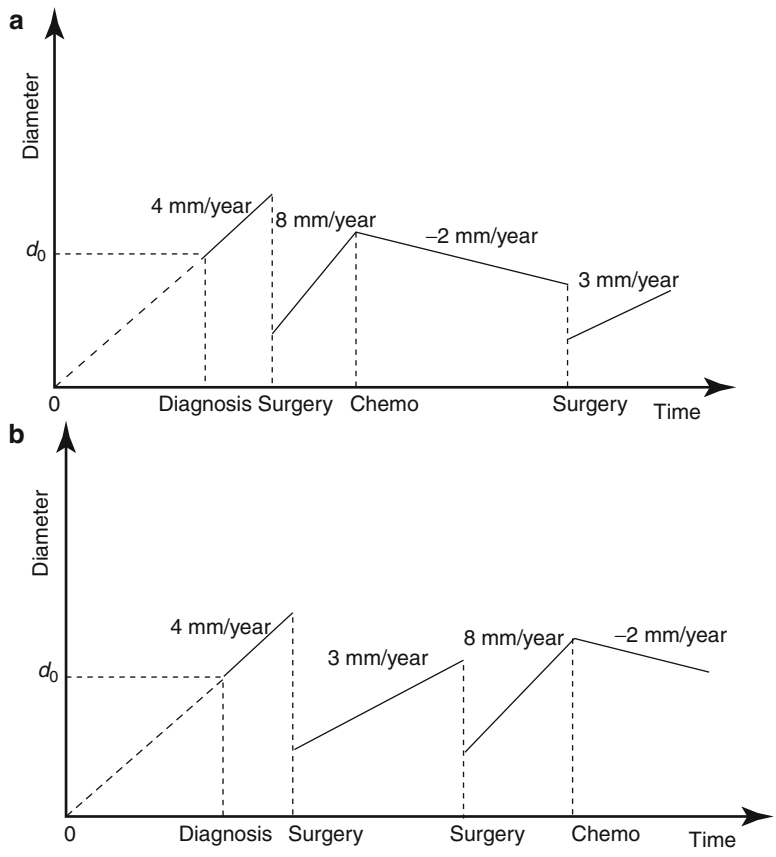

Fig. 17.3 Fitting treatment to individual dynamics. (**a**) After initial surgery, a patient exhibiting a VDE above 6 mm/year (and/or a substantial residual volume) is offered chemotherapy, with the aim to stabilize the volume (VDE = 0 mm/year) or even to shrink the residue (negative VDE), leading eventually to a second surgery. (**b**) A patient with a lower postoperative VDE can be clinically and radiologically watched, with the hope that brain plasticity will occur in these very slow-growing tumors, thus enabling in a second surgery to push the resection ahead in areas found to be eloquent at the functional mapping performed on first operation. During follow-up after second surgery, VDE becomes higher than 6 mm/year, and chemotherapy is administered, with the aim to slow down the growth rates

for each patient dynamics and adapted, all along the course of the disease, to dynamic changes.

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Part IV

Natural History and Spontaneous Prognostic Factors

Natural History and Spontaneous Prognostic Factors

18

Roberta Rudà, Luca Bertero, and Riccardo Soffietti

Abstract

Low-grade gliomas (LGGs) are a group of tumors with distinct clinical, histological, and molecular characteristics. The most common presenting symptom is represented by seizures that in MRI era occur in 70–90 % of patients with low-grade gliomas and are medically refractory in about 50 % of cases. LGGs typically affect young adults and are rare in elderly patients (>60 years). Occasionally, grade II glioma is discovered incidentally on brain imaging.

The natural history and patterns of care of LGGs have changed over time with an increase of survival, which is, at least in part, due to the earlier diagnosis afforded by CT and MRI. Overall the 5-year survival rates reported in recent randomized trials are in the order of 64–68 %. A number of retrospective and a few prospective series have evaluated variables of potential prognostic significance in patients with LGG. Some of these factors have been fully validated: age >40 years, presence of neurological deficits and/or absence of seizures at onset, low performance status (Karnofsky < 70), preoperative tumor diameter > 4–6 cm, astrocytoma as histology, while others still need validation (Table 18.1). Among molecular markers, 1p-19q codeletion and IDH1 mutation are the most important prognostic factors.

Based on the prognostic factors that emerged as significant after multivariate analysis among large, randomized multicenter trials, several prognostic scoring systems have been developed to identify subgroups of patients with different outcome (so-called low- and high-risk groups).

Keywords

Natural history • Prognostic factors • Age • Clinical presentation • Neuroimaging findings • Histology • Molecular markers • Prognostic scores

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Introduction

Low-grade gliomas (LGGs) are a group of tumors with distinct clinical, histological, and molecular characteristics. Although it is accepted that all

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grade I and II lesions according to WHO are low grade, the so-called diffuse LGGs include only grade II tumors that share similar invasive and malignant potential [1, 2]. Patients with LGG may survive for relatively long periods [3] but often progress to higher grade tumors, which are associated with neurological disability and invariably fatal. Thus, reference to these lesions as benign gliomas has generally been abandoned. The widespread availability of MRI has resulted in an earlier diagnosis for many patients who have few or no symptoms and a normal neurologic examination.

The optimal management of patients with LGG is still controversial: for physicians caring for patients with LGG, the challenge lies between providing too much therapy too early or too little too late [4].

Some clinico-radiological and pathological factors are clearly correlated with outcome, while others are still a matter of discussion. In the last decade, molecular biology is being increasingly helpful in identifying subsets of LGGs with better prognosis and increased chance of responding to therapy; additionally, advanced neuroimaging seems to allow a better prediction of malignant transformation, thus contributing to a better prediction of outcome.

Natural History

The most common presenting symptom is represented by seizures that in MRI era occur in 70–90 % of patients with low-grade gliomas and are medically refractory in about 50 % of cases [5]. There is no clear association between severity of epilepsy and behavior of the tumor. The advent of CT and MR imaging has shortened the duration and reduced the severity of symptoms at diagnosis. In a series of adult low-grade gliomas, diagnosed between 1979 and 1995, 78 % had seizures at presentation, 31 % focal neurologic deficits, 29 % headache, 11 % cognitive or behavioral changes, and 9 % papilledema, and the median interval between symptom onset and first imaging was 2 months [6].

Focal deficits, altered mental status, or increased intracranial pressure can still be presenting symptoms, especially in diffuse tumors.

Low-grade gliomas typically affect young adults; they are thought to be rare in elderly patients (>60 years), but very few specific studies have been performed in this population [7–10]. About 8–10 % of histologically verified LGGs occur at 60 years of age or greater [9, 11, 12]. This observation could be somewhat underestimated if one considers that elderly patients (particularly the very old) are less likely to undergo a biopsy when a suspicion of LGG is found on MRI.

In particular Kaloshi et al. [9] have compared the clinical, radiologic, pathologic, and therapeutic data of a series of 62 patients older than 60 years to those of 704 younger patients. The comparison between older and younger groups showed that elderly patients more often presented with a clinical deficit and a lower Karnofsky performance status. The lower frequency of seizures in the elderly (47 % vs. 85 %) could be due to a more aggressive tumor growth favoring deficits over seizures. On MRI the mean tumor diameter was significantly larger, the tumors more often infiltrated both hemispheres through the corpus callosum, and contrast enhancement was about twice more frequent in the older than in the younger group. The histologic diagnosis was similar, including the ratio of oligodendroglial versus astrocytic tumors, and the same was observed for 1p/19q codeletion. Not surprisingly, the older population had a lower rate of resection and radiation therapy; conversely some series did not find differences in the rate of aggressive resections (≥90 %) between the two age groups [13].

Rarely a grade II glioma is discovered incidentally on brain imaging although the detection of incidental gliomas will likely increase the access to brain imaging worldwide. The rate of incidental WHO grade II gliomas is in the order of 3–4.9 % [14–17]. Incidental grade II gliomas differ from symptomatic tumors in several aspects [17, 18]. There is a female predominance, younger age, and smaller tumor volume, being the tumors limited to one lobe in most cases, with rare involvement of corpus callosum and extremely rare contrast enhancement on MRI. These data could suggest that in the natural history of gliomas incidental grade II gliomas may represent an ear-

lier step of symptomatic GIIG. It has been suggested that an incidental discovery could be associated with a longer survival [17, 18].

The natural history and patterns of care of LGGs have changed over time with an increase of survival, which is, at least in part, due to the earlier diagnosis afforded by CT and MRI. Overall, the 5-year survival rates reported in recent randomized trials are in the order of 64–68 % [19, 20]. Up to 25 % of patients survive for 20 years [3]. Nonetheless, the natural history of LGGs is one of progressive growth [21, 22] and eventual malignant transformation (50–70 %) [6, 23–25]. The great variability in outcome for individual patients with LGG (survival ranging from less than 2 years to more than 20 years) depends on the combination of clinico-radiological, pathological, and molecular factors.

A number of retrospective and a few prospective series have evaluated variables of potential prognostic significance in patients with LGG (Tables 18.1 and 18.2).

Table 18.1 Spontaneous clinical and neuroimaging factors of unfavorable prognostic significance in low-grade gliomas

Fully validated
Age > 40 years
Presence of neurological deficits and/or absence of seizures at onset
Low performance status (Karnofsky < 70)
Preoperative tumor diameter > 4–6 cm
Astrocytoma as histology
Needing validation
Presence of contrast enhancement on MRI
High speed of volumetric increase or velocity of diametric expansion (VDE) on MRI
Elevated cerebral blood volume values (rCBV) on MRI
High uptake of methionine on PET

Prognostic Factors

Age

Younger age is a well-established prognostic factor for survival [6, 26–28]. Early in the 1980s Laws et al. [23], in a retrospective study involving 461 patients with LGGs treated at the Mayo Clinic, found that patients who were younger than 20 years had a 5-year survival of more than 80 %, with a progressive decrease in survival from 60–35 % for those in the 20–50 years age group and of less than 30 % for those in the over 50 age group. The linear functional relationship between age and prognosis has been confirmed in large datasets from prospective randomized trials [13, 29, 30]. A cut point at 40 years has been more commonly found, but in the clinical practice this should not be interpreted as an absolute cutoff value. A reluctance to undertake large resections (thus undersampling a higher grade component of the tumor) in older patients could potentially contribute to a worse prognosis, even in patients with an imaging appearance of a low-grade tumor. On the other hand, tumor biology may differ in older patients, the tumors being inherently more aggressive. In this regard, it has been reported that the proliferative index is higher and malignant transformation more frequent among patients with astrocytomas with an age >40–45 years [31]. Moreover, the proportion of gemistocytes in astrocytic tumors that could be associated with a more aggressive behavior increases with age [32]. Ultimately, although the biology behind the association of older age and worse outcome is still unclear, a possibility is that an age-dependent impairment of DNA repair mechanisms and the resulting acquisition of mutations may promote rapid progression after transformation occurs [33].

Table 18.2 Prognostic value of molecular markers in low-grade gliomas

Markers	Method of assessment	Prognostic value
TP53 mutation	PCR and immunohistochemistry	Minimal/absent
1p/19q codeletion	PCR, FISH, MLPA, CGH	Prognostically favorable, not predictive with regard to radiotherapy or chemotherapy
MGMT promoter methylation	Methylation-specific PCR	Probably different with regard to treatment received
IDH-1 mutation	PCR or immunohistochemistry	Prognostically favorable
BRAF mutation	PCR	Unknown

Clinical Presentation

Clinical presentation is another strong prognostic factor, whether expressed as the presence of seizures, absence of neurological deficits, or good performance status [23, 24, 34–38]. These factors are interrelated, e.g., neurologically intact patients who present with isolated seizures have a better performance status and prognosis. Moreover, patients who present with seizures tend to be younger and have smaller tumors than those without seizures [39–41]. It has been hypothesized that LGGs associated with epilepsy might differ biologically from LGGs of patients presenting with neurological deficits [42]. The duration of longest lasting symptom before diagnosis has been suggested as an independent predictor of time to recurrence [43]. The presence of an abnormal mini-mental state examination (MMSE) has been found as a strong predictor of poorer progression-free and overall survival in a large dataset of patients treated with adjuvant radiotherapys [44, 45].

Structural Neuroimaging Findings

Conventional neuroimaging findings have some prognostic importance. A tumor diameter >4–6 cm [38, 46, 47] and a tumor crossing the midline [38] correlate with a short progression-free and overall survival. The actual criteria for assessment of tumors crossing the midline were left to the discretion of the investigators: nonetheless, this measure reflects both mass effect and infiltration of midline structures (corpus callosum). Several investigators in the 1990s [25, 48, 49] have reported a tendency for larger tumors to behave differently with an earlier recurrence risk and/or greater tendency toward malignant transformation.

Tumor volume measurement on MRI has been increasingly used to study the relationships with outcome. Kreth et al. [49] found preoperative tumor volume greater than 20 ml to be of unfavorable prognostic significance, with the presence of midline shift being correlated with volume. In a recent study of hemispheric LGGs [50] greater preoperative volume was significantly associated with shorter malignant progression-free survival.

Growth rates, measured with different methods, are inversely correlated with survival [21, 51] and early malignant transformation occurrence [22, 52, 53]. Among 143 consecutive patients with LGGs in adults, a median survival of 5.16 years was associated with a growth rate of 8 mm/year or more compared with a median survival of >15 years with a growth rate of less than 8 mm/year [51]. Other investigators have demonstrated that sequential measurement of LGG volume, by allowing a precise determination of growth rates, permits the identification of patients whose tumors are at high risk for an early malignant transformation [53]. Six-month tumor growth may also predict outcome in patients with LGGs [52]. It must be said that the lack of widespread availability of volumetric assessments preclude their implementation in clinical trials at present [54], although progress in imaging software is likely to make routine implementation possible in the near future.

The prognostic implication of contrast enhancement on either CT or MRI remains controversial. Contrast enhancement occurs when the blood–brain barrier is disrupted, and lack of contrast enhancement more often suggests a low-grade glioma diagnosis [55]. However, contrast enhancement is reported in 15–50 % of patients with low-grade gliomas [19, 49, 56–58]. The finding that contrast enhancement is more commonly seen in high-grade gliomas has led many authors to infer that contrast-enhancing low-grade gliomas represent a more malignant subset of low-grade gliomas. Most articles on contrast enhancement and low-grade gliomas derive from series in which both CT and MRI were used and have concentrated on the relationships with survival, while there is paucity of information regarding the association with tumor recurrence and malignant transformation. The presence of contrast enhancement has been reported either as a negative factor for survival [28, 35, 59–61] or as without prognostic significance [6, 36, 62, 63]. Two recent papers [57, 58] have analyzed the prognostic significance of contrast enhancement in the MRI era, and the results are still somewhat different. In the experience of Chaichana et al. [57], at John Hopkins, on 189 patients with

LGGs who underwent surgical resection, preoperative contrast enhancement was independently associated with decreased survival, increased recurrence, and a trend toward increased malignant transformation in multivariate analysis. Five-year overall survival, progression-free survival and malignancy-free survival rates for patients with contrast-enhancing versus non-enhancing tumors were 70 % versus 85 %, 32 % versus 49 %, and 74 % versus 90 %, respectively. Notably in this series patterns of survival, recurrence, and malignant transformation were not significantly different between contrast-enhancing fibrillary astrocytomas and contrast-enhancing oligodendrogliomas. Pallud et al. [58] reviewed 927 histologically proven (either after biopsy or resection) WHO grade II gliomas in the French Glioma Database and found that the presence of contrast enhancement was not significantly associated with a worse prognosis in multivariate analysis: median survival and surviving rates at 5, 10, and 15 years were 11.9 years, 79.1, 68.5, and 27.8 % for patients with contrast enhancement compared to 12.7 years, 83.2, 60.3, and 44.3 % for those without contrast enhancement. Conversely, in univariate analysis, of a nodular-like pattern and of progressive contrast enhancement over time were statistically associated with survival.

Overall, the persistent discrepancies among the different series can be accounted by several factors, such as a different rate of sampling errors leading to the inclusion of a variable percentages of high-grade tumors and the absence of reproducible criteria to quantify the different degrees and characteristics of contrast enhancement prospectively. In this regard, it has been suggested that a quantification of the volume of contrast enhancement at baseline MRI could identify individuals at high risk for transformation [64].

Physiologic Neuroimaging Findings

The emergence of physiological imaging techniques has added new perspectives for the prediction of outcome and malignant transformation in LGGs. Proton MR spectroscopy allows the quantification of cellular metabolite levels. Normalized creatine/phosphocreatine levels of LGGs are a significant prognostic factor for progression-free survival as well as time to malignant transformation [65].

Measurement of relative cerebral blood volume (rCBV) derived from dynamic susceptibility-weighted perfusion contrast-enhanced MR imaging (DSC-MRI) could predict tumor behavior: a low rCBV correlates with longer PFS and OS [66, 67]. A longitudinal magnetic resonance perfusion imaging study was performed on conservatively treated LGGs to determine whether rCBV changes precede malignant transformation as defined by conventional MR imaging [68]. In patients with non-transforming LGGs, the rCBV remained relatively stable and increased to only 1.52 of normal tissue over a mean follow-up of 23 months. In contrast, patients with transforming LGGs showed a continuous increase in rCBV up to the point of transformation when contrast enhancement became apparent on conventional MRI. The mean rCBV was 5.36 at transformation and showed a significant increase from the initial study to 6 and 12 months before transformation. The measurement of rCBV correlates well with time to progression among low-grade astrocytomas, while it is not useful in oligodendrogliomas, as these tumors have an abundant vasculature which is not a sign of malignant evolution.

PET Findings

PET with FDG is of limited value for prognostic purposes since LGGs show a low FDG uptake compared to the normal cortex. Conversely, PET with amino acid tracers is more useful, as the uptake of tracers is increased in approximately two-thirds of patients with LGGs, and correlates with the proliferative activity of tumor cells. A low uptake of ^{11}C-methionine is correlated with longer survival [69, 70]. Recently Smits et al. [71] have reported that among high-risk patients with LGG (as defined by the presence of 3–5 unfavorable clinical prognostic factors), those

with high Met uptake had a worse outcome than patients with low Met uptake. Overall, the uptake of Met is physiologically relatively higher in low-grade oligodendrogliomas compared to astrocytomas: thus, the prognostic value of PET Met seems restricted to astrocytomas.

PET with 18F-FET (18-fluoroethyltirosine) is similar to PET Met and has been reported a similar prognostic value [72]. In 33 patients with LGGs, absence of 18F-FET uptake in areas of focal MRI signal changes was correlated with a better prognosis with little tendency to tumor progression, whereas increased 18F-FET uptake was related to further tumor progression, especially when the MRI signal changes were diffuse.

The prognostic value of uptake on 18F-FLT (18F-fluorothymidine) PET is still unknown.

Histology and Proliferation Markers

Oligodendrogliomas have a better prognosis than astrocytomas, being oligoastrocytomas in between [6, 19, 29, 38, 73]. The median survival for patients with oligodendrogliomas is typically 10–15 years compared to 5–10 years for those with astrocytomas. Among diffuse astrocytomas, the gemistocytic variant has been associated with a poorer outcome [74]. Some investigators [75] have associated the clinical behavior and rates of proliferation of low-grade astrocytomas with a different cellular lineage: slow growing, cortically based astrocytomas would be associated with a type 1 (protoplasmic) astrocytic lineage, whereas white matter astrocytomas would express antigens consistent with a type 2 (fibrillary) astrocytic lineage.

The difficulties in predicting the prognosis of low-grade gliomas have led to the increasing use of proliferation markers as an adjunct to routine histological techniques. Historically, different methods have been used to estimate the proliferative activity of low-grade gliomas: silver staining of nuclear organizer regions (AgNORs) (a measure of ribosomal gene activity that correlates with the degree of tumor malignancy [76]); evaluation of proliferating cell nuclear antigen (PCNA) [77]; analysis of the S-phase fraction by flow cytometry [78, 79]; immunohistochemical investigation of bromodeoxyuridine and iododeoxyuridine labeling index [80, 81]. However, the commonly used technique up to date is the immunohistochemical evaluation of the MIB-1 monoclonal antibody to the Ki-67 nuclear antigen to stain cells undergoing active division. Several studies have reported an association between high Ki-67 labeling index (≥3–5 %) and shorter survival [82–84], even if the independent prognostic value of Ki-67 has not yet been proved. Still unresolved issues are the best techniques to be employed, the interobserver subjectivity, the heterogeneity of Ki-67 within the tumor specimen, and the variability between cutoff points in the different studies.

Molecular Factors

Positive TP53 mutation status (but not P53 overexpression/accumulation) was suggested to be an independent unfavorable predictor of progression-free and overall survival [85], but in contemporary studies from the German Glioma Network, the P53 status was not associated with progression-free survival in tumors managed by surgery alone [86]. Accordingly, at present there is no need to know the P53 status for any clinical decision.

1p deletion or 1p/19q codeletion, which are commonly associated with the oligodendroglial phenotype, predict longer overall survival [45, 87–91]. 1p/19q codeletion does not confer any prognostic advantage in terms of progression-free survival in patients with LGGs who received surgery alone [86, 92]. Conversely, it could predict better response and longer progression-free survival in temozolomide-treated patients [9, 22].

MGMT promoter methylation could influence differently the progression-free survival depending on the treatment received, being a negative prognostic factor in patients (with astrocytomas) treated by surgery alone [93] and a positive prognostic factor in patients receiving temozolomide [94, 95].

IDH-1 mutation is the strongest prognostic factor in low-grade gliomas: in particular patients without IDH-1 mutations (30–40 %) have a poorer survival compared to those with the mutation

(60–70 %) [86, 96, 97]. Conversely the predictive value of IDH-1 mutation respective to response to nonsurgical treatments is still to be proven [98].

BRAF mutations are rare in diffuse low-grade gliomas; conversely, an activating point mutation, BRAF V600E, is found in approximately 60–70 % of pleomorphic xanthoastrocytomas and 20 % of gangliogliomas [99]. So far, there is no data on a prognostic relevance of BRAF mutations. Recently, the activation of the Akt-mTOR pathway has been suggested to correlate with a poorer outcome [100].

Prognostic Scoring Systems

Based on the prognostic factors that emerged as significant after multivariate analysis among large, randomized multicenter trials, several prognostic scoring systems have been developed to identify subgroups of patients with different outcome (so-called low- and high-risk groups).

In the EORTC analysis, [38] age >40 years, astrocytic tumor type, tumor size >6 cm tumor crossing the midline and neurological deficits at diagnosis had an independent negative prognostic value. A favorable prognostic score was defined as no more than two of these adverse factors and was associated with a median survival of 7.7 years, while the presence of 3–5 adverse factors was associated with a median survival of 3.2 years only. The EORTC prognostic score has been recently validated in the NCCTG database in the USA and has yielded similar results [45]. However, in the American dataset the histology and tumor size had the maximal importance.

Another LGG preoperative prognostic scoring system has been developed at UCSF [46], based upon the sum of points given to the presence of four significant adverse prognostic factors (1 point per factor), location of tumor in presumed eloquent location, KPS score ≤ 80, age > 50 years, and tumor diameter > 4 cm. Tumors with scores 0 or 1 had a 97 % 5-year survival compared to 56 % for those with score 4. This scoring system accurately predicted overall survival and progression-free survival in a multi-institutional population of patients [101].

Conclusions

Significant challenges remain in the management of low-grade gliomas, in particular the substantial clinical and biological heterogeneity that still exists. There is need for further investigation to better define the different tumor subsets. In the last decade, molecular markers have been increasingly introduced into the design of clinical trials in an effort to define more homogeneous patient populations which are more likely to respond to treatment in a uniform manner. 1p/19q codeletion has been used as a stratification factor in the EORTC 22033–26033 and RTOG 0424 ongoing trials, together with the most important clinical factors (age, Karnofsky, extent of resection). The hope for the future is to be able to increasingly develop personalized treatment approaches in the daily clinical practice.

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Part V

Functional Assessment and Interaction with the Brain

Language and Other Cognitive Evaluations

19

Sylvie Moritz-Gasser and Guillaume Herbet

Abstract

Adults harboring a diffuse low-grade glioma (DLGG) present most of the time without evident cognitive disorders. Nevertheless, extensive and specific cognitive assessments often highlight disorders in cognitive functioning, especially concerning memory, attentional resources, and information processing speed, which may affect negatively quality of life. Therefore, whatever may be the chosen therapeutic option, cognitive functioning must be assessed longitudinally in all patients. Such a longitudinal assessment may provide significant information about tumor progression, on the one hand, and allow to put the bases of a cognitive rehabilitation program if needed, on the other hand.

We report here a proposal of language and other cognitive evaluations, these latter encompassing attention, memory, executive functioning, but also social cognition, in the context of patient care (perioperative evaluations) and in the context of longitudinal follow-up. This proposal is based on our clinical practice with DLGG patients, keeping in mind that cognitive functions interact with each other. Finally, we underline that a relevant cognitive evaluation should encompass both objective and subjective scales and be associated with a psychological support.

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Diffuse low-grade glioma • Cognitive functioning • Assessments • Quality of life

Introduction

World Health Organization diffuse low-grade glioma (DLGG) is a precancerous, invasive, and slow-growing brain tumor, occurring mainly in young adults, most frequently after an inaugural seizure. Given this slow-growing character, patients with DLGG present most of the time without evident cognitive disorders, even when the tumor is located in functional areas for cognitive functioning, thanks to brain plasticity which allows cerebral functional reorganization. Nevertheless, extensive and specific cognitive assessments often highlight disorders in cognitive functioning, especially concerning memory, attentional resources, and information processing speed. These disorders, which may be caused by the tumor itself but also by tumor-related epilepsy and by treatments [1], affect negatively patients' quality of life (QoL) [2]. Therefore, whatever the therapeutic option (surgery, radiotherapy, chemotherapy), cognitive functioning must be assessed longitudinally in all patients. Moreover, it seems that the longitudinal assessment of cognitive functioning might provide significant information about tumor progression [3, 4] and then might contribute to a better prediction of patient's survival [5, 6] as well as may help clinicians in the selection of the most appropriate treatment to propose to the patient.

What Are Cognitive Functions?

Cognitive functions interact with each other and encompass the so-called higher functions, that is, language, memory, attention, and executive functions, to which we may add social cognition, and more "basic" functions: visuospatial orientation, sensorimotor functions, praxis, and gnosis. Each cognitive function does not work in an isolated manner; cognitive functioning is possible thanks to the interaction between the different functions. This assertion is particularly true for language processing because an efficient language functioning implies the integrity of attentional, executive, and memory functions. As a consequence, if dividing cognitive functioning in several subsystems is irreplaceable in order to understand the mechanisms involved in information processing, and thus the mechanisms damaged in clinical practice, this division is quite artificial since all cognitive functions participate, with different degrees, in an efficient cognitive processing, whatever its modality. Therefore, when one studies a peculiar cognitive function, for example, language, one has to keep in mind that other functions are involved in the function studied. These considerations are essential in cognitive functioning assessment and even more in cognitive rehabilitation.

Language

Although language is the mean to express our thought and to communicate, it is not a simple tool: it also allows conceptualizing the world and structuring thought. In other words, language is not reducible to speech, which is the motor planning and act of spoken language.

Language is processed following two poles, a productive one and a receptive one, in two modalities, spoken and written. There are different levels of processing, phonological, lexical, semantic, and syntactic, to which must be added the level of communicating acts, called pragmatic level, which allows us understanding, particularly regarding implicit and metaphoric language.

Schematically, language processing involves a set of parallel processes interacting together, at different levels (Fig. 19.1). As mentioned above, to be efficient, these parallel processes depend on the integrity of other cognitive functions, namely, attention, executive functions, and memory.

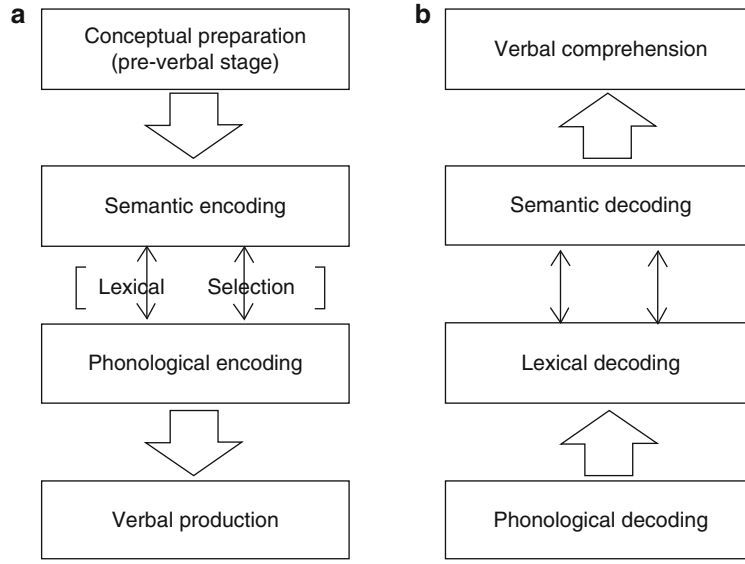

Fig. 19.1 Schematic model of (**a**) spoken word production and (**b**) spoken word comprehension

Memory

Thanks to numerous works in cognitive neuropsychology for over 30 years, consensual views and models of memory functioning have emerged. It is believed that memory is not a unitary system but that it is composed of multiple independent systems nonetheless working in a relative interactive manner. A classical and well-accepted distinction is the one made between short-term/working memory and long-term memory. The former is involved in the temporary maintenance of information and its mental manipulation [7]. Numerous activities of daily life are constrained by the proper functioning of this memory. This can range from basic activities such as keeping in mind a telephone number to more complex cognitive tasks such as comprehension during reading, mental calculation, and problem solving. Long-term memory is divided in two non-completely segregated subsystems [8]. It includes semantic and episodic memories. The former is highly involved in accessing the meaning of words, objects, people, and facts but also in the apprehension of the whole world. The latter has an essential role in encoding and storing new information in a spatial and temporal given context. It is the basis on which our autobiographic memory (personal facts) is built.

Attention

Attentional system is rooted in a long tradition in neuropsychology. It has been the subject of extensive experimental investigations during the last century. However, the term "attention" remains difficult to define because it involves several phenomena. Numerous cognitive or anatomo-functional models have been proposed in the past. The more consensual in clinical neuropsychology is perhaps the model by van Zomeren and Brouwer [9]. According to these authors, the attentional system is characterized by two axes, which are themselves partitioned into two subcomponents. The first axe corresponds to attentional intensity. It covers the notion of attentional arousal, vigilance, and sustained attention. The second, the selectivity axe, includes the functions of selective attention (the capacity to select and orientate its attention on relevant information and to maintain it) and divided attention (the capacity to allocate attention on several sources of information). All the processes would be under the control of a more globalizing entity, namely, the supervision attentional system, particularly involved during goal-directed behaviors (i.e., executive functioning).

Attention functions are crucial because they are the prerequisite to the functioning of all other cognitive functions.

Executive Functions

Executive functions are defined as the set of processes which allows cognitive and behavior control. They are particularly involved when the subject has to adapt himself to a new or complex situation (carrying out no procedural tasks; problem solving). Even though the term "executive functions" usually refers to a unitary concept, it includes a large number of sub-processes. Among these, the most prominent processes are action initiation, planning, organization, cognitive flexibility, cognitive control, emotion control, or conflict monitoring. It is believed that their coordination makes possible the success of self-generated action or behavior [10] and efficiency in dealing with the environment intentionally. In the case of severe executive functioning disturbances [11], patients behave as if they were completely subject to environment (e.g., imitation behavior, lack of control and disinhibition, stereotypic actions, or perseverations).

Social Cognition

Social cognition encompasses all psychological processes involved in the comprehension and the regulation of social behaviors. It includes a number of skills in which theory of mind (ToM) and empathy are the most representative. The former is referred to a unique form of metacognitive ability, which makes it possible to attribute mental states to oneself or others, like intentions, emotions, motives, or beliefs [12]. ToM allows establishing causal links between behaviors and the hypothetical psychological reasons which have induced them [13]. For this reason, such a brain function is thought as one of the pedestal on which social cognition is supported, authorizing successful social relations and behaviors. As for empathy, it can be defined briefly as the ability to recognize and share an emotional experience [14, 15].

These two social cognitive functions are very important for the appropriateness of behavior. For example, ToM disturbance is the cognitive landmark of some neuropsychiatric or neurodevelopmental condition like schizophrenia or autism spectrum disorders [16, 17]. A severe lack of empathy characterizes psychopathy or antisocial personality disorders [18].

Effects of Therapeutic Strategies on Cognitive Functioning

As mentioned above, surgery, radiotherapy, and/or chemotherapy constitute the main therapeutic options that may be proposed to DLGG patients, often completed by antiepileptic drugs. The effects of these different treatments on cognitive functions have been reported in several studies (see Table 19.1 for an overview).

Concerning surgery, provided that tumor removal is performed in awaken conditions, allowing intraoperative brain mapping and maximal resection according to functional boundaries (see Chaps. 23 and 24), a transient worsening in cognitive processing is often observed. Most of these cognitive deficits (see below) are resolved within 3 months [19], thanks to brain plasticity [20] which is presumably potentiated by the surgical act itself and by individualized speech-therapy rehabilitation.

Regarding radiotherapy, several studies showed that DLGG patients who received local radiotherapy experienced late-delayed (mean of 12 years after diagnosis) treatment-related disorders in cognitive functioning, especially in attentional functioning. Of note, these disorders were regularly associated with radiological abnormalities, compared with patients who were radiotherapy naïve [21, 22]. Moreover, a phase III trial showed that early radiotherapy has no impact on overall survival in DLGG patients [23]. Therefore, authors suggest that deferring radiotherapy treatment might be the most beneficial strategy to cognitive status.

Concerning chemotherapy, some authors have recently suggested that it might be a valuable therapeutic alternative in the management of patients with DLGG considered as initially inoperable because of an extensive involvement of eloquent areas or because of invasion of contralateral hemisphere [24]. Interestingly, patients who benefited from this therapeutic strategy, namely, neo-adjuvant chemotherapy followed by surgical resection after tumor shrinkage, presented with only slight cognitive disturbances, mostly related to glioma location [25].

Table 19.1 Effects of therapeutic strategies on cognitive functioning

	Therapeutic strategy		
	Awaken surgery	Radiotherapy	Chemotherapy
Effect on cognitive functioning	Transient disorders mostly resolving within 3 months	Early and late-delayed disorders	Slight disorders mostly related to tumor location

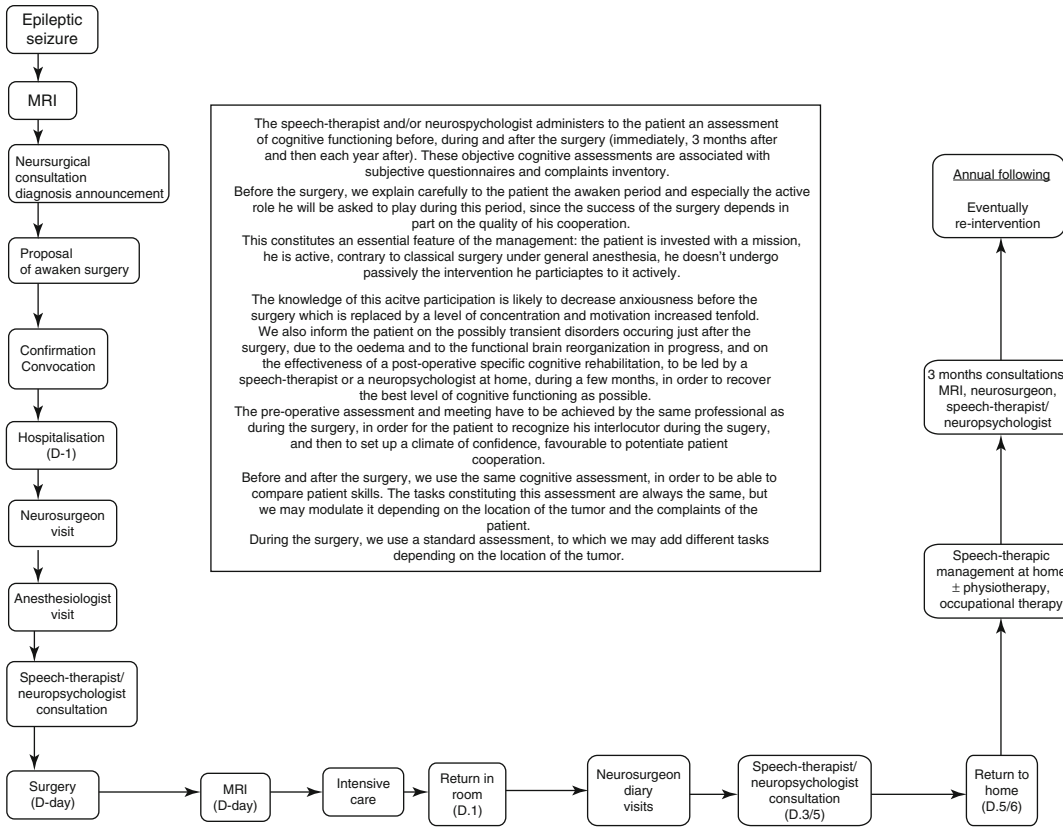

Fig. 19.2 Longitudinal management of DLGG patients undergoing a surgical resection in awaken conditions

Surgical Management of DLGG Patient

In any case, without treatment, malignant degeneration is invariably observed in DLGG. According to the guidelines of the European Association of Neuro-Oncology, surgical resection is now considered as the first therapeutic option for DLGG patients [26]. The extent of resection has been demonstrated to have a significant impact on the natural history of the disease, by delaying malignant transformation and increasing overall survival [27, 28]. Given that this lesion is preferentially located in brain areas involved in sensorimotor and language functions [29], this surgical management has to be led in awaken conditions, in order to check online patients' functioning (for which a significant interindividual variability has extensively been reported) [30]. This therapeutic (and not "wait and see") attitude allows achieving a challenge with two antagonist goals: to maximize the extent of resection while preserving functional areas, in order to increase patients' survival without inducing a worsening of their QoL – or even by improving it [31].

Therefore, the surgical management of DLGG patients has to be highly controlled and to follow a sequential succession of therapeutic stages well defined, which begins at the moment of the diagnosis and never ends (see Fig. 19.2). This dynamic

strategy encompasses the involvement of a pluridisciplinary team, which in the perioperative period is constituted by the neurosurgeon, anesthesiologist, speech therapist and/or neuropsychologist, and nurses.

The speech therapist and/or neuropsychologist has an important role to play in this management. They have to assess cognitive functioning of patients at different perioperative times, in order to highlight their cognitive status and the efficiency of their own brain plasticity. To achieve this assessment, not only several objective tests but also subjective questionnaires and complaints inventory may be used. Moreover, the speech therapist and/or neuropsychologist, who will be near the patient during surgery, has to explain clearly the modalities of the awaken surgical procedure as well as the active role of the patient, crucial to allow the neurosurgeon to achieve a successful resection. Indeed, the patient should be concentrated and motivated during all the awaken period, to enable the neurosurgeon to establish relevant anatomo-functional correlations. This latter aspect of the management is as important as the assessment of cognitive functioning.

Choice of Cognitive Tests

It is obvious that the choice of cognitive tests depends on the goal of the assessment, that is, patient care (including perioperative evaluations) versus patient longitudinal follow-up. Cognitive functioning assessment in the context of patient care must take into account several constraints: temporal constraints, on the one hand, especially concerning intraoperative assessments, and physical and psychological associated signs, on the other hand, due to the tumor itself but also to reactive psychological distress and treatments – especially antiepileptic drugs (AED). Fatigue is the most reported physical associated sign, and it is correlated with reduced concentration, motivation, and activity. There seems to be no relation between fatigue and tumor laterality or between fatigue and type of neurosurgical intervention (biopsy versus resection). On the other hand, fatigue is associated with AED use [32]. Psychological distress and mood disturbances associated with the disease may also lead to attention disorders and decreased motivation and, thus, affect cognitive functioning [33]. In the context of patient care, we should get the most sensitive information as possible about cognitive functioning, independently of disorders induced by physical and psychological associated signs. For instance, in the context of preoperative examination, we have to assess *present* (i.e., the day just before the surgery) patient cognitive functioning in order to understand his own brain functional organization and the efficiency of brain plasticity, to give in a way certain clues concerning the possible extent of resection. This preoperative assessment must be performed in a given time, keeping in mind the understandable psychological consequences induced by the prospect of the imminent brain surgery. Now, extensive cognitive assessments are time-consuming and may fatigue the patient entailing biased results. Thus, cognitive assessments in the context of patient care should be in the same time individualized, sensitive, relevant, but not too long (ideally, each assessment should not exceed 1 h).

Cognitive functioning assessment in the context of longitudinal follow-up should be more extensive, because we are facing neither temporal constraints nor acute psychological distress. In this setting, the use of comprehensive series of tests, possibly administered during several sessions to avoid fatigue, is relevant and of great interest for many reasons: to understand accurately patients' cognitive functioning, to put the bases of a possible cognitive rehabilitation, and to control its effects periodically. Of course, the tests used in this context have to be sensitive, valid, and reliable. Moreover, longitudinal cognitive assessments should be administered with a sufficient delay between each other (at least 6 months), in order to avoid practice effects.

Cognitive Evaluation in the Context of Patient Care: Perioperative Assessment

The surgical management of a DLGG patient is highly controlled, and it always follows the same temporal organization (see Fig. 19.2). Cognitive evaluations are administered 3 times perioperatively: the day before surgery, during surgery, and 3–5 days after surgery. Then, cognitive functioning is assessed periodically 3 months after surgery and each year (for an overview, see Table 19.2).

Preoperative and Immediate Postoperative Assessments

The same assessments (except subjective questionnaire and complaints inventory which concerns only the preoperative assessment) are administered to the patient the day before the surgery and 3–5 days after. These assessments have to be very sensitive in order to understand one's individual cognitive functioning, the effects of the tumor on neurocognition, the efficiency of functional brain plasticity (preoperative time), and the immediate effects of the surgery and surrounding edema on cognitive processing (postoperative time), with the goal to establish an individualized program of cognitive rehabilitation.

Preoperative meeting allows explaining to the patient the surgical procedure and the importance of his active participation, answering to his questions, and ensuring him that his present cognitive functioning will be at least preserved or even improved after surgery. Nevertheless, the patient and close parents must be informed that transient disorders (sometimes impressive) are frequently observed immediately after surgery, due to the resection, surrounding edema, and brain reorganization in progress. We must explain to the patient the transient character of most of these disorders, which will resolve in a few weeks, thanks to brain reorganization. Moreover, the patient must be informed that he will benefit from a specific cognitive rehabilitation performed at home by a speech therapist and/or neuropsychologist during at least 3 months, in order to potentiate this spontaneous brain reorganization and then to maximize the recovering of the best level of QoL.

Subjective Questionnaire and Complaints Inventory

Most of the time, before surgery, DLGG patients do not report cognitive symptoms, or only mild ones, which do not interfere with their daily life. Nevertheless, an extensive and specific cognitive evaluation highlights most of the time slight cognitive deficits, especially concerning working memory and speed of processing [34, 35].

Indeed, if brain plasticity allows an efficient reorganization, thanks to the slow-growing character of DLGG, such a brain functioning implies consequently a new functional network entailing presumably a higher cognitive cost in information processing. Moreover, there are frequent discrepancies between objective disorders revealed by cognitive tasks and subjective disorders experienced by patients in their daily life. That is the reason why it is very important to ask the patient, before any objective assessment, about his complaints. Questions are very simple:

- Do you have complaints concerning your cognitive functioning?
- Are you working full time?
- If no, what are the reasons of decreasing your time of work?
- Is it difficult to mobilize your attention?
- Did you note any difficulties in elaborating projects, understanding orders, being concentrated on a task, and participating in a conversation?
- Do you feel an important fatigue during or after such cognitive tasks?

It may be useful to ask the same questions to the close relations in order to compare subjective complaints and observed disorders.

Table 19.2 Overview of (A) language assessments and (B) other cognitive assessments

Context and goal of the assessment: language	
Patient care (perioperative assessments)	Patient longitudinal follow-up
Part A	
Pre- and postoperative standard assessment	*Some or all of the following tasks*
Subjective questionnaire/complaints inventory	Subjective questionnaire/complaints inventory
Handedness	Handedness
Fluency/informativity (spontaneous speech)	Fluency/informativity (spontaneous speech)
Timed naming task	Timed naming task
Fluency task	Fluency task
Timed semantic association task	Timed semantic association task
Timed reading task	Timed reading task
Additional tasks depending on tumor location	BDAE [40, 41]
Metaphoric language	Repetition
Repetition	Lexicality judgment
Reading, writing	Reading/writing
Intraoperative standard assessment	Token test [55]
Counting task	Information, similarities, vocabulary (WAIS 4) [56]
Naming task	Metaphoric/implicit language, prosody
Additional tasks depending on tumor location	Communication
Semantic association task	
Reading	
Repetition	
Double task	
Part B	
Pre- and postoperative standard assessment	*Intellectual functioning*
Subjective questionnaire/complaints inventory	Verbal comprehension, perceptive organization, working memory, processing speed (WAIS 4) [56]
Speed of information processing	
Working memory	*Verbal and nonverbal memory*
Executive functioning (flexibility, inhibition)	Short-term and working memory (digit span test) [63]
Motor and reflexive praxis	Episodic memory (RL/RI 16) [64]
Additional tasks depending on tumor location	*Praxis*
Visuospatial cognition	Motor, ideomotor, reflexive, constructive
Social cognition	*Visual gnosis*
Intraoperatve assessment depending on tumor location	V.O.S.P. [65]
Voluntary movement	*Somatognosis*
Visuospatial cognition	Naming body parts
Visual fields	*Visuospatial cognition*
Dual task	Line bisection, bell test [66]
Social cognition (studies in progress)	*Attention*
	Sustained attention, divided attention (T.E.A.) [67]
	Executive functions
	Motor and verbal inhibition (Go-nogo and Stroop tests) [67, 68], shifting (T.M.T.) [69], visuospatial planning (Rey's Figure) [70], dual tasks (personal material)
	Social cognition
	Theory of mind, social and moral reasoning, empathy (personal material)
	Emotion
	Facial emotion recognition (Ekman's facial emotion recognition task) [71]

Language Evaluation

We use always the same battery of tests to assess language processing before and immediately after surgery, whatever the location of the DLGG. This gold standard assessment begins with the Edinburgh inventory [36] in order to specify patient handedness and is then constituted by:

- An evaluation of the level of fluency and informativity of spontaneous speech
- A timed naming task (DO 80), which consists in naming 80 black and white pictures [37]
- A fluency task (semantic and phonological), which consists in producing the highest number of words belonging to a given semantic category or beginning by a given letter, during 2 min [38]
- A timed nonverbal semantic association task (PPTT), which consists in matching two semantically related pictures [39]
- A timed reading task, in which the patient is asked to decide if presented sentences are correct or incorrect, from a phonological and semantic point of view (personal material)

The naming, semantic, and reading tasks are presented on a computer screen.

It is worth noting that this assessment does not encompass a whole evaluation of language (e.g., BDAE [40, 41]). Indeed, we made the choice not to include this kind of whole examination, because we never observed, after more than 200 DLGG patients evaluated since 5 years, aphasic disturbances demonstrated by such a test. In other words, whole language evaluations such as BDAE (for relevant they are concerning other etiologies) are not sensitive enough for slight language disorders in DLGG patients, especially in the immediate preoperative period.

Finally, we added a nonverbal semantic association task, because we estimated that the sole naming task did not bring enough information on semantic processing.

Our assessment presents several advantages. Firstly, it is short (less than 1 h); it allows us to study language functioning at all levels of processing (phonological, lexical, semantic, syntactic) and in both modalities (written and spoken). Moreover, given that preoperative assessment provides clues concerning the efficiency of functional reorganization and then concerning the possible extent of resection, we have to select preoperatively some tasks that may be used easily intraoperatively, namely, simple and sensitive tasks.

Of note, each task is timed: this procedure allows highlighting slowness in language processing. We give the same significance in responses accuracy as in responses time. A good response is an accurate one produced in a given time. Indeed, as mentioned, DLGG patient presents frequently with slowness in cognitive processing. In a recent study, we showed that there might be a link between naming speed and QoL [42]. Indeed, the return to professional activities after surgery seems to be correlated with lexical access speed (see Fig. 19.3). Therefore, in addition to the assessment of responses accuracy, we consider that the measurement of responses times should be systematically included in language evaluations.

Depending on tumor location, we may add some tasks to this basic language assessment: comprehension of metaphoric language, repetition of words and pseudo-words, and reading and writing of words and pseudo-words.

Other Cognitive Evaluations

As concerning language, a short but sensitive evaluation of other cognitive functions is proposed before and immediately after surgery. This evaluation always includes an assessment of the speed of information processing, working memory, and, at the level of executive functioning, verbal or graphic auto-generation, cognitive flexibility, and inhibition. Motor and reflexive praxis are also systematically reviewed. Depending on tumor location, visuospatial cognition, social cognition, and emotion, including facial emotion recognition and theory of mind may be added to this basic evaluation. Postoperatively, if the patient does not reach his presurgical neurocognitive baseline, a cognitive rehabilitation may be prescribed. Interestingly, patients may sometimes improve their performances on some tests despite the resection. This can be explained, at least partly, by the lifting of the mass effect possibly applied by the tumor on brain tissue or by the decrease of functional interferences induced by the DLGG within neural networks (see Chap. 21).

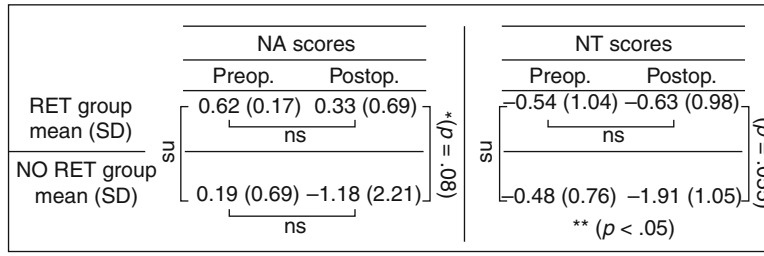

Fig. 19.3 Relations between naming performances and return to professional activities. In a recent study [42], we analyzed the correlation between naming skills, in terms of accuracy and speed of lexical access, and the return to prior professional activities, after awaken surgery in DLGG patients. Twelve patients were included in the study, six of them returned to their prior job in the year following surgery (RET group), whereas the other six were not able to resume their prior professional activities during this year (no RET group). Mean and standard deviations are reported in this table for each group, concerning naming accuracy (NA) and naming time (NT), preoperatively and postoperatively (between 6 and 12 months). Statistical analyses showed that the only significant difference was found between NT scores pre- and postoperatively, in the no RET group. In other words, patients who had not recovered their preoperative level of lexical access speed (and not only lexical access accuracy) were not able to return to their professional activities in the year following surgery, contrary to patients who did recover their preoperative level of lexical access speed

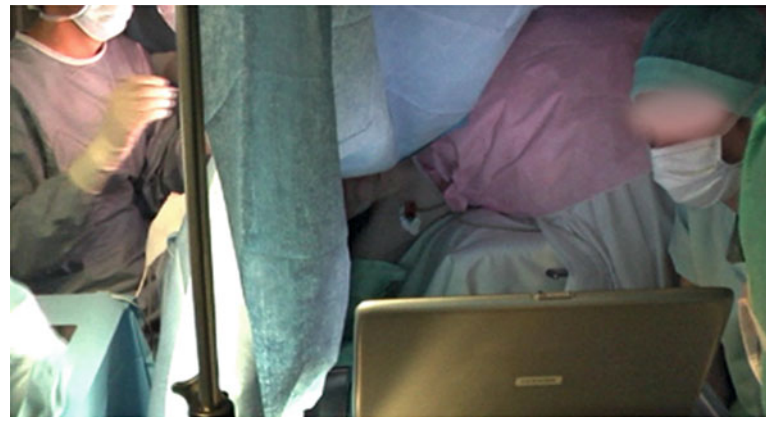

Fig. 19.4 Positioning in the operating theater, showing the relative positions of the patient, the neurosurgeon, the speech therapist/neuropsychologist, and the computer screen

Intraoperative Assessment

Language

During surgery, we assess patient cognitive and sensorimotor functioning, while the neurosurgeon applies direct electrical stimulations (DES), at the cortical and subcortical level (see Chaps. 23 and 24). The role of the speech therapist and/or neuropsychologist is, in addition to motivate the patient and to explain him what he is asked to do, to note, analyze, and interpret the most precisely and quickly as possible each disorder observed as well as to transmit this interpretation to the neurosurgeon – in order for him to perform a relevant cortico-subcortical individual brain mapping.

Moreover, to assess objectively the patient skills, the speech therapist/neuropsychologist is never informed about when and where the DES are applied. With the aim to interpret the most accurately as possible patient behavior, we chose to always use the same intraoperative assessment, namely:

- A counting task, from 1 to 10, in loop, in order to map brain areas involved in the motor implementation of automatic language (i.e., speech)
- The naming task DO 80 (with only the items passed the day before)

Our clinical practice shows that this simple assessment is, on the one hand, perfectly adapted to the surgical theater and patient positioning constraints (Fig. 19.4) and, on the other hand, very sensitive in highlighting language disorders, at all levels of processing.

Table 19.3 Different kinds of disorders observed during DES, reflecting the level of processing concerned

Effect of DES (naming task DO 80)	Level of processing
Speech arrest	Motor programming
Anomia	Semantic or phonological encoding, lexical access
Semantic paraphasia	Semantic encoding, lexical access
Phonologic paraphasia	Phonological encoding
Dysarthria	Motor programming
Perseveration	Inhibitory mechanisms
Increased delay of response	Lexical access

Indeed, given the briefness of the effect of DES on brain functioning (about 4 s), we can only use tasks which necessitate very short responses while providing valid clues on patient cognitive functioning. The DO 80 meets all these requirements. Each language disorder observed may be linked to a level of processing (Table 19.3), allowing an accurate brain mapping and, therefore, providing to the neurosurgeon the functional boundaries of the resection.

It is worth noting that, given the fatigue and the possible anxiety related to surgical conditions, it is not always easy to differentiate a disorder due to the effect of the DES from a disorder due to attentional disorders or simply fatigue. Thus, we always search for reproducibility when a disorder is observed during DES: a given brain area is considered as critical or belonging to a functional network, only when three consecutive DES of this area entail exactly the same disorder. The awaken period is over when all functional boundaries are reached, in other words when a reproducible disorder is reported for each stimulated area, both at cortical and subcortical levels. Before putting to sleep again the patient, we administer a last time some items of the task, in order for him to be sure that his language skills are not damaged and then that if there are disorders in language processing after his awakening, it would be transitory.

Depending on tumor location, we may add other tasks to this standard evaluation:

- The nonverbal semantic association task PPTT

This task is very informative regarding the assessment of the level of integrity of the semantic system, independently of verbal processing. For instance, it may be useful to specify a verbal semantic disorder observed during the naming test, by allowing differentiating a whole disorder of the semantic store from a simple deficit in accessing this store:

- Reading tasks, repetition tasks, and calculation tasks

Other Cognitive Evaluations

Monitoring Voluntary Movement to Prevent Motor or Motor Cognition Disturbances

Movement, when it is voluntary (i.e., the consequence of an endogenous activity and not the automatic reaction to environmental stimulation like reflex), is the result of a set of sophisticated processes (intention to act, planning, initiation, action control). Using a simple motor task through the surgery allows controlling *a minima* these different aspects of voluntary movement. Classically, we ask the patient to make a regular movement of the upper limb: lower the arm and open the hand, then raise the arm and close the hand (the lower limb may be also concerned or both at the same time depending on the location of the lesion). In addition, to check the velocity and the accuracy with which the movement is performed through the surgery, this simple test enables to functionally map under electrical stimulation crucial areas for motor cognition. Depending on the brain area stimulated, several clinical manifestations may occur. For example, whereas electrostimulation of the supplementary motor area can induce a transient motor initiation disturbance (the patient is able to initiate the movement but at the cost of a very important effort, as if it lifted a very heavy dumbbell), electrostimulation of primary motor cortex will stop the movement.

It is often useful to ask the patient occasionally to perform motor praxis and to control fine motor skills (e.g., strumming) or reflexive praxis (e.g., imitation of meaningless movements) to evaluate in this latter case complex movement planning.

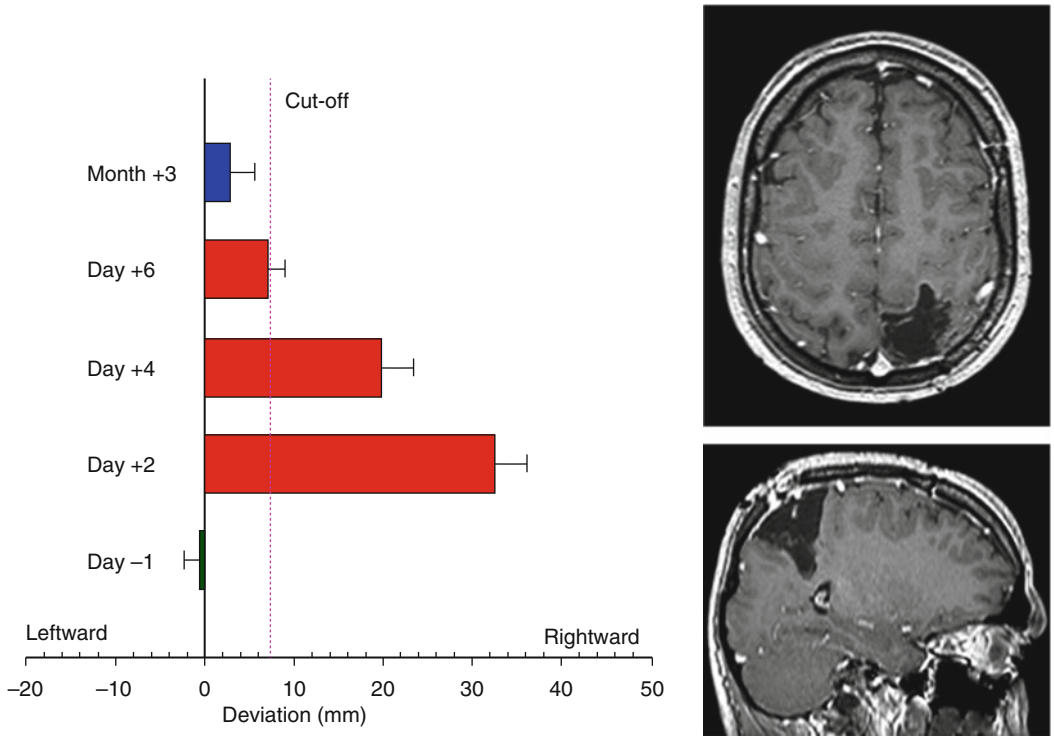

Fig. 19.5 Longitudinal performances of a patient harboring a glioma in the right parietal lobule. This patient was operated on under local anesthesia. Line bisection test was used during the surgery to map the functional networks for visuospatial cognition. Although patient presented a severe spatial neglect 2 days after surgery (Day +2), it has already begun to decline during following evaluation (Day +4). Six days after (Day +6), performances were located just at the level of pathological threshold. Three months after, performances reached approximately the preoperative level, demonstrating that eloquent structures, especially the subcortical connectivity (i.e., superior longitudinal fascicule in this patient), were preserved thanks to intaoperative testing (Unpublished personal data)

Mapping Visuospatial Cognition to Prevent Unilateral Spatial Neglect

Unilateral spatial neglect (USN) is a dramatic neuropsychological condition characterized by a failure to explore the side of the space contralateral to the damaged hemisphere, leading to a loss of consciousness of it [43]. It occurs mainly after a right lesion (especially when the lesion involves the temporoparietal and posterior frontal cortices but also basal ganglia or thalamus) and sometimes after a left posterior lesion [44]. When this deficit does not resolve, it has a major impact on QoL by depriving the patient to resume a normal socio-professional life.

A gold standard test to evaluate visuospatial cognition is the bisection line test [45]. During surgery, patient is asked to separate a line in two identical segments. If, during the time of the stimulation, an objective rightward deviation is observed in the case of a right lesion (a deviation over the range of 7–10 %, depending on the accuracy in which the patient performs the task), the brain area inhibited by electrostimulation is considered as critical for visuospatial cognition. As a consequence, this area will be preserved. Thanks to this method, easy to implement during surgery and very sensible in our experience, none of our patients have ever presented a long-term USN, although it is possible to observe it in the immediate postoperative phase – but transitorily (Fig. 19.5). Sometimes it disappears within a few hours, demonstrating that the neural network underlying visuospatial cognition has been completely preserved [46].

Mapping Visual Pathways to Prevent Homonymous Hemianopsia (HH)

Patients with visual field defect – homonymous hemianopsia – have a poor functional outcome [47]. In general, driving is prohibited and some activities such as reading or others become arduous [48]. To prevent this decrease of QoL and to avoid the occurrence of this visual deficit after surgery, we set a simple test to assess visual fields during resection. Patient is asked to name successively two pictures presented in two given visual quadrants. The position of the pictures is determined by the laterality of the lesion (left or right) and its location within the hemisphere. For example, in the case of a right mesio-temporal DLGG, we must preserve the left inferior visual quadrant to avoid a permanent hemianopsia, and then one picture will be placed in the left inferior quadrant and the other in the right superior quadrant. The second picture will serve as a very useful control in order to make the difference between visual, language, or sometimes gnosic disturbances (in the case, e.g., of a left temporo-parieto-occipital tumor).

More often, electrostimulation of visual pathways or related structures induce subjective complaints from patient (perception of a shadow, phosphenes). But when it is not the case, some clinical indicators are very useful as the amplitude of visual saccade (an increase indicating an insidious occurrence of visual disturbance) or the increase of naming reaction times in the corresponding visual field. This qualitative interpretation of visual behavior requires a great expertise and experience.

Dual Task as an Ongoing Measure of Executive and Attentional Processes During Surgery

Many activities in daily life involve the performance of two or several tasks simultaneously (e.g., making a call and working on his personal computer; driving his car and talking with his neighbor). This higher capacity requires allocating concurrently attention among several tasks [49]. A simple way to evaluate this important function during surgery is to ask the patient to perform a regular movement of the upper limb combined with a naming task in a coordinated manner. Although the qualitative analyses may be difficult at the end of the awake period because of fatigue, some manifestations are highly suggestive. For instance, we can mention several phenomena including temporary desynchronization/lack of coordination; an inability to perform the two tasks at the same time while performing the tasks serially remains possible or the sudden stop of one task. These manifestations may be explained at least in part by a disturbance of neural networks subserving divided attention, working memory (maintenance of task goals), or executive functions (planning and coordination).

The Future of Intraoperative Assessment: Toward the Mapping of Social Cognition

So far, intraoperative assessment has mainly concerned the functions that have been described above. Nevertheless, in some very specific cases (e.g., right frontal tumor infiltrating the contralateral hemisphere via the anterior part of the corpus callosum), we can use tasks that are designed to assess some aspects of social cognition as theory of mind, a function crucial for normal social interaction and functioning, as mentioned above. However, it remains difficult for the moment to generalize this type of evaluation during awaken surgery for several reasons. Firstly, this type of function is really complex and multidimensional, and then it is difficult to select appropriate and sensitive tasks, particularly given the constraints related to the surgical theater. Secondly, in our experience, only few patients present with this kind of deficit. In any case, the fact remains that when this happens, it can be dramatic for the patient and his family. For example, we report the case of a teacher patient who, after a right fronto-temporo-insular tumor, presented an important disturbance of theory of mind. This deficit was manifested by the incapacity to identify precisely the intentions of his students and resulted in a pseudo-psychiatric syndrome. The patient had indeed begun to construct delusional beliefs such as “my students ask me questions to try to find my fault.” Another example is that of a patient who experienced severe difficulty in recognizing

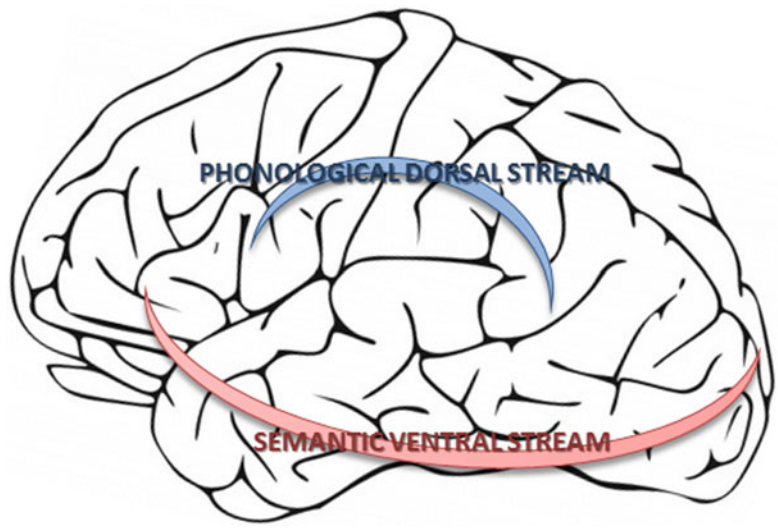

Fig. 19.6 Schematic representation of the phonological dorsal stream (*blue*), underlain by the left superior longitudinal fascicle, and the semantic ventral stream (*red*), underlain by the left inferior fronto-occipital fascicle

emotional and affective states of her young child following a left paralimbic glioma. Neurocognitive examination confirmed objective deficits in, respectively, facial emotion recognition and affective theory of mind (unpublished personal data).

These two extreme but rare cases illustrate very well the need to adapt tests to surgery in order to prevent the occurrence of this kind of socially disabling deficit. However, before implementing them, we must understand the conditions of occurrence of these disorders and identify the brain regions likely to induce them. In other words, we have to identify the anatomical regions of susceptibility, reluctant to functional plasticity, by the means of probabilistic anatomo-functional maps. Part of our research is focused on this important issue.

Anatomo-Functional Correlations

In addition to the evident clinical interest of awaking the patient in order to achieve an extensive resection while preserving functional networks, this procedure provides the opportunity to establish reproducible and accurate anatomo-functional correlations. Among them, our clinical practice allows highlighting, in agreement with current cognitive models of language processing [50], the subcortical anatomical correlates of the phonological dorsal and semantic ventral streams. Indeed, on the one hand, stimulating the left superior longitudinal fascicle (SLF) in its medial part (arcuate fascicle) always induces phonological disorders, whereas stimulating this fascicle in its anterior lateral part always induces articulatory disorders. On the other hand, stimulating the left inferior fronto-occipital fascicle (IFOF), whatever the location of the DES, always induces semantic disorders [51]. Therefore, these white matter bundles are always preserved in their main part since they underlie the phonological dorsal stream (SLF) and the semantic ventral stream (IFOF), respectively – from our point of view based on clinical analysis (see Fig. 19.6).

Concerning visuospatial cognition, eloquent sites are regularly found when electrical stimulations are applied to the supramarginal gyrus, to the posterior part of superior temporal gyrus and, to a lesser extent, to the posterior part of inferior frontal gyrus. In the same way, stimulation of superior longitudinal fascicule reproduces also the clinical signs of USN, demonstrating the essential role of the fronto-parietal connectivity and the absolute necessity to preserve it.

Cognitive Disorders Following Surgery

Most of the time, the immediate postoperative assessment (between 3 and 5 days after the sur-

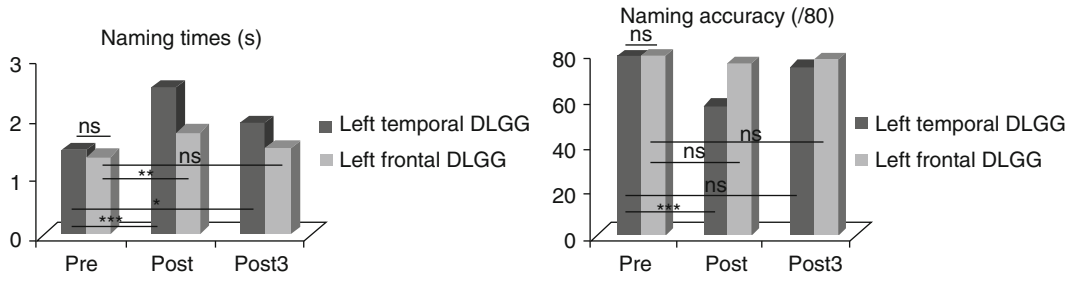

Fig. 19.7 Naming skills in left temporal DLGG patients ($n=8$) and left frontal DLGG patients ($n=8$). We report in this figure the mean scores in naming times and naming accuracy for 16 patients (Non-published personal data). In the immediate postoperative period, naming times are significantly increased, whatever the location of the DLGG is (temporal: $p<0.001$; frontal: $p=0.03$). This slowness resolves at 3 months for left frontal DLGG patients ($p=0.52$). If naming times tend to decrease in left temporal DLGG patients, they remain significantly increased compared with preoperative evaluation ($p=0.047$). Concerning naming accuracy, the right table shows clearly that, except concerning the difference between preoperative and immediate postoperative assessments of left temporal DLGG patients ($p=0.01$), no significant worsening is observed postoperatively for both populations (temporal: $p=0.13$; frontal: $p=0.36$). Thus, recovering one's preoperative level of naming accuracy does not mean necessarily recovering its speed. These results underline the significance of measuring naming times in addition to naming accuracy

gery) highlights disorders related to the brain area which was removed. Nevertheless, these disorders are mainly transient, due to the postoperative edema, maximal about 3 days after surgery. Moreover, the surgery itself induces processes of functional reorganization, which may spoil transiently the functioning of a given functional network. Apart from "site-specific" disorders (e.g., articulatory or initiation disorders after a resection close to motor planning areas), we may observe different kinds of language and other cognitive disorders. Thus, immediate postoperative clinical presentations are various and may go from slight disorders to broad impairments in different cognitive functions. In any case, patients present always with slowness in information processing and attentional disturbances.

For instance, if we do not observe systematically a deficit in naming accuracy postoperatively, an increase of naming latencies is always highlighted in the immediate postoperative evaluation. We must underline that recovering a normal speed in naming, thanks to cognitive rehabilitation (among other things), is often difficult and costly in terms of cognitive resources. In any case, even if it is not impossible, since lexical access speed seems to be recovered most of the time 3 months after the surgery (see Fig. 19.7), it is always much more difficult rather than recovering a normal level of naming accuracy. This postoperative dissociation between accuracy and speed of processing is besides observed in each task, in various degrees.

This slowness in information processing is likely to be related to a disorder in working memory and executive functioning rather than to a global psychomotor slowness.

Considering that, as mentioned above, language functioning has been checked at the end of awaken period, we are able to ensure the patient that immediate postoperative disorders are transient, even if sometimes impressive. Nevertheless, in order to potentiate spontaneous functional reorganization and thus to recover the best level of cognitive functioning in a short delay, all patients benefit from a specific and intensive program of cognitive rehabilitation, performed immediately after their return to home by a speech therapist specialized in this management (see Chap. 30).

Three months after surgery, patient neurocognition is reevaluated. This assessment highlights the level of recovering and thus the efficiency of brain plasticity and speech-therapy manage-

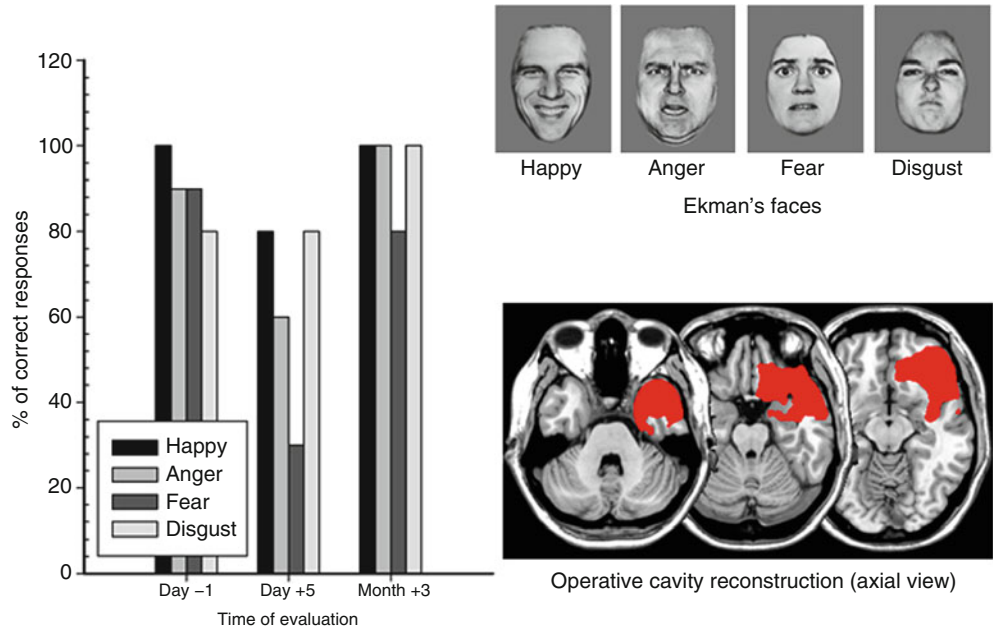

Fig. 19.8 Longitudinal performances in recognizing basic facial emotions of a patient harboring a fronto-temporo-insular tumor. Results of this patient show a transient emotion recognition deficit, particularly for "fear" and in a lesser extent to "anger," immediately after surgery (Day +5)

ment. Concerning language functioning, we always observe a clear improvement compared with immediate postoperative skills. Most of the time, the preoperative level is reached (see Fig. 19.7).

Concerning other cognitive functions, attention, working memory, and executive functions, disturbances are common in the postoperative phase, requiring a following specific cognitive treatment, although they are not systematic [31, 52, 53]. Depending on tumor location, specific deficits can be observed, notably in the domain of social cognition and emotion. For example, resection of DLGG in the insular and the amygdala regions induces more often facial emotion recognition impairment, concerning disgust and fear, respectively (Fig. 19.8). When the lesion involves the posterior part of right inferior frontal gyrus, recognition of "happy" emotion becomes sometimes transiently very difficult. Recognition of complex affective mental states (affective theory of mind) may have also been disturbed (unpublished personal data). Another example is the problem of comprehension of intentions following medial frontal areas removal, including the most anterior part of cingulate gyrus.

If the glioma is located in the inferior parietal lobule, or more generally at the level of the temporoparietal junction, transient spatial neglect may be observed (Fig. 19.5). This is also the case, to a lesser extent, when the resection involves the posterior part of inferior frontal gyrus.

Following a temporo-mesial lobectomy, transient severe disorders of anterograde memory may be observed. In some case, learning and retention of any new information is impossible. Although some degrees of slight disturbances may persist at 3 months, the initial dramatic disorders disappear almost completely in most cases.

Cognitive Evaluations in the Context of Longitudinal Follow-Up

Whatever the therapeutic strategies, and whatever the location of the lesion, the assessment of DLGG patients' cognitive functioning must

absolutely be included in the context of medical longitudinal follow-up, for many reasons (for an overview, see Table 19.2).

Firstly, our clinical practice shows that extensive and specific assessments always highlight at least slight disorders in cognitive functioning. Secondly, these cognitive assessments might provide significant information about tumor progression. Thirdly, the moment of these evaluations allows patients to set out cognitive complaints which cannot always be demonstrated by the tests proposed. Fourth, the highlighting of cognitive disorders during these assessments may lead the clinician to propose an individualized program of cognitive rehabilitation to the patient.

Thus, cognitive evaluation in the context of patient longitudinal follow-up must begin at the moment of the diagnosis, and it never ends. In this setting, in opposition to the perioperative context, the use of comprehensive series of tests, possibly administered during several sessions to avoid fatigue, is relevant and of great interest. Follow-up evaluations should not have to be administered more than twice a year, with the goal to avoid practice effects. As far as possible, we may use standardized tests, but we can also use personal non-standardized specific tasks, which allow the comparison with patient-specific skills between them over time.

We insist on the fact that these extensive follow-up evaluations should be performed in all patients harboring a DLGG and must begin at the moment of the diagnosis, even for patients who are candidate to a surgical management. In this latter case, the first evaluation will take place far before the surgery and will not replace the immediate preoperative and postoperative assessments described above.

Subjective Questionnaire and Complaints Inventory

In addition to the complaints collection, standardized subjective questionnaires and scales may be used in order to assess the quantitative and qualitative level of communication, from the patient and close-related point of view [54].

Language Evaluation

In addition to the battery of tests presented in section "Language Evaluation," we propose to assess language extensively, at all levels of processing, by administrating some or all of the following tests:

- Boston Diagnostic Aphasia Examination (BDAE) [40, 41], in order to have a baseline score at disposal
- Repetition of words and pseudo-words (phonological level)
- Lexicality judgment (lexical level)
- Reading and writing of words, irregular words, and pseudo-words (phonological, lexical, and semantic levels)
- Token test [55] (syntactic level)
- Information, similarities, and vocabulary tasks from the WAIS 4 [56] (semantic level)
- Comprehension of metaphoric and implicit language, and comprehension and production of prosody [57] (pragmatic level)
- Objective assessment of the qualitative and quantitative level of daily communication [58].

Other Cognitive Evaluations

In this setting, an extensive cognitive examination is proposed. It is thought to assess all domains of cognitive and intellectual functions. Depending on tumor location, we may focus more specifically on cognitive functions, for example, numeric or visuospatial cognition, if the tumors involve the left or the right posterior parietal cortex, respectively. However, an important basic assessment is common to all patients, whatever the location of the DLGG (see Table 19.2).

It is worth noting that, in the context of longitudinal follow-up, the first neurocognitive assessment is a crucial step in cognitive care because it will serve as a reference point for subsequent years.

Finally, we may add to these extensive assessments a questionnaire concerning QoL, in order to assess the impact of the DLGG and the therapeutic strategies on daily life [59, 60].

Psychological Support

If one of the fundamental roles of the speech therapist/neuropsychologist is to assess as accurately as possible the neurocognitive functioning, as mentioned above, this is only a part of the management. The diagnostic announcement and the prospect of neurosurgical management may induce psychological distress. The loss of self-esteem is, for example, a fairly common problem after surgery. Moreover, even if the cognitive loss is minimal, it may be experienced as dramatic and may have a large rent on the psychosocial functioning and QoL of the patient (and its family) and, finally, it may lead to a depressive state. About this, it is well known that depression has detrimental effects on cognitive processes, in terms notably of memory by damaging natural plasticity (e.g., hippocampal plasticity) in healthy individuals [61, 62]. Another problem induced by depression is the inactivity and apathy often associated. After brain surgery, it is essential for the patient to resume quickly his daily activities (e.g., reading, crafts, and outings). These environmental stimulations are needed to recover a normal functioning as quickly as possible, by stimulating neural networks. Therefore, we have to take into account affective states and psychological distress, to be listening in to patients' complaints and to carefully deal with them. Actually, we have absolutely to keep in mind that, even if cognitive status is widely correlated with QoL, especially with return to work, recovering a satisfactory level of QoL is not reducible to the recovery of a normal cognitive status.

Conclusion

In the context of perioperative patient care as well as in longitudinal follow-up, language and other cognitive evaluations are crucial in the management of DLGG patients. The neuropsychologist and the speech therapist, working in the same state of mind, have an essential role. They establish a real therapeutic alliance with the patient, which never ends. In the setting of longitudinal follow-up, extensive assessments of all the domains of cognitive functioning may be administered periodically, during several sessions if needed, to avoid fatigue. In the context of neurosurgical procedure, accurate and sensitive evaluations of neurocognitive functioning are administered before, during, and after surgery, in order to assess the impact of the DLGG on cognitive functions and the efficiency of one's brain plasticity – and then to allow a maximum resection while preserving functional networks. Whatever the context of evaluation is, its results allow to establish the bases of a relevant individual program of cognitive rehabilitation (when needed). In addition to these objective assessments, we include psychological support in patient care. Indeed, to resume a normal life is not reducible, from far, to the sole recovery of a satisfactory level of cognitive functioning, even if cognitive functioning contributes for a large part in QoL. Thus, in addition to objective cognitive assessments, subjective questionnaires, complaints collection, and psychological support should always be a part of DLGG patients care. Consequently, institutions and neurosurgeons should absolutely pay interest in these aspects of the management of DLGG patients, which are often neglected in practice.

If the management of these patients has dramatically improved in recent years, there is a long way to go. Some issues need to be further addressed. This is, for example, the case of possible changes of personal identity or personality after surgery. Indeed, it is not uncommon to observe some modifications in behaviors or more generally in decision making in multiple settings (the patient being aware of this or not). This may range from simple irritability, easily manageable, to more problematic manifestations, such as lack or exacerbation of empathy, emotional indifference, changes in sexual conducts, or others – sometimes related to social cognition disturbances. Although important behavior changes are rather the exception than the rule, it is nevertheless crucial to understand their conditions of occurrence. This challenging work can be accomplished in systematizing some types of psychological (e.g., premorbid

personality) and cognitive (e.g., social cognition and emotions) evaluations before and after surgery. It would be useful to create appropriate tests to assess some significant aspects during surgery, at least for the high-risk situations. Moreover, from an ethical point of view, even if social and behavioral functions are difficult to assess during surgery given operative constraints, we think that these questions must be integrated in the discussion around the benefit-to-risk of DLGG surgery.

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Functional MR and Diffusion MR Imaging in Diffuse Low-Grade Gliomas

20

Alberto Bizzi

Abstract

In the last decade, functional MR imaging and MR tractography with diffusion tensor imaging have changed the way to evaluate patients with glioma before surgery, in particular when the tumor is infiltrating eloquent brain structures. Mapping of cortical sites and white matter pathways may improve presurgical planning and surgical targeting with neuronavigational devices, and it may reduce intraoperative time. Clinical use of these advanced MR imaging tools is growing in importance, and exams in patients are increasingly being requested by neurosurgeons worldwide. Applications and current limitations of fMRI and MR diffusion tractography are discussed focusing on the sensorimotor, speech, and visuospatial networks.

Keywords

Functional MR imaging • MR tractography • Diffusion MR imaging • Low-grade glioma • Language network • Visuospatial network

Introduction

The development of functional magnetic resonance imaging (fMRI) with blood oxygenation level-dependent (BOLD) contrast in 1990 [1] and of MR diffusion tensor imaging (DTI) in 1994 [2] has opened a new era in mapping brain function. Since the first MR images of brain activity in the human visual cortex by Belliveau et al. in 1991 [3], fMRI has become the most used and valuable in vivo research imaging tool in modern cognitive neuroscience. fMRI is currently used by investigators in many scientific disciplines as diverse as cognitive neuroscience, psychology, neurosurgery, psychiatry, linguistics, and neuroeconomics. The unique capability of diffusion imaging to virtually dissect major white matter tracts has attracted the attention of neurosurgeons since the very early attempts of performing MR tractography [4–6]. The number of fMRI and DTI tractography clinical studies requested by neurosurgeons is increasing rapidly.

The main aims of therapeutic strategies in neuro-oncology are to increase median survival

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and to improve quality of life. In particular, in diffuse low-grade gliomas (LGG), the specific target of surgery is to resect maximal tumor volume while avoiding postoperative neurological deficits. Overall survival of LGG at 5 and 8 years is improved when volume resection is above the critical threshold of 90 % [7]. In order to accomplish this target, it is mandatory to safeguard indispensable gray and white matter components of eloquent networks that are located in the proximity of the glioma. The aims of imaging are to map networks of basic human functions that are indispensable (i.e., eloquent) to the patient to maintain a good quality of life and to assess the spatial relationship of the tumor with the essential components of each eloquent network. Currently, there are three networks that are considerate eloquent: sensorimotor, speech, and visuospatial. In the future, other important human functions such as memory, emotion, and decision-making eventually will be mappable in the clinic. It has been shown that mapping the network of interest improves presurgical evaluation, surgical planning, and intraoperative targeting with neuronavigational devices. Currently intraoperative direct cortical and subcortical electrostimulation mapping (ESM) remains the reference method to map eloquent function. Intraoperative ESM has significantly improved the survival rate of patients undergoing resection of low-grade gliomas [8]. It has been shown that integration of fMRI and diffusion tractograms into navigational devices in the operating room may decrease the time needed to map eloquent cortex with intraoperative direct ESM [9].

Clinical use of fMRI and diffusion MR tractography in neuro-oncology is the first clinical application and is at the forefront of methods development. In 2007, the American Medical Association (AMA) has definitely recognized that use of fMRI for presurgical mapping is clinically valuable, and it has established current procedures terminology (CPT) codes for reimbursement fees [10]. At a time when personalized therapy is considered the best option to defeat gliomas, fMRI and diffusion imaging have the potential to improve our understanding of the interaction between the disease, the host, and adaptive changes in brain function.

In this chapter, we will focus on clinical surgical applications of fMRI and DTI with particular emphasis on clinically feasible and available methods, practical clinical indications, interpretation of the results, and validation studies. Current critical issues will be addressed to offer to the general reader a perspective of method developments that are expected to enter the clinic in the next years.

Methods and Clinical Imaging Protocols

Functional MR Imaging

FMRI is the most popular functional neuroimaging method in clinical practice and in neuroscience. FMRI is noninvasive, and it is now widely accessible in both clinical and research settings. Most fMRI studies in humans are performed using BOLD signal. It measures changes in blood oxyhemoglobin/deoxyhemoglobin ratio, and it is not a direct marker of neuronal activity. Despite many advances in the last two decades, BOLD fMRI is far from being able to map neural activity due to several inherent limitations. In the human brain, there are about 90,000–100,000 neurons under a cortical surface of about 1 mm^2. Its spatial resolution is better than other functional imaging methods; however, the typical unfiltered voxel size is in the order of 12 ml, and it contains about 1.2 million neurons, $1–2 \times 10^{10}$ synapses, 6 km of dendrites, and 60 km of axons. The temporal resolution is also limited: a whole brain volume acquisition takes about 3 s. The BOLD signal may be primarily originated by changes in excitation-inhibition balance occurring in brain tissue at rest and during execution of a task. Logothetis et al. have associated increasing oxyhemoglobin concentration to a change in local field potentials in the dendrites of postsynaptic neurons rather than changes in firing (action potential) of groups of neurons [11–14]. This balance may be controlled by neuromodulation more than by changes in spiking rate of a small set of neurons. As a consequence, BOLD fMRI is strongly modulated by attention.

For clinical applications, most centers use task-based fMRI; few centers have recently included in their protocol a resting state fMRI sequence to measure functional connectivity (fcMRI). Mapping of the sensorimotor functional network with fcMRI has been tested for presurgical mapping [15]; there is also great interest to use fcMRI to disclose adaptive changes in brain function occurring in patients harboring slow-growing gliomas [16, 17].

Functional MRI is a reliable method when the workflow runs smoothly and the multiple steps have been extensively explained to the patient in order to obtain optimal cooperation. It is important to emphasize that patients should be able to perform well the task of interest. Patients harboring a slow-growing glioma are usually in their forties and otherwise healthy; thus, they are usually motivated and cooperative. Instructions of each task should be explained slowly to the patient with preliminary training. Patients usually are more anxious than healthy peers, especially when the fMRI study is performed only a few days before brain surgery. The examiner should test carefully patient's compliance and proficiency, since underperformance or dropout might result in decreased or even absent BOLD response. The task should be adjusted to patient's skills when focal neurological deficits are present. Overlearning the task should be avoided. Online acquisition of behavioral data attesting subjects performance during the study is desirable.

For clinical studies, block paradigms are generally well suited and preferable than event-related ones. Block design paradigms are statistically more robust, but they are restrained because they measure average brain activity over a relatively long (about 20 s) span time. The number of cortical activated sites and their location depends on the condition that is chosen to evaluate the function and the network of interest. The control condition will have to include all brain processes that are associated with the function of interest but are not considered object of investigation. Silent tasks are usually preferred because motion artifacts are reduced [18]. Stimulus input may be visual or acoustic. The former is preferable when evaluating a mass in the temporal and parietal lobes because speech-related BOLD signal will not be confused with activity in primary auditory areas.

Variability of clinical fMRI results among multiple sites is an important factor to consider when planning surgery in a patient with glioma. Data quality and robustness of the task are the most important factors with an impact on reliability, on intrasite, and intersite reproducibility of fMRI results. Data quality should be measured periodically with the following quantitative parameters: percent signal change, contrast to noise ratio, and head motion. Motor paradigms are more robust than those with sensitive stimuli and cognitive tasks [19]. The multiple steps of the procedure should be standardized. In order to localize the cortical sites, the center of mass of the area with BOLD response rather than peak activation should be measured since it is less influenced by noise [20].

Diffusion Tensor Imaging and Tractography

DTI measures the dispersion of the random walks (Brownian motion) of water molecules within brain microstructure over a time interval, usually a few tens of milliseconds [2]. DTI models the dispersion of water molecules using a Gaussian distribution. In three dimensions, the Gaussian distribution has ellipsoidal contours, so the assumption in DTI is that the scatter pattern has an ellipsoidal shape. In the white matter, axon bundles are orderly oriented [21]; thus, they have a big effect on the shape of dispersion of water molecules. Diffusivity of water molecules varies with the tissue's orientation (anisotropic diffusion). DTI measures the diffusivities (eigenvalues) of water molecules along the three orthogonal axes of an ellipsoid and their average (mean diffusivity). Water diffuses fastest along the axial orientation of the bundles (axial diffusivity) and slowest along the cross-sectional plane (radial diffusivity). Fractional anisotropy (FA) is an index that measures eccentricity of the displacement of water molecules. In the healthy human brain, probably the most relevant factor affecting

FA is the intravoxel orientation coherence of white matter fibers [22]. FA has rapidly become a very popular surrogate marker of white matter integrity; however, it lacks specificity due to few inherent limitations of DTI tractography. There are three main imaging output of DTI MR imaging: quantitative parametric maps displayed in gray scale (i.e., FA maps), color maps showing the principal orientation of water diffusivity, and three-dimensional maps showing results of virtual dissection of tracts with streamline tracking methods (i.e., tractography).

Diffusion MR tractography has the potential to provide unique information about connectional anatomy and pathology-induced changes. The aim of tractography or fiber tracking is to infer the three-dimensional trajectories of white matter bundles by piecing together discrete estimates of the underlying continuous fiber orientation field measured noninvasively with DTI data [5, 6]. Fiber-tracking algorithms can be broadly classified into two types: deterministic and probabilistic. Several DTI-based tractography atlases for virtual in vivo dissection of the main human white matter tracts have been published [23, 24].

DTI has quickly become popular in neuroscience because it can be used as a probe into tissue microstructure at a spatial resolution that is in the order of microns, far below the current spatial MR imaging resolution. DTI quantifies diffusion anisotropy in white matter, and it provides an estimate of the principal direction of axon bundles. The DTI model is robust; however, it has several inherent limitations. One key limitation is that DTI fails to estimate bundle configuration when there are crossing, bending, or fanning bundles within the voxel. The diameter size of axons is about 20,000–200 times smaller compared to the voxel size of state-of-the-art diffusion MR imaging: 0.1–10 μm versus 2 mm. Thus, voxels contain thousands of axon bundles which can adopt a wide range of complex configurations. DTI results can be misleading when dispersion of water molecules is significantly different from what predicted with the Gaussian model.

New advanced diffusion parametric (i.e., model based) and nonparametric (i.e., Q-ball imaging, diffusion spectrum imaging, spherical deconvolution (SD)) models have been developed to resolve two major limitations of DTI: the multiple bundle orientation problem and the tract-specific characterization paradox [25]. High angular resolution diffusion imaging (HARDI) acquisition schemes have been developed to better characterize water molecule displacement in voxels with multiple bundle populations. It has been estimated that about 90 % of white matter voxels contain crossing bundles [26]. While DTI measures the orientation of the principal diffusivity (i.e., one bundle orientation for voxel), nonparametric HARDI methods provide information on the number of bundle orientations, their orientation, and the weight of each bundle component. SD methods are being used in an increasing number of clinical studies as their acquisition requirements (i.e., number of diffusion gradients and b-values) are similar to and feasible with DTI protocols. A limitation of SD is its susceptibility to noise. Both deterministic and probabilistic approaches can be applied to DTI and HARDI dataset.

In conclusion, new diffusion imaging techniques and models are being developed for resolving the crossing fiber population problem in each voxel. We predict that these methods will have a great impact especially in diffusion imaging of brain tumors. However, each new method has pros and cons that require new strategies to exploit the complex information they provide. New challenges must be met before the new HARDI methods can be routinely applied in clinical practice and replace DTI that is currently user-friendly and widely available.

Clinically Feasible Brain Mapping Imaging Protocols

The suggested imaging protocol includes two conventional morphologic imaging sequences (volumetric T1-weighted MPRAGE and 3D-FLAIR), few runs of the BOLD-echo planar imaging (EPI) sequence acquired during execution of the tasks of choice, and one diffusion (DTI or HARDI) sequence for tractography. Whole brain coverage is mandatory. For fMRI of speech, it is recommended to implement the same tasks that will be used for intraoperative direct ESM. The study can

be acquired on clinical 1.5 T MR units; however, it would greatly benefit from acquisition at higher magnetic field strength (i.e., 3.0 T and beyond). Adequate spatial resolution for fMRI is about 2*2*3 mm^3 voxel size; for DTI, it would be preferable to use 1.5 or at least 2.0 mm isotropic voxel size. Gradient diffusion-weighting is achieved with b-values in the range of 1,000–3,000 ms/m^2. SD can be performed on DTI datasets acquired at 3.0 T with *b* value > 1,500 and 2.0 mm isotropic voxel size. Each run for the fMRI study will last about 4 min; DTI acquisition with at least 32 or 64 gradient directions will last about 7 or 14 min, respectively. Total scan time of a fMRI/DTI study should not run above 45 min.

Mapping Functional Networks

Clinical feasibility and validation studies published in the last 15 years have provided evidence that nodes and pathways of three main eloquent networks can be mapped with advanced MR methods. They are the sensorimotor, speech, and visuospatial networks.

The following structures are the target of presurgical mapping of the motor system: primary motor cortex, supplementary motor area, and corticospinal tract (CST). The targets of mapping speech are anterior productive areas (dorsal premotor cortex (BA9/46), ventral part of the precentral gyrus (BA6), pars opercularis (BA44)), and posterior receptive areas (superior temporal sulcus (BA22/42) and inferior parietal lobule (BA40)), the arcuate fasciculus (AF), and the inferior frontal-occipital fasciculus (IFOF) in the dominant cerebral hemisphere. The main targets in the visuospatial system are cortical areas in the frontal and parietal lobes, the superior longitudinal fasciculus components (SLF-I, SLF-II, and SLF-III), and the IFOF in the nondominant hemisphere.

Sensorimotor

The most robust and commonly used task is finger tapping that is alternating tapping of the fingers of each hand in a block designed paradigm. Mapping of the motor network identifies cortical motor sites in the precentral cortex (primary area), postcentral cortex (primary sensitive area), and supplemental motor area (SMA) in the superior frontal gyrus (SFG) of the contralateral cerebral hemisphere. The entire homunculus can be easily mapped on each side; however, in the interest of time mapping is limited to the hand, foot, and lips (tongue).

fMRI mapping is also used to delineate seed regions for DTI tractography of the CST and projections to the SMA. MR tractography is unique to demonstrate the relationship between a glioma and the CST (Figs. 20.1 and 20.2). MR DTI tractography of the CST has been validated by multiple investigators [27–29]. With deterministic DTI, only CST trajectories projecting to the hand knob area can be visualized while it is unusual to visualize trajectories projecting to the foot and mouth areas. Probabilistic DTI and Q-ball tractography may show trajectories with fewer false-negative streamlines (Henry RG, personal communication). Within the tumor, it is often difficult to identify reliable streamlines, even when using decreasing threshold to FA = 0.1.

Language

Mapping the network involved in speech production, perception, and comprehension is a hot topic in neurosurgery and clinical neuroscience. In the past century, until the advent of modern functional imaging (positron emission tomography, magnetoencephalography, and fMRI) classical theories of language adopted compartmentalization into independent stations as a paradigm. This approach is unfortunate and outdated because it gives the impression that multiple language processes occur serially or at least without much interaction. Modern theories suggest that high cognitive systems are organized in widespread, segregated, and overlapping networks [30]. Recently Hickok and Poeppel have proposed a model of the language network with two broad processing streams connecting an anterior language center located in the inferior frontal opercular region (Broca territory) with a posterior center located in the posterior

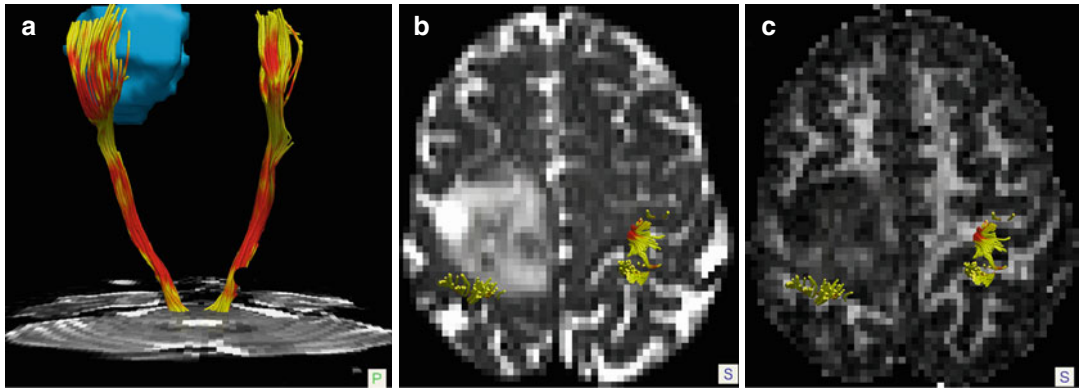

Fig. 20.1 MR diffusion tensor imaging (DTI) tractography in a 41-year-old male with oligoastrocytoma WHO-II infiltrating the left precentral and prefrontal gyrus. Posterior view (**a**) of the trajectories of the corticospinal tracts (CST) in scalar code with fractional anisotropy (FA) values overlaid over axial T2-weighted MR image at the level of the pons. Views from above of the CST overlaid over axial T2-weighted (**b**) and FA map (**c**) at the level of the primary motor cortex. This case is a typical example of the pearls and limitations of deterministic DTI tractography. MR tractography is the best technique available to show in vivo that the glioma (bright on T2WI and dark on FA map) is displacing the dorsal segment of the left CST posterolaterally. However, note that only CST trajectories projecting to the hand knob area can be visualized on both sides. With deterministic DTI, it is unusual to visualize trajectories projecting to the foot and mouth areas. In addition on the lesion side, there are fewer streamlines due to decreased FA caused by the infiltrating glioma. Note heterogeneity of FA within the lesion suggesting that few streamlines may be spared within the tumor. DTI dataset was acquired at 3.0 T (Siemens Verio) with 2.0*2.0*2.0 mm^3 spatial resolution, *b* value = 1,500, 64 gradient directions. Tensor was calculated with tortoise; tractography was performed with trackvis using FA > 0.15 and <35° angle thresholds. Tracking was performed with trackvis using two ROIs delineated in the precentral gyrus (seed) and pons (target)

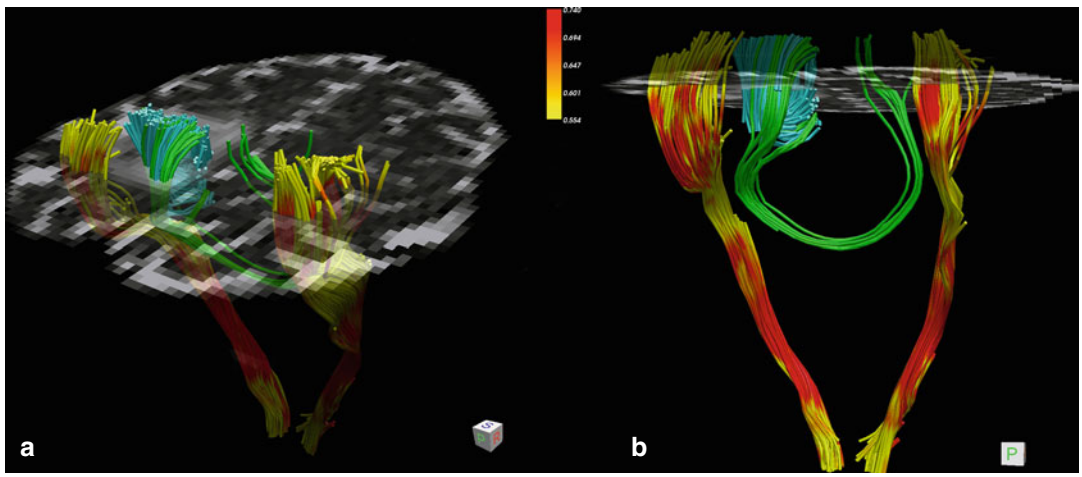

Fig. 20.2 MR diffusion tensor imaging (DTI) tractography in a 41-year-old male with oligoastrocytoma WHO-II infiltrating the left precentral and prefrontal gyrus. Posterior oblique view (**a**) and posterior view (**b**) of the trajectories of the corticospinal tracts (CST) in scalar code with fractional anisotropy (FA) values overlaid over axial T2-weighted MR image al the level of the primary motor cortex. Fiber tracking with seed ROI delineated within the glioma identified arcuate trajectories (in *cyan*) projecting to the ipsilateral supplementary motor area (SMA) and trajectories (in *green*) coursing in the corpus callosum and projecting to the contralateral SMA. At the usual threshold (FA > 0.15), DTI did not identified additional CST trajectories. Intraoperative electrical stimulation mapping (ESM) validated the trajectories of the left CST and M1 projections to the ipsilateral and contralateral SMA

temporal (Wernicke territory) and inferior parietal (Geschwind territory) regions [31, 32]. According to this dual system model, a stream running dorsally to the sylvian fissure via the AF is involved in mapping sound onto articulatory-based representation, whereas a stream coursing ventrally in the temporal lobe, temporal stem, and orbital part of the frontal lobe is involved in mapping sound onto meaning. According to this model, language emerges through the interaction of multiple neocortical nodes in the frontal operculum, inferior parietal lobule, and temporal lobe of the dominant hemisphere.

A dual stream system has also important implications for understanding the adaptive processes that are in action for preserving function, while slow-growing gliomas are infiltrating brain tissue. A parallel processing system with dorsal and ventral pathways would offer many options for compensatory and adaptive (plasticity) mechanisms. The dual model should help to understand the complexity of aphasic syndromes and mechanisms of recovery from aphasia [33].

The Dorsal Language Pathway

In humans, the AF and the SLF have long been considered synonymous, and the names have been used interchangeably. Early DTI MR tractography studies have contributed to this confusion in terminology. DTI MR tractography studies are showing that the anatomy of the perisylvian dorsal pathway is more complex than previously thought. In humans, the AF connects the posterior superior and middle temporal gyri with the neocortex in the anterior (pars opercularis, BA44) and posterior (ventral precentral gyrus, BA6) banks of the ventral precentral sulcus. Catani et al. [34] virtually dissected three segments of the AF using two-ROIs seeding with a deterministic approach: a direct pathway connecting "Broca territory" with "Wernicke territory" (long and medial segment) and an indirect pathway subdivided into two segments: an anterior segment connecting the premotor cortex (ventral and dorsal BA6) with the inferior parietal lobule and a posterior segment connecting the inferior parietal lobule with the posterior temporal region (middle temporal gyrus (MTG)/superior temporal sulcus (STS)). Postmortem dissection in human has confirmed the existence of the three segments of the AF [35]. According to deterministic DTI MR tractography results, the AF does not project into the pars triangularis (BA45) and orbitalis (BA47) of the inferior frontal gyrus (IFG) [36]. With more advanced diffusion imaging methods (i.e., SD and HARDI), the AF streamlines may extend into pars triangularis and orbitalis.

In the monkey, the SLF comprises three segments (SLF I, II, and III) connecting parietal-occipital cortex with prefrontal areas. Recently, the three SLF components have been identified also in humans using SD [37]. The anterior segment of the AF is equivalent of SLF-III.

The Ventral Language Pathway

The effective course of the ventral routes is still unclear and matter of a hot debate [33, 38, 39]. Some authors have suggested that the IFOF, uncinate fasciculus (UF), and inferior longitudinal fasciculus (ILF) are components of the ventral pathways, whereas other investigators claim that the bundles coursing along the extreme capsule (EmC) between the insular cortex and the claustrum are involved [40]. The UF, IFOF, and EmC enter the temporal stem, a narrow band of white matter concentrating long and short association bundles coursing immediately above the middle cerebral artery [41]. The temporal stem provides a route for tumor spread, and it could be the critical segment that connects the temporal and the frontal lobes [42]. It seems to be the preferential pathway for seizure spread via the UF from the hippocampal formation and via the IFOF for visual hallucinations. Theoretically, a complete surgical resection of the temporal stem in the dominant hemisphere should disconnect the ventral language stream connecting sites in the temporal and frontal lobes. Because the temporal isthmus is very deep, not easily accessible, and contains tracts and arteries that must be preserved, complete resection of diffuse insular gliomas infiltrating the stem is difficult.

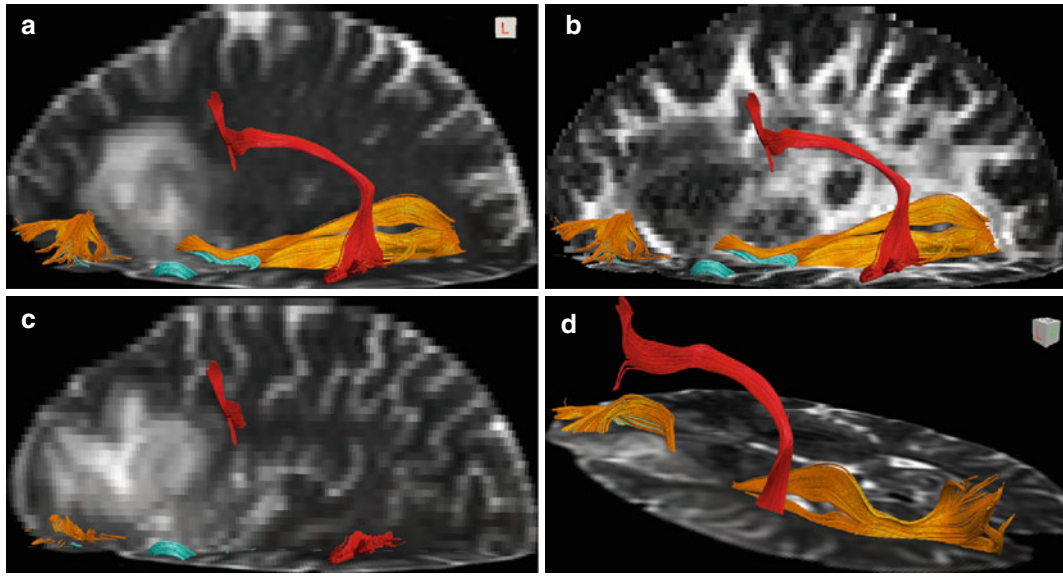

Fig. 20.3 MR diffusion tractography in 31-year-old male with oligodendroglioma. Left lateral view of the left arcuate fasciculus (AF) overlaid over sagittal and axial T2-weighted (**a**, **c**, **d**) MR images and FA maps (**b**). The tumor has displaced posteriorly the direct segment of the AF (in *red*), displaced medially the frontal components of the IFOF (in *orange*) and UF (in *cyan*). Despite tumor infiltration and decreased FA in the temporal stem, DTI was still able to reconstruct the trajectories of the IFOF and UF and show the relationship between the gloom and the tracts. Sparing of the dorsal and ventral language pathways despite heavy gloom infiltration of Broca area and anterior insula may explain preserved language function on the left hemisphere with no language deficits (see also [45])

A brief description of the tracts of the ventral pathway virtually dissectible with DTI MR tractography has been provided with a 3D atlas [23, 43]. The *IFOF* is an associative bundle with long and short fibers that connects the ventral part of the occipital lobe with the medial part of the temporal lobe and the orbitofrontal cortex. Along his course in the temporal lobe, the IFOF runs parallel and medially to the ILF before it enters the extreme and external capsules [44]. In the temporal stem, the IFOF runs dorsally and medial to the uncinatus and projects to the IFG in pars orbitalis and triangularis (Fig. 20.3). Its functions may be related to reading, attention, and visual processing. The *UF* is an associative hooklike-shaped tract connecting the anterior temporal lobe with the gyrus rectus and the medial and posterior orbitofrontal cortex [44]. It was first described by Dejerine [46], and it has three parts: the frontal and temporal extensions merging at the isthmus. In the temporal pole, the UF is lateral to the amygdala and hippocampus, then curves upward, passing above and behind the trunk of the middle cerebral artery. From the temporal stem, the UF continues into the medial and posterior orbital gyri. The dorsal part of the UF is crossing bundles emerging from pars opercularis and projecting to the SMA, a frontal intralobar tract recently described with MR tractography [24, 47, 48]. The UF is considered to belong to the limbic system, but its functions are still poorly understood. The ILF is an associative bundle with long and short fibers connecting the occipital and temporal lobes. The long fibers connect the amygdala and hippocampus to the visual areas [44]. The ILF is involved in face recognition, visual perception, reading, visual memory, and other functions related to language.

In the monkey, the EmC connects superior and middle temporal gyri and rostral part of the insula with the pars triangularis (BA45) and pars orbitalis (BA47) [49]. This ventral pathway may contain mono- and polysynaptic bundles that relay in the insula and claustrum (acting as association

centers). Recently, axonal autoradiographic tracing studies in the macaque monkey have demonstrated two direct streams of fibers that originate from the posterior temporal and parietal cortex and project to the ventrolateral frontal cortex [49]. The ventral temporo-frontal axons course via the extreme capsule and target most strongly pars triangularis (BA45) with a more modest termination in pars opercularis (BA44). By contrast, a dorsal stream of axons originate in the inferior parietal lobule (IPL) and the adjacent caudal STS then course via a simple AF to target both BA44 and BA45. The most rostral part of the IPL is preferentially linked with the ventral precentral gyrus (VPCG, BA6) that controls the orofacial musculature. These results are consistent with DTI studies of language in humans [40, 50].

Current Issues in Mapping Speech

Several important issues are the focus of current basic and clinical research: function, importance, vulnerability, and indispensability of each tract in reference to network functionality. Four main techniques are currently available to map the tracts: axonal label tracing methods in monkeys, ex vivo postmortem dissections [51], in vivo MR tractography with diffusion tensor imaging (DTI), and intraoperative direct subcortical ESM in humans. Despite current limitations of each technique, the concordance between the four methods is good.

Revisitation with the aid of MR imaging of the lesion studies made by Paul Broca in 1861 has led to reevaluation of long accepted theories about the cortical sites involved in speech production. MR imaging of Leborgne's and Lelong's autopsy brains revealed large infarcts extending far beyond the classic area described by Broca and involving also the anterior part of the insula and the AF [52]. Gliomas infiltrating the frontal opercular region offer a unique opportunity to identify structures that are essential for speech production. In a study on 19 right-handed patients, it was shown that gliomas growing in the ventrolateral aspect of the left frontal lobe may cause mild to moderate speech deficits. Gliomas growing in the left VPCG were much more likely to cause speech deficits than gliomas infiltrating the IFG, including Broca area. MR DTI tractography was valuable to demonstrate that lesion extension to the AF was a requisite for the appearance of aphasia in brain tumor patients [45]. Patients with glioma infiltrating either the IFG or the VPCG without involvement of the AF-direct segment did not show conduction aphasia (Figs. 20.3, 20.4, and 20.5). A prominent role for the insula in speech production has been suggested by an MRI study in 25 stroke patients with a deficit in motor planning of articulatory movements [53]. All patients with the deficit had lesions that included a discrete region of the dominant precentral gyrus of the insula, but not all had a lesion in pars opercularis. This area was completely spared in other 19 stroke patients without these articulation deficits. fMRI studies have confirmed the important role of the insula for motor planning of speech. However, patients with diffuse LGG infiltrating the insula, the temporal stem, and the anterior temporal region have normal scores on language tests despite large tumor size (Bizzi A, manuscript is in preparation).

Intraoperative ESM provides functional information about a bundle when stimulation causes arrest of speech. However, ESM is not accurate to determining the distance of the tract from the point of electrical stimulation. ESM cannot identify the tract's origin and termination sites. Diffusion imaging tractography does not provide functional information, but it is the method of choice to identify tract trajectory. Thus, MR tractography provides unique and valuable information for neurosurgery; it may facilitate and reduce time of ESM in the operating room. Several ESM studies have validated MR tractography results suggesting that the dorsal pathway is involved in phonological processes whereas the ventral pathway in semantics processes. Direct ESM of the left AF induces speech arrest and phonemic paraphasias [54, 55]. Direct ESM of the left IFOF causes semantic paraphasias in response to picture naming [56].

Although language lateralization has been well established, its anatomical basis is not fully understood. In particular, the role of the IFOF, ILF, and UF in maintaining the integrity and functionality of the language network has been elusive.

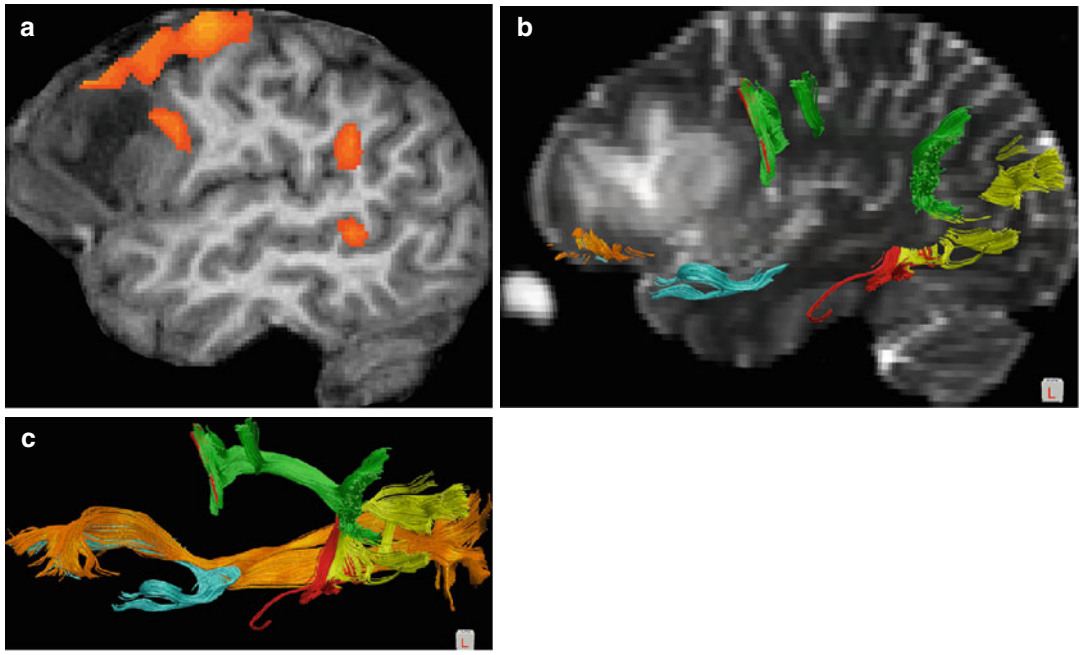

Fig. 20.4 MR diffusion tractography in 31-year-old male with oligodendroglioma infiltrating Broca area. Patient had normal language performance on Aphasie Achten test. Functional MRI during verb generation evoked dominant BOLD responses (FDR < 0.001) in the anterior (pars opercularis and middle frontal gyrus) and posterior (angular gyrus and middle temporal gyrus) classic speech cortical sites (**a**). In addition, there is a cluster of BOLD response in the ipsilateral dorsal prefrontal gyrus. fMRI is overlaid over a left-sided sagittal T1-weighted MR image. Left lateral view (**b**) of the left arcuate fasciculus (AF) overlaid over sagittal T2-weighted MR images. The trajectories of the inferior frontal-occipital fasciculus (IFOF, in *orange*), uncinate fasciculus (UF, in *cyan*), and three segments of the left arcuate fasciculus (AF) are illustrated: anterior (in *green*), direct (in *red*), and posterior (in *yellow*). Left lateral view (**c**) of the trajectories of the dorsal and ventral language pathways. Note preservation of the anatomo-functional relationship between MR functional and tractography data: AF projections to the cortex match with clusters of fMRI activity in the anterior and posterior language sites. The tumor has displaced posteriorly the rostral parts of the anterior and direct segments

Intraoperative direct cortical and subcortical ESM data seem to support the hypothesis that the IFOF in the dominant hemisphere is likely implicated in language specialization [56, 57]. Interestingly, in another intraoperative study, subcortical ESM of the left ILF never elicited any language disturbances [58]. In addition, all patients recovered following a transient postoperative language deficit, despite the resection of at least one part of the ILF. This study seems to suggest that the ILF may not be indispensable for language. On the contrary, evidence for a role of the ILF in object naming was provided by the single case of a patient with glioma infiltration of the left temporal lobe and IFOF disruption prior to tumor invasion [59]. Surgical resection of the UF (either in its frontal or temporal extensions) may have long-lasting consequences for famous face naming [60]. Bello et al. have suggested that it is part of a circuitry involved in the retrieval of word form for proper names.

Visuospatial Attention

Difficulty to test cognitive functions other than speech in the operating room has led to neglect the functional importance of the right hemisphere that plays a role in managing visual interaction with the environment. Great progresses in understanding the visuospatial attention network have been made in the last few years, driven by fMRI clinical research studies [61], new lesion-based DTI studies, and intraoperative direct cortical and subcortical ESM [62].

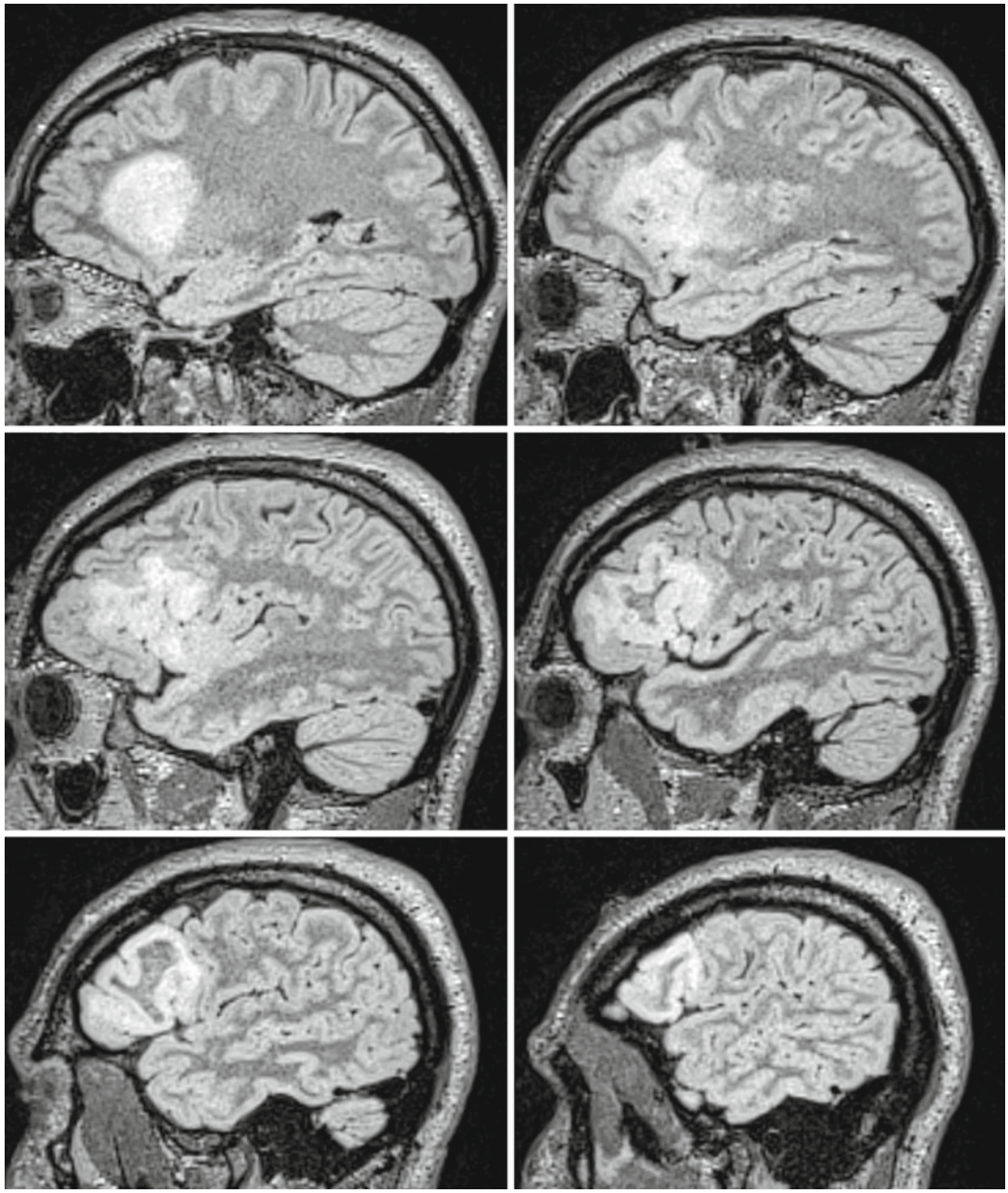

Fig. 20.5 Consecutive sagittal FLAIR MR images in 31-year-old male with oligodendroglioma WHO-II showing infiltration of the anterior part of the left insula, pars opercularis and triangularis (i.e., Broca area), with sparing of pars orbitalis of the left inferior frontal gyrus. There is no evidence of extension in the adjacent orbital gyri, ventral part of the precentral gyrus (VPCG), and middle frontal gyrus

One of the most complex and fascinating neurological syndromes is unilateral neglect. Stroke patients with lesions including the right supramarginal gyrus (SMG) often exhibit left unilateral neglect [63]: they will typically ignore people, objects, their own body parts, or events occurring to their left visual hemifield. Patients with neglect can see things in their left visual field if their

attention is actively drawn to them. There is a marked difference in prevalence between neglect of the left side of space and neglect of the right side. The incidence of unilateral left spatial neglect is much higher. One possible explanation could be that patients with right unilateral neglect (due to a lesion in the left parietal lobe) may recover far more quickly [64]. Spatial awareness of the patient is evaluated by neurologists with the line bisection test or the star cancellation task. The line bisection task is also used for fMRI studies.

Visual interaction with the environment is managed through a complex bilateral fronto-parietal network. Anterior nodes in the dorsal and ventral prefrontal regions (i.e., IFG and frontal eye fields in the middle frontal gyrus (MFG)) are connected with posterior nodes in the temporal-parietal junction and parietal lobes (i.e., SMG and superior parietal lobule). The nodes of the network are connected by long-range association pathways running in the SLF [37]. The SLF-I is the most dorsal component, and it projects anteriorly into the supplementary motor area, posteriorly into the superior parietal lobule. The SLF-III is the most ventral of the three components, and it projects into pars opercularis of the IFG and posteriorly into the SMG. Nodes of the dorsal and ventral component of the network appear to be connected by the SLF-II that is the major of the three components. The SLF-II projects anteriorly to the lateral aspect of the SFG and MFG, posteriorly to the inferior parietal lobule. During performance of a spatial orienting attention fMRI task, the above nodes show increased BOLD response [61].

Most severe and persistent signs of left neglect typically occur after lesions involving both gray and white matter in the right parietal lobe. Left unilateral transitory spatial neglect due to small ischemic white matter lesions in the SLF-III has been reported in a single case report [65]. There is evidence that additional parallel ventral pathways running in the IFOF and ILF may play an important role in the network since left unilateral neglect has been reported also in stroke patients with lesions involving these pathways [66, 67]. The nodes described above also connect through the corpus callosum.

Mapping of the visuospatial network is sometimes requested when a glioma is located in the parietal lobe, generally on the right side. Unilateral spatial neglect is rare in patients with diffuse low-grade gliomas, in part due to possible adaptive functional reorganization of the network occurring during brain infiltration by a slow-growing lesion. Understanding of the many interactions of the visuospatial network with other attentional processes is growing rapidly. Hopefully, new treatment strategies will soon have an impact on rehabilitation of visuospatial attention in brain-damaged patients.

Clinical Indications

There are several indications to request a clinical fMRI with DTI study when an eloquent functional network is located in the proximity of a mass: (a) presurgical evaluation in order to determine the distance of the mass boundaries from eloquent sites and tracts, (b) surgical safeguarding of eloquent structures that may be located within the mass or encountered along the surgical trajectory of choice, and (c) assessment of the dominant hemisphere for language (and memory) in left-handed patients and in those with a negative score in the Edinburgh Handedness Inventory test.

The CST, AF, IFOF, and the optic radiations (Fig. 20.6) are the most clinically relevant and often requested tracts by neurosurgeons.

Critical Issues and Challenges in Slow-Growing Gliomas

Many preliminary studies with MR tractography have raised great expectations about the ability of the technique to depict the tumor-induced changes. A critical issue with MR tractography and diffusion imaging in general is that the occurrence of a focal lesion may affect not only white matter bundles but also the diffusion signal properties that we exploit to perform fiber tracking. Gliomas may infiltrate, swell, or destroy bundles of axons in white matter [69]. The increased amount of free water associated with diffuse

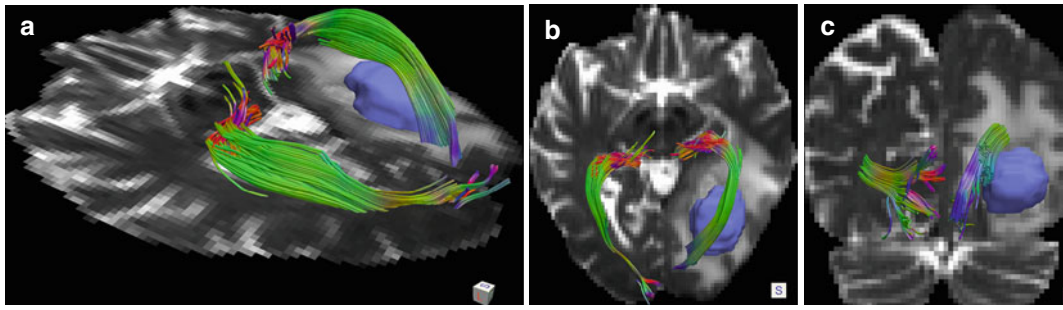

Fig. 20.6 MR diffusion tractography in a 76-year-old male with glioblastoma multiforme in the deep white matter of the right occipital lobe. Left lateral oblique view (**a**), view from above (**b**), and posterior view (**c**) of the trajectories (orientation color encoded) of the left and right optic radiations (OR) overlaid over axial and coronal T2-weighted MR images. The mass is in *blue*, surrounded by extensive vasogenic edema. The tumor has dislocated dorsally and medially the right OR. Demonstration of the relationship of the tumor with the OR trajectories was very valuable for presurgical planning. DTI dataset was acquired at 3.0 T (Siemens Verio) with 2.0*2.0*2.0 mm^3 spatial resolution, *b* value = 1,500, 64 gradient directions, and processed with spherical deconvolution algorithm [68]. Tracking was performed with trackvis using two ROI delineated in the lateral geniculate body (seed) and ipsilateral occipital white matter (target)

LGG artificially decreases FA values used for tracking. Several authors have proposed lowering the threshold from 0.15 to 0.1 in order to increase sensitivity [29]. Mass effect associated with gliomas may displace and compress adjacent white matter tracts. Vasogenic edema associated with high-grade gliomas may disaggregate the microstructure of compact bundles. Bundles floated with excessive amount of free water in areas of vasogenic edema may show symptoms of decreased conduction velocity, but they may be still viable and recover their function after steroid therapy [45].

Diffusion anisotropy is typically reduced in areas infiltrated or destroyed by the tumor. In these areas, diffusion imaging detects loss of anisotropy with true negative tractography results. Displacement and compression by mass effect are less severe fiber architectural changes. Color-coded maps and tractography in particular are very valuable to depict tracts that are still morphological intact but displaced. Radial diffusivity is often decreased in compressed tracts leading to effective increase FA [70]. Thus, mass effect may turn out to facilitate tracking of bundles whose trajectories are not easily demonstrated under physiologic circumstances (Fig. 20.6). On the contrary, excessive free water associated with vasogenic edema may artificially decrease FA of viable bundles in peritumoral areas leading to false-negative tractography results [45, 71, 72].

It is important to remark that streamlines in tractograms are virtual estimation of the orientation of white matter bundles. The estimate depends on diffusion measurements of the microstructural tissue properties. The degree of uncertainty of the estimate is reduced in anatomical and pathological conditions: in voxels with more than one bundle (such as in the deep fronto-parieto-temporal white matter at the crossroad between the corticospinal tract, corpus callosum, and SLF) and in voxels with increased free water content secondary to tumor infiltration or edema leading to apparent reduced anisotropy. The former type of challenge can be overcome with advanced diffusion methods such as HARDI [73] and constrained SD [74] which have the ability to extract multiple orientations of fibers in voxels containing more than one bundle. For instance, SD provides better results than DTI to identify the trajectories of the optic radiation, a relatively thin bundle at its origin near the lateral geniculate nucleus, on the healthy and pathological side (Fig. 20.6). The latter type of challenge can be overcome with advanced imaging methods able to separating tract diffusion properties from interstitial free water. Implementation of new advanced methods such as CHARMED [75], AxCaliber

[76], ActiveX [77], and Noddi [78] should offer a new class of microstructural tissue parameters, such as mean axonal diameter, that may give a more specific estimation of regional changes than current parameters derived from DTI. In the near future, clinical implementation of the new methods in patients with glioma may have the potential to provide tract trajectories that will be more precise, reproducible, and less user dependent.

Interpretation of Clinical Studies

Interpretation of fMRI and DTI studies requires a high degree of experience. The neuroradiologist must have knowledge of nodes and pathways that constitute a functional network. She or he must understand which sites and tracts are likely to be considered eloquent structures according to intraoperative ESM outcome studies [79]. fMRI and DTI results must be evaluated together in a congruent manner. Interpretation of conventional MR imaging results should guide interpretation of functional data.

Interpretation begins at the MR console where the DICOM images must be inspected for quality, motion, and EPI artifacts. If needed, the acquisition should be repeated after adjustments to reduce artifacts are taken. The data preprocessing pipeline should include current state-of-the-art steps suggested above.

On fMRI statistical maps, the location of positive and negative cortical sites must be accurately identified. The spatial extension of the BOLD response in each location must be evaluated at different statistical thresholds to choose the optimal value for each individual. Spatial extent of the BOLD response can be highly variable among subjects, since it depends on the quality and quantity of signal acquired, rather than on intrinsic boundaries of brain function [80]. The following language tasks are the most clinical relevant: denomination (picture naming), verb generation, and sentence comprehension.

Object naming evaluates prosody production. Look for activation in the frontal opercular region (pars opercularis and BA6) and in the posterior temporal cortex (MTG and STS). This task is good to evoke BOLD response in the classical areas of the language network that are connected by the AF and IFOF. The AF mediates phonologic processes, while the IFOF mediates semantic processes. In a good quality fMRI study, additional areas are usually activated in pre-SMA and SMA (prosody planning), in the face area of the premotor cortex (motor planning), and in the face and hand areas of the postcentral gyrus (sensitive component). Lateralization of the BOLD response in the premotor cortex correlates well with the Edinburgh Handedness Inventory test. Finally, look for clusters of BOLD response in the fusiform gyri bilaterally and in the contralateral cerebellar dentate nucleus.

The BOLD response evoked by the verb generation task is broader than picture naming. The former task is performed mainly for language lateralization purposes. The association between the semantic component and utilization of the presented object is strong. The BOLD response in the frontal operculum is quite precise and accurate: it locates in the anterior or posterior banks of the ventral precentral sulcus, and it usually varies little among individuals. The location of the fMRI cluster around pars opercularis is very useful to identify variants of sulci anatomy, and it is very useful for intraoperative ESM [81]. A strong BOLD response is also evoked in the middle frontal gyrus (BA9/46), SMA, STS, and fusiform gyri bilaterally and in the contralateral cerebellar dentate nucleus.

Other language tasks are occasionally used in the clinics. Verbal fluency (word generation) is good for determining language lateralization with a robust BOLD response in the frontal operculum and MFG (BA9/46); however it is less clinically valuable. Sentence or word comprehension evokes BOLD response in the posterior temporal lobes, they are not very useful for language lateralization, and they are usually performed for research purposes.

Interpretation of DTI results begins with inspection of color-coded maps. These maps show the main orientation of bundles for each voxel, and they are very handy for a preliminary assessment of the spatial relationship between the mass and the tracts of interest. The CST runs

cranio-caudally from the primary motor cortex to the medulla; therefore, it is blue along most of its course. In the centrum semiovale and at the level of the corona radiata, it runs between the SLF (laterally) and the corpus callosum (medially).

The AF has a characteristic C shape. On axial color maps, the AF runs lateral to the corona radiata, medial to the body of the corpus callosum. On FA maps, the color of the AF changes according to its orientation in space: it looks green in the centrum semiovale and in the temporal lobe, blue at the level of the FA isthmus, and red when it projects to the cortex in the posterior bank of the precentral sulcus, inferior parietal lobule, and STS.

The IFOF is a thick association tract that on sagittal images has the shape of a bow tie. On color-coded maps, it looks green on most of its course. The anterior extension projects to pars orbitalis and triangularis of the IFG; the posterior extension projects to the superior parietal lobule and to the occipital lobe. When your neurosurgeon knows white matter tract anatomy very well, color-coded maps are a marvelous tool, and it is almost all you have to offer him for presurgical planning. Tractography is a great research tool that at time is very clinically valuable to understand the relationship of tumor infiltration with the patient's functional performance (Figs. 20.3, 20.4, and 20.5). However, it is labor intensive and very much user dependent. Accuracy of tractography results depends on multiple factors: type of diffusion imaging acquisition, pre-processing, diffusion model, fiber-tracking algorithm, and trajectory reconstruction strategies. It is mandatory to understand the multiple factors involved before attempting virtual dissection of any major tract with tractography.

Correct terminology is important when neuroradiologists describe tractography results. Use words as streamlines and trajectories; avoid axons, bundles, and even tracts because these words are more appropriate for description of biological structures than maps. It is also worth it to repeat MR tractography that cannot determine the direction of a trajectory; it cannot provide information about the function of a tract. Fiber tracking may not be feasible in few glioma cases; in others, tractography results might appear inaccurate to the experienced anatomist showing false-negative and positive streamlines; thus, their predictive value may be elusive. Most of the studies published in the literature so far are feasibility studies that have used a priori anatomy knowledge to preliminary validate the results of tractography. However, validation with an appropriate reference index is a critical step in order to use tractography for presurgical mapping.

Imaging Integration in the Operating Room

Diffusion MR tractography has recently emerged as a valuable clinical tool for presurgical planning [4, 82, 83] and intraoperative imaging-guided navigation in the operating room [84].

Three-dimensional objects of preoperative virtually dissected tracts can be reliably integrated into a standard neuronavigation system, allowing for intraoperative visualization and localization of the main tracts [85]. MR tractography may show the relationship of the mass to the virtually dissected AF. Virtual dissection of the three segments of the AF may show whether the mass has partially interrupted or only displaced the tracts. Display of MR tractography results may also be useful in the operating room when the neurosurgeon is approaching an important bundle and he wants to refresh his anatomical orientation in the operating field and consider whether to use subcortical ESM to test the functional relevance of a specific tract [29].

Modern cognitive models of language have shown that there is a lot of redundancy in the language network. It is of paramount importance to identify those bundles that if severed may cause permanent language deficits. Definition of which tracts are indispensable (eloquent) and will have to be absolutely spared during resection remains an important issue.

The long list of important limitations must be well understood before the results of presurgical MR tractography dissections can be safely exported to the operating room. It will have to be considered that resection of infiltrated tracts that appear interrupted on DTI MR tractography (false-negative

results) may result in postoperative neurologic deficits. On the contrary, it is not yet established whether resection of redundant tracts that may be anatomically intact within the tumor boundary will cause permanent postoperative deficits. Severing of which of the many language connections in the dominant hemisphere will cause aphasia remains to be determined [56].

Methods Limitations

Functional MR Imaging

There are several limitations inherent to the fMRI technique. There are important differences in the time course of electrophysiological and hemodynamic responses. This uncertainty of neurovascular coupling is a big challenge and a potential source of error in interpreting clinical fMRI studies. The BOLD response often localizes in the adjacent sulcus, and it can be few millimeters away from the electrophysiologically functioning cortex. Localization of an important part of the BOLD signal with the draining vasculature is very well known and studied [86]. Not least, it should be kept in mind that activation of an area detected by fMRI and PET predominantly reflects the input to the area and the corresponding changes in information processing rather than output (i.e., neuronal firing) from that area [13].

Diffusion Imaging

Three main types of limitations will be considered: (a) inherent to the DTI model, (b) associated with tractography algorithms, and (c) related to tumor invasion.

The most important limitation of DTI is that only one orientation is measurable within each voxel. The measured eigenvector is the average of the orientations of all bundles included in that voxel. Since the voxel size is much bigger than the axonal bundles contained within it, DTI cannot resolve multiple bundle orientations and in particular crossing bundles. The limitation of DTI in detecting crossing fibers has been addressed with the development of more sophisticated imaging acquisition schemes and HARDI [87].

Currently tractography is a user-dependent method. Limitations of tractography performed with the deterministic approach motivated the development of probabilistic tracking algorithms [88]. Limitations of early fiber-tracking algorithms have been addressed with more sophisticated reconstruction schemes such as SD [68].

Another limitation of the MR tractography is associated with the presence of a tumor. Abnormally elevated diffusivity and free water content leads to inaccurate FA measurements. This artifact is quite relevant for the study of gliomas because it may cause false-negative results in areas of T2-signal abnormality due to interstitial (Figs. 20.1 and 20.2) and vasogenic edema (Fig. 20.6). This issue is being addressed by multiple investigators who are developing new multi-compartments models such as CHARMED [75] and Noddi [78] that are capable of measuring diffusivity in compartments containing viable brain structures, tumor, and free water.

Despite several challenges and limitations inherent to current diffusion imaging methods [88], the information provided by DTI and tractography has not been available before. At the current state of the art, it is good enough to be used in the clinic. Useful imperfect and user-dependent tests are used in the clinic everyday all the time. The challenges, limitations, and pitfalls [89] should be understood carefully before interpreting the results of planning.

Conclusions

fMRI and DTI provide unique information that has been changing presurgical evaluation of patients with slow-growing gliomas and in particular when the mass is located in eloquent areas. fMRI is mainly used to identify cortical motor and language sites and their relationship with tumor boundaries. fMRI is also preferred to Wada test in order to assess laterality of the language network. Virtual dissection of the major white matter tracts with the deterministic DTI method should be used only as a road map for presurgical planning and as guidance for intraoperative subcortical ESM. In

the near future, more advanced diffusion imaging models and methods will be implemented to meet the challenges of presurgical planning in brain tumor patients.

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Tortoise software v. 1.3.0 (https://science.nichd.nih.gov/confluence/display/nihpd/TORTOISE) and Trackvis software v. 0.5.2.1 (www.trackvis.org) were used to process DTI datasets and generate virtual dissections of the white matter tracts displayed on Figs. 20.1, 20.2, 20.3, 20.4, 20.5, and 20.6.

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Magnetoencephalography, Functional Connectivity, and Neural Network Topology in Diffuse Low-Grade Gliomas

21

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Abstract

Structural as well as functional connectivity of the cerebral network may be affected by the presence of a brain tumor. Magnetoencephalography (MEG) is one of the methods to study functional connectivity. Within predefined, classical frequency bands, various resting-state network characteristics can be studied. These characteristics enable us to describe a network in terms of synchronization, clustering coefficient, and so-called small worldness. These phenomena appear to be correlated with cognitive functioning and with the occurrence of epileptic seizures in low-grade glioma patients. A better understanding of the relation between the presence of a glioma and the disruption of the neural network will in the future contribute to the planning of surgery and will make it possible to study neural plasticity.

Keywords

Low-grade glioma • Functional connectivity • Neural networks • Epilepsy • Cognitive functioning • Magnetoencephalograpy

Introduction

Much of what we know about the correlations between brain function and neuroanatomy is based on clinical observations. In the past, the neurological deficit demonstrated by a patient suffering from a focal brain lesion led to conclusions on the specialized function of that particular brain area. But the more complex a function, the more brain areas will be involved. The notion that a certain degree of brain dysfunction exists (e.g., diminished attention or altered emotion) does not allow conclusions on the precise location or extension of a lesion, because lesions interfere not only with local cortical functioning but also with the connections between cortical areas. In other words, a focal cerebral lesion may cause a focal deficit but may also interfere with connections between widely distributed cortical areas. Thus, focal lesions cause large-scale alterations in network architecture and connectivity.

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Structural, Functional, and Effective Connectivity

Connectivity, in the context of the cerebral network, can be described as structural, functional, or effective [1, 2]. Structural connectivity refers to anatomical connections. Functional connectivity is a more complex conception. It denotes any statistical association or dependency between two different parts of the nervous system. Effective connectivity means that directed or causal relationships between elements exist. When the comparison with the European railway network is made, structural connectivity can be displayed by a map that contains all the local and international railways and gives an overview of the physical "connections" between railway stations. However, such a map will not provide travelers with information on time schedules, duration of the trip, or railway stations where trains have to be changed. And such information on "functional connectivity" is indispensable when planning a journey. Effective connectivity means that a person who travels from A to B will be able to carry out an action in B, for example, meeting another person or attending a conference, which would not have been possible if the journey would not have been made. It also implies that traveling from A to B is not always the same as traveling from B to A.

Optimal knowledge not only on the precise location of eloquent areas but also on the connectivity between these areas (referred to as the "connectome") will contribute to the possibilities of the neurosurgeon to achieve resections of low-grade gliomas (LGGs) that are as extensive as possible. As has been demonstrated by Pallud and coworkers [3], conventional MRI underestimates the actual extent of LGGs. The outcome of surgery might improve when the resection is extended beyond the region of MR-defined abnormalities, while still avoiding critical functional areas (or "functional hubs" in network terminology). Yordanova et al. [4] explored this concept. They analyzed the outcome of awake surgery with intraoperative functional electrostimulation mapping in 15 patients who underwent a so-called supratotal resection of an LGG located in noneloquent areas in the left dominant hemisphere. The results, with regard to recurrence rate, anaplastic transformation, and the need for adjuvant treatment, were better than in a group of comparable LGG patients who underwent "only" a total, and not a supratotal, LGG resection, because in these patients the lesion involved eloquent areas. This study is a plea for an extension of surgical resection beyond the borders of MR-defined abnormalities. Apart from that, the study also showed that 60 % of patients who underwent a supratotal resection had a transient, postoperative clinical worsening, particularly of language function, but they all recovered their preoperative neurological status within a few weeks after surgery. Moreover, it appeared that epileptic seizures were under control after surgery, allowing a decrease or even cessation of antiepileptic treatment. It seems obvious that the favorable results of this study should be ascribed to the use of intraoperative mapping (and, of course, to the technical skills of the surgeons). However, intraoperative electrical stimulation, although being the gold standard for the localization of functional areas, has its limitations [5]. It is a time-consuming invasive procedure, and the number of tasks is limited as patients who undergo an awake procedure tend to get fatigued. Moreover, there is an increased risk of epileptic seizures. Therefore, the question arises if the use of techniques that preoperatively provide insight into functional relations between brain areas may further contribute to the improvement of survival with preservation of function.

Techniques Employed to Measure Connectivity in the Brain

The mapping of structural networks in the human brain should lead to a "connectome" [6], which could be conceived as the description of all structural elements in the nervous system. Structural mapping can be done by means of diffusion tensor imaging (DTI), an MRI technique that makes it possible to tract myelinated nerve fibers. Diffusion spectrum imaging (DSI) is another MRI technique, which is very laborious, but has

the additional capability to resolve multiple directions of diffusion within the white matter. The advantage over DTI is that it visualizes more details of the cerebral network. Alternative techniques to reconstruct structural brain networks from MRI data in individual subjects have recently been introduced [7]

However, we need other techniques to elucidate how this structural network architecture supports neurophysiological interaction. Functional MRI (fMRI) is such a technique. It relies on hemodynamic correlates of neuronal activity. It has good spatial resolution, but – in contrast to electrophysiological techniques – it has poor temporal resolution.

Electroencephalography (EEG) and magnetoencephalography (MEG) do not rely on hemodynamic or metabolic fluctuations. These techniques provide us with a direct reflection of neuronal activity. For that reason, they have a much better temporal resolution than fMRI. On the other hand, their spatial resolution is in the order of millimeters or even centimeters. However, due to new advanced technical possibilities, this will probably improve in the near future [8, 9].

MEG measures brain activity by detecting very small perturbations in the extracranial magnetic field that are generated by the electromagnetic activity of populations of neural cells.

An advantage of the use of MEG over the use of fMRI in patients with brain tumors is that MEG is not influenced by the alterations in metabolism or blood flow of a specific brain area by the tumor [10, 11].

With MEG it is possible to study functional connectivity. All regions of the brain show oscillatory electrical activity, and the correlation between these oscillations in different brain areas is a measure of functional interaction between these respective areas. These relations can be studied during specific tasks but also at rest.

Which oncological mechanisms make that LGGs may affect this functional connectivity? Some LGGs show predominantly "invasive" behavior, whereas others have a more "proliferative" growth. [4]. Proliferative growth may result in local compression of brain structures, and infiltrative growth may cause destruction of tissue (cortical tissue, but also myelinated fiber tracks). Both types of growth will interfere with local connectivity of brain areas, and these alterations may be observed both during activity as well as during resting state. Measurement of resting-state connectivity has the obvious advantage that it can be done without cooperation of the patient [5].

MEG measures magnetic flow within the brain, and this results in one time series per MEG sensor. The skull and the scalp have no influence on the magnetic field patterns and, for that reason, the MEG signal is less transformed than the EEG signal. Another advantage of MEG over EEG is that it does not require a reference electrode, which makes it a more straightforward technique than EEG. With MEG it is more or less possible to "look through the skull." This makes that MEG – in this respect – is comparable to corticography. MEG and EEG both have the disadvantage of volume conduction, which means that signals that are picked up by different electrodes or sensors may originate from the same source. Volume conduction could lead to an erroneous interpretation of similarity between signals, suggesting a certain degree of connectivity that, in fact, does not exist. Meanwhile, several analyzing tools have been developed to overcome this disadvantage. Later in this chapter, we will give some more detailed information on these improvements.

MEG signals, just like EEG signals, are categorized within frequency bands, which are defined as follows: delta (0.5–4 Hz), theta (4–8 Hz), lower alpha (8–10 Hz), upper alpha (10–13 Hz), beta (13–30 Hz), lower gamma (30–45 Hz), and upper gamma (55–80 Hz). Within these predefined frequency bands, the correlation between time series of different MEG channels can be calculated. A measure for the functional connectivity between these individual channels is the synchronization likelihood (SL) [12]. This measure takes both linear and nonlinear coupling between time series into account and varies between 0 (total absence of synchronization) and 1 (total synchronization). Making use of this measure, it was possible to demonstrate an increase in theta band synchronization during the retention interval of a memory task in healthy volunteers [13].

Impact of Brain Tumors on Functional Connectivity and Network Architecture

One of our first studies in patients with brain tumors was aimed at three questions [14]. (1) Is there a loss of functional connectivity in these patients using the SL applied to MEG signals? (2) If there is loss of functional connectivity, is this restricted to the region of the tumor or does it extend beyond these margins, especially to the contralateral hemisphere? (3) Is such loss of functional connectivity particularly found in the gamma band, which is a relevant frequency band for cognitive processes?

We demonstrated that there was loss of functional connectivity in brain tumor patients in comparison to control subjects. Moreover, in these brain tumor patients there was no difference between the lesioned and the non-lesioned hemisphere, which indicates that loss of connectivity was also present in the non-lesioned hemisphere. When the SL was computed in the gamma band, it appeared that the patient group as a whole again showed a decreased connectivity in comparison to control subjects. However, there was a wide variance in the patient group, with some patients showing no loss of connectivity at all, whereas other patients had an almost complete loss of connectivity in the beta frequency range. It is of interest to mention that abnormal findings were more frequently present in patients with left hemispheric tumors. Of course, it should be kept in mind that the global loss of connectivity that we observed in these patients could be a consequence of the presence of the tumor but that most patients had also received radiotherapy and were being treated with antiepileptic drugs (AEDs), both factors that have an established influence on cognitive functioning and, therefore, may also be supposed to affect connectivity [15, 16].

A next study addressed the question whether the network architecture – again within the predefined frequency bands – might be modified [17] by the presence of a brain tumor. This architecture was estimated from graph analysis, applied to the MEG recordings of the same group of 17 brain tumor patients. After conversion of the SL matrix to a graph, two parameters were used to quantify the network: the clustering coefficient (C) and the characteristic path length (L). The computation of these two measures is described in Stam et al. [18], and an overview of graph analysis is given by Stam and Reijneveld [19]. These two parameters characterize the architecture of a network in terms of "regular" and "random." In short, the significance of these measures can be summarized as follows: C is defined as "the likelihood that neighbors of a vertex are also connected." To compute the clustering coefficient of a certain vertex, the first thing that has to be done is to determine to which other vertices this one vertex is directly connected. These vertices, which are all one vertex away, can be defined as the neighbors. The clustering coefficient is the ratio of all existing edges between these neighbors and the maximum possible number of edges between the neighbors. This means that C ranges from 0 to 1. When the clustering coefficient, as described above, is computed for all vertices of the network and then averaged, we have the disposal of a measure for the tendency of the network elements to form local clusters.

L is defined as "the average of the shortest distance between pairs of nodes counted in the number of edges." Or, in other words, "how many steps does it take on average to get from a particular node to any other node in the network?" This measure indicates how well the network elements are integrated.

Referring to the earlier-mentioned metaphor of the European railway system, this theory means that traveling between cities in various countries is easier when long-distance (high speed) trains do not stop too often (low L), and when – starting from the railway stations where the long-distance trains do stop – local stopping trains frequently leave to various small interconnected stations (high C).

Using these two measures, we can resume the theory as follows: regular networks are very clustered (high C), but it takes a lot of steps to get from one side of the graph to the other (high L). In contrast, random networks lack this high clustering (low C) and their path length is short (low L). In our study, brain tumor patients were found to have val-

ues for local and long-distance couplings that differed significantly from the values of healthy controls. With regard to the local couplings, increases in alpha, theta, and delta bands were found. For the long-distance couplings a significant decrease in beta and an increase in alpha and delta bands were observed. The main results of this study confirmed the previous findings with respect to an altered functional connectivity of the brain in tumor patients. These alterations involve also intrahemispheric connectivity. Further, the effects differ for the various frequency bands, with predominance for a decrease in high-frequency bands for long-distance connections and an increase in low-frequency bands for local connectivity. It is very difficult to give an unambiguous interpretation of these results, as the significance of the various frequency bands for local and long-distance connectivity remains largely obscure. The most affected long-distance couplings appeared to be the frontoparietal interactions with decrease in both the gamma and beta synchrony and an increase in synchrony for the delta band. This is relevant with respect to the study of Halgren et al. [20], who found that in normal subjects working memory and direct attentional tasks involve transient synchronization between frontal and parietal regions.

The Small-World Phenomenon

In our above-mentioned study [17], an attempt was made to analyze the architectural properties of the cerebral network, according to the pioneering work of Watts and Strogatz [21]. We made use of the parameters "C" and "L." Watts and Strogatz were the first to show that networks (or "graphs") which combine a high number of local (short-distance) connections with a few (random) long-distance connections are characterized by a high *C* and a low *L*. Networks like this are optimal and are usually referred to as "small-world networks."

Network (or graph) theory originates from the fields of mathematics and sociology. The combination of these two has led to methods of analyzing all kinds of networks, including railway systems but also the human brain, by representing these networks in an abstract, theoretical figure called a "graph." The challenge of the study of networks is to find the universal parameters according to which all kinds of biological and social networks – including neural networks –can be defined. In general, networks combine two concepts: integration and segregation [22]. And the most optimal functioning networks have so-called small-world characteristics, referring to an architecture that combines high clustering with long-distance connections. As pointed out above, the consequence of such a structure is that parts of the network that are seemingly remote from each other can be coupled through a few steps. This phenomenon in which both integration and segregation are present and which is characteristic for a complex network has only recently been described.

Watts and Strogatz provided an apparently simple way of modeling small-world networks. They proposed a very simple model of a one-dimensional network on a ring as depicted in Fig. 21.1. In the "regular" network, each node (or vertex) is only connected to its neighbors.

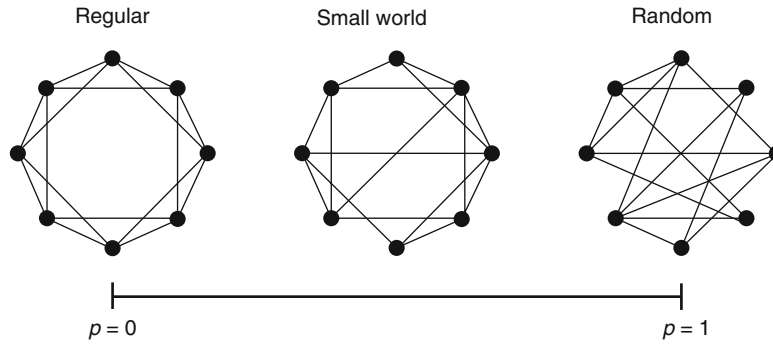

Fig. 21.1 Three network types as described by Watts and Strogatz [21]. At the one end of the spectrum is the regular or ordered network. At the other end is the random network. In between the so-called small-world network (From Heimans and Reijneveld [23])

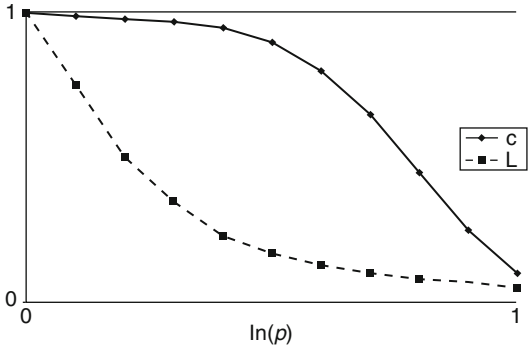

Fig. 21.2 Graph illustrating different types of networks according to graph theory. When $p=0$, the network is completely regular with high C and high L. When p is only slightly higher than 0 (which is the case when only a few edges are randomly rewired), the path length L drops sharply, while C hardly changes. At the other end of the graph the network is completely random ($p = 1$, low C, low L)

The number of neighbors (k) represents the degree of the network. Next, a few nodes are chosen at random (with likelihood "p"), and these nodes are connected to other nodes (also chosen randomly) by means of connections (or edges). Now, with increasing p, more edges become reconnected, and finally, when $p=1$, the network is completely random. This model makes it possible to study all types of networks, ranging from completely regular ($p=0$) to completely random ($p=1$).

The intermediate between random and regular network architecture explains the small-world phenomenon. As mentioned above, "C" and "L" are the two crucial measures for the classification of a network on the continuum of regular to random. Small-world properties already occur when p is only slightly higher than 0: now "L" drops sharply, but "C" hardly changes (Fig. 21.2). This means that a regular network, in which only a few connections are randomly rewired, suddenly combines a high clustering with a short path length. This is – in short – the small-world phenomenon. The measures C and L make it possible to define the index of "small worldness" [24]. Almost all existing networks, varying from railway networks to the World Wide Web and from social to neural networks, show some degree of small worldness.

In a study in 28 healthy volunteers [25], the correlation between resting-state small-world network topology (measured with MEG) and cognitive performance was studied. A higher "small-world index," defined as the ratio between normalized clustering and path length, in the theta and lower gamma bands appeared to be related to a better cognitive performance. The clustering coefficient in the delta and theta bands was also positively correlated to global cognitive functioning. Moreover, the study showed that there was a gender difference: the female brain has shorter path length than the male brain, which indicates that the female brain has a more efficient network architecture. In a resting-state fMRI study [26], an association between intelligence and a shorter path length was found, in particular for the highly connected default mode network.

We have looked at the changes in functional connectivity due to surgical treatment in 15 brain tumor patients [27]. The patients had various tumor histologies (low-grade gliomas, high-grade gliomas, and meningeomas) and all underwent maximal debulking of tumor. After tumor resection, functional connectivity appeared to have changed in a complex way. There was a decrease in long-distance interhemispheric connectivity in the theta band. Other patient-related factors or tumor- or treatment-related factors had no influence on this change, so the effect must be attributed to the surgery itself or to the effects of surgery, that is, reduction of tumor volume and edema and, subsequently, reduction of compression of brain tissue. The decrease in interhemispheric connectivity was most prominent in patients who were free of epileptic seizures, postoperatively. Again, it is impossible to give a proper and fully unequivocal explanation of these observed phenomena with the current knowledge.

It is of importance to note that, in this particular study, we made use of the so-called phase lag index (PLI) as a measure for functional connectivity. This measure is extensively described by Stam et al. [28, 29] and can be defined as the asymmetry of the distribution of instantaneous phase differences between two MEG signals. Hereby, the assumption is that the presence of a consistent, nonzero phase lag between two time

series cannot be explained solely by volume conduction. This means that real interactions between two areas are more likely than volume conduction effects when this parameter is used, and in this respect, the PLI is superior to the SL which was used in former studies

Correlation Between Network Disturbances and Clinical Functioning in Low-Grade Glioma Patients

It has been shown that resting-state connectivity, as registered with MEG, is a reliable indication of neuropsychological functioning in patients with Alzheimer's disease [18].

We investigated network functions in relation to cognitive functioning in low-grade glioma patients [30]. This study will now be described more extensively, because it produced results that are pivotal to the question whether MEG may have a function in the study of connectivity in LGG patients. Our hypothesis was that changes in functional connectivity are the intermediate between the impact of the tumor and the antitumor and antiepileptic treatment, on the one hand (this could be defined as "input"), and cognitive performance (defined as "output"), on the other hand. This interaction is illustrated in Fig. 21.3. Seventeen LGG patients were selected for participation in the study. The patients had shown no radiological or clinical signs of tumor progression during the previous 6 months. The results obtained in these patients were compared with the results in healthy controls (relatives of the participating patients or staff members of our hospital). Cognitive performance was measured by an extensive standard testing battery, containing tests that reflect a wide variety of cognitive abilities, such as psychomotor function, executive (frontal) function, (selective) attention, mental processing speed, mental control, verbal learning, organization, memory, mental concentration, information processing, and flexibility of verbal thought processes. It took between 60 and 120 min to complete this assessment. The data that were collected in this way were reduced to six cognitive domains (which are commonly used in neuropsychological research): (1) information processing speed, (2) psychomotor functioning, (3) attention, (4) verbal memory, (5) working memory, and (6) executive functioning.

For the MEG recordings, we used a 151-channel whole-head MEG system (CTF Systems Inc., Port

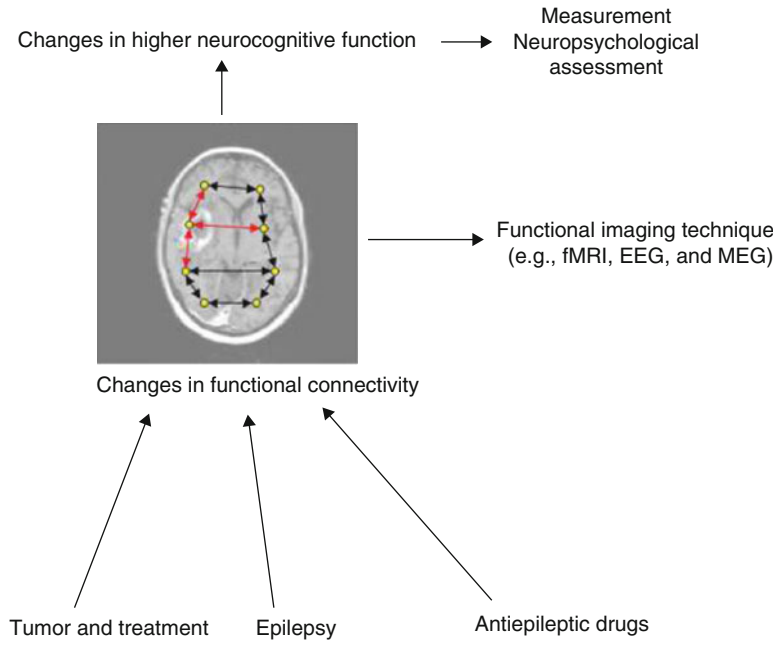

Fig. 21.3 Diagram of the relationship between tumor-related factors, cognitive functions, and functional connectivity in LGG patients (From Bosma et al. [30])

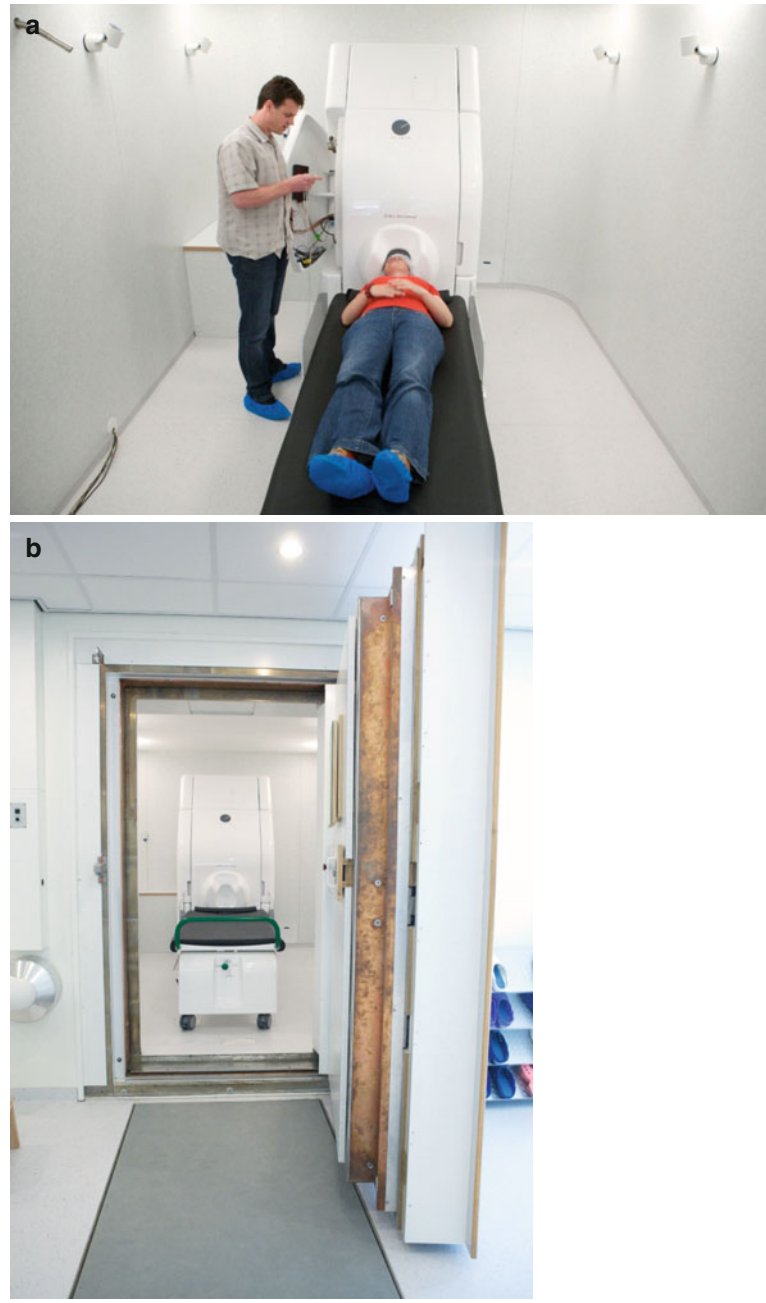

Fig. 21.4 (**a**, **b**) The MEG system as used in the Department of Clinical Neurophysiology of the VU Medical Center

Coquitlam, BC, Canada) which was mounted in a shielded room and where the patient was sitting in an upright position (Fig. 21.4). The recording was performed during a no-task, eyes-closed resting state. From the files, four artifact-free samples of 13 s were selected by visual analysis. Magnetic field frequencies between 0.5 and 80 Hz were recorded. Just like in previous studies, the SL was used as a measure of statistical interdependencies between time series from different MEG sensors, and values were calculated for every possible pairwise combination of MEG sensors for all frequency bands. MEG sensors were grouped according to their location in central, frontal, occipital, parietal, and temporal areas.

Subsequently, three types of SL averages were calculated: (1) five interhemispheric between-area SLs, (2) eight (four per hemisphere) long-distance intrahemispheric SLs (frontotemporal, frontoparietal, parieto-occipital, and temporo-occipital), and (3) ten (five per hemisphere) within-area local SLs. The latter were a measure for short-distance connectivity, while the first two SL types can be regarded as a measure for long-distance connectivity.

The patient group did not differ from the control group with respect to age, gender, and educational level. For the patients, the mean time between diagnosis and this study was 8 years, with a range of 1–19 years. Seven patients had been treated with radiotherapy, and two patients had received prior chemotherapy (the combination of procarbazine, lomustine, and vincristine). Sixteen of the 17 patients used antiepileptic drugs, and of these, six were free of seizures.

Regarding cognitive functioning, we found – as could be expected from the results of one of our previous studies [15] – that patients performed poorer than control subjects. This was the case for the overall measure of cognition as well as for psychomotor function, working memory, information processing speed, and attention. There were no significant differences for the domains of verbal memory and executive functioning. Regarding the functional connectivity outcome, it appeared that long-distance functional connectivity was abnormally increased in the patient group in comparison to the controls. This was the case for a total number of eight connections in the delta, theta, and lower gamma bands. A significantly decreased long-distance connectivity in patients was observed for the intertemporal connectivity in the delta band and for the interoccipital connectivity in the lower alpha band.

Obviously, the most important question was if there was an association between the cognitive outcome and the outcome of the connectivity studies. With regard to the previously mentioned disturbed cognitive domains, post hoc regression analysis showed that an increase in long-distance and short-distance synchronization for the delta band in the left frontotemporal region, the right frontoparietal region, the right parieto-occipital, and the left temporal region was associated with decreased working memory. Also within the delta band, it appeared that decreased attention was associated with an increase in connectivity in the left frontotemporal, the left temporo-occipital, the right parieto-occipital, the right temporo-occipital, the interoccipital, the interparietal, the (intraregional) left temporal, and the (intraregional) right occipital regions. Decreased working memory was associated with increased connectivity in the theta band in the left frontotemporal region, whereas decreased attentional tasks were associated with increased connectivity in the theta band in the left frontotemporal, left temporo-occipital, interfrontal, interoccipital, and interparietal regions. In the lower gamma band, an increase in synchronization in the left frontotemporal and left temporo-occipital regions was associated with diminished attentional tasks. In the upper gamma band, an increase in synchronization in the right temporo-occipital and the left frontal regions was associated with a decrease in information processing. Within this same upper gamma band, an increased synchronization in the left temporo-occipital region was associated with worsening in attentional tasks. The only association between an increase in synchronization and better cognitive functioning was found in the delta band in the right frontal region: a better working memory was correlated with increase in synchronization.

How should these findings be interpreted? It seems clear that diminished cognitive performance (especially in working memory and attentional tasks) is associated with pathologically increased connectivity, above all in the lower-frequency bands (although the same was observed in the lower and upper gamma bands), for a number of long-distance connections. It could be speculated that these changes indicate a compensatory mechanism in LGG patients: they might need an increase of synchronization in order to compensate for their diminished cognitive performance. But it might also indicate a "disinhibition": local inhibiting connections are disrupted, and this leads to slowing down and higher amplitude of oscillations and subsequently to increase of cortico-cortical connections. Anyhow, these findings strongly suggest that changes in resting-state

synchronization are not simply an epiphenomenon, but may be relevant for the observed cognitive impairments, although the causal relation of the association remains unclear.

It must be stressed that the findings of this study regarding functional connectivity are not completely consistent with the findings of our earlier study [17], which is cited above. In that study we reported an increase in the low-frequency bands for the short-distance connections and a decrease in high-frequency bands for the long-distance connections. In that same study a decrease in the lower alpha band and an increase in the lower gamma band were found. It is – again – not clear how these conflicting results should be explained, but at least differences in the patient populations of both studies (LGG patients versus patients with a mixture of primary intracranial tumors) may lie at the root of these divergent findings.

Another study [31] explored further details of network architecture in that same LGG patient group, and, moreover, it explored correlations with cognitive functioning. The PLI, which was also used in the aforementioned study by Douw et al. [27], was used to detect synchronous neural activity and to generate connectivity matrices that could be converted into graphs. As mentioned before, the main advantage of the use of PLI in this type of research is that it is less sensitive to volume conduction than other parameters of functional connectivity, and it therefore measures "true interactions." Our hypothesis was that functional connectivity in LGG patients – expressed by the PLI – is diminished in comparison to healthy controls. Secondly, we expected to find a loss of small-world characteristics (by computing the clustering coefficient (*C*), the path length (*L*), and the "small worldness" (S) which is based on the trade-off between high local clustering and short path length) in the neural network of LGG patients, with subsequent loss of cognitive performance. PLI values were calculated for the same pairwise combinations of MEG sensors and for the same frequency bands as was performed for calculation of SL values in the previous study.

It appeared that higher synchronization was present in the theta frequency band for short-distance connections and for interhemispheric connectivity. For all frequency bands, the value of the clustering coefficient was higher than in random networks for both the patient group as well as for the control group. Differences between patients and controls were found within the theta, the beta, and the upper gamma bands. In the theta band, the clustering coefficient was significantly higher in the patient group than in the control group. In the beta band, the opposite was the case. Moreover, the patient group showed a lower small worldness than the control group.

In the delta and lower alpha bands, interactions between network characteristics and cognitive functioning (attentional tasks and executive functioning) were observed. In the delta band, a longer path length was associated with poorer executive and attentional functioning, whereas a higher degree correlation (which indicates if the degree of a vertex is influenced by the degree of the vertices to which it is connected) was associated with better attentional functioning. In the lower alpha band, an interaction existed between network characteristics and verbal memory: increases in clustering coefficient and path length were associated with diminished verbal memory.

Summarizing the results of this study, we showed that there was a higher synchronization in the theta frequency band in LGG patients in comparison to controls and that there were differences in network organization. Moreover, these changes appeared to be associated with cognitive performance. The calculation of the PLI pointed to an increased theta band functional connectivity in LGG patients, and because PLI is hardly affected by volume conduction, this should be regarded as a robust finding. This increased theta band synchronization has also been observed in other brain diseases, such as autism spectrum disorders and Alzheimer's disease.

Local clustering, pointing at a small-world organization, was increased in the lower frequency bands in LGG patients, whereas there was a decrease of local clustering in the high-frequency bands.

Summarizing these results, it appears to be clear that network configuration and functional connectivity are related to cerebral performance

in LGG patients. Both increased and decreased connectivity for the various frequency bands may be demonstrated in the same patient, and these findings may again differ for long-distance and short-distance connections. The significance of many of these details still has to be elucidated, and it is obvious that the unraveling of these questions will take a lot of time and a lot of effort, but it is also becoming clear that the use of network parameters, as described above, will enable us to understand more about plasticity of the brain and about the way that primary brain tumors interfere with the network and with both local and general functional connectivity.

Assessment of Functional Connectivity and Clinical Applications

The aforementioned studies were primarily aimed at the clarification of general network disruption by a localized lesion. A more direct approach to the use of MEG in neurosurgical practice was performed by Ganslandt et al. [32]. In this study, MEG was preoperatively used to identify the sensorimotor cortex. The MEG results were superimposed onto a three-dimensional MRI. The term "magnetic source imaging" was used for this technique, and a total of 50 patients with a variety of intracranial tumors, all located in the central region were examined and operated. The authors were able to distinguish sensory-evoked fields and motor-evoked fields in all patients. Sensory-evoked fields were monitored by applying tactile stimuli to the thumb, the index finger, and the little finger on the side contralateral to the lesion. For the registration of motor-evoked fields, patients were asked to perform repetitive tapping with the contralateral index finger. Subsequently, the calculated cortical fields were used for image fusion with MRI. The procedure in which preoperative MEG findings are used for the optimization of the neuronavigation procedure is referred to as "functional neuronavigation." The main advantage of this procedure is that it allows preoperative assessment of the relation of the tumor with the sensorimotor cortex and thus makes it possible to predict the type of surgery needed in an individual case. The authors conclude their paper with the question if the clinical benefits of the study can justify the costs. They point to the fact that intraoperatively SSEP data can be obtained faster and with lower costs.

The quantitative comparison between preoperative magnetic source (MS) imaging and intraoperative sensory and motor mapping has been studied by Schiffbauer and colleagues [33]. They found an MS imaging source localization accuracy of 12.5 mm and considered this to be "…a reasonable starting point in the preoperative planning of surgical approaches, resection strategies, and treatment options." MS imaging is considered as an aid to intraoperative cortical mapping in patients with intra-axial brain tumors.

An MEG-based approach to study the relation of changes in resting-state functional connectivity with structural lesions was performed in 15 patients with unilateral lesions, one patient with bilateral lesions and 14 healthy control subjects [34]. Detailed analyses of connectivity were focused on the alpha frequency band. Significantly lower connectivity values were found in brain areas that were disconnected in comparison to contralateral tissue. This decrease was only observed in regions corresponding to a clinically functional deficit, rather than in the entire area of the tumor extension. These regions with diminished functional connectivity could reliably be resected. Moreover, patients in whom no connectivity deficit could be demonstrated or patients with increased connectivity in the tumor area suffered from transient or permanent neurological deficits after the resection. It is interesting to note that all patients, in comparison to control subjects, showed areas of scattered or diffuse decreased connectivity, but these were unrelated to tumor location, tumor volume, or clinical deficits. This is in accordance with our own findings [14].

In a more recent study, this resting-state functional connectivity concept was further explored [5]. In 57 patients with a brain tumor near or within motor, sensory, or language areas, resting-state MEG recordings were performed. Functional connectivity was estimated by means of "imaginary coherence." This is a measure that reduces the chance of overestimating the magnitude of connectivity as a

consequence of crosstalk between voxels or common references. This method, like the PLI, makes use of the fact that phase similarities between time series that arise from a common reference or that result from volume conduction occur with zero time delay. The mean imaginary coherence between voxels in and around brain tumors and the rest of the brain was compared to the mean imaginary coherence between contralateral voxels and the rest of the brain. A second comparison was made between the local cortical connectivity pattern in the tumor area and the results of intraoperative electrical stimulation. A very important finding of this study was that the cortical maps that were obtained with MEG and that showed decreased resting-state connectivity (this was the case in 7 out of 57 patients) had a negative predictive value of 100 % for the absence of functioning eloquent cortex. On the other hand, if the functional connectivity in the tumor area was increased (which was the case in 42 out of 57 patients), a positive predictive value of 64 % for the identification of language, motor, or sensory cortex by means of intraoperative electrical stimulation was calculated. These results are encouraging and will stimulate further research on clinical applications of functional connectivity.

The potential clinical significance of these findings is obvious, but also limited at this point in time. Based on the preoperative MEG findings, individual risk profiles can be calculated before operation, and this may have consequences for the planning of the surgical procedure. The extent of resection of low-grade gliomas still depends mainly on the results of intraoperative functional mapping, which includes also the stimulation of subcortical (white matter) structures [35, 36], but the role of preoperative assessment of functional connectivity will certainly become more important in the near future.

Epilepsy in Low-Grade Gliomas and Networks

Epilepsy is the most common symptom in LGG patients, and it accounts for a significant negative attribution to quality of life, especially when seizure freedom is not achieved with antiepileptic drugs [16]. Tumor resection may contribute to seizure control. In a recently published review on 773 patients from 20 studies [37], gross total tumor resection (compared to partial resection) appeared to be the variable that was most predictive of seizure freedom. Another variable that indicated a favorable outcome of seizure control was a duration of seizures of less than 1 year. The presence of medically refractory epilepsy before surgery as well as the presence of simple partial seizures was associated with a poorer outcome. Consequences of these observations are obvious: surgery should be aimed, whenever possible, at gross total resection, and operation should be performed as early as possible.

There is accumulating evidence that, apart from the ictal onset zone, the neural networks surrounding this zone and parts of the network even lying further remote are pivotal in the initiation, and particularly in the propagation of seizures [19, 38, 39]. It is hypothesized that a loss of small-world network characteristics renders the brain more prone to seizures [39]. Also, the presence of essential clusters of connections in the network ("hubs") may play a role in the initiation and propagation of seizures [40]. The question arises whether assessment of functional connectivity and neural network architecture may contribute to the planning of the surgical procedure of patients with LGG who also suffer from epilepsy.

What do we know about neural network configuration and epilepsy? The paroxysmal phenomenon of epilepsy is related to hyperexcitation of neurons, and this leads to synchronization of large neuronal networks during the seizure [41]. Various features of the cerebral network determine to what extent the network facilitates this synchronization. The more random a network, the more susceptible it is to whole system synchronization [42].

Forty-one patients with chronic localization-related epilepsy who were analyzed with fMRI with a silent-word generation paradigm appeared to show a disruption of both local segregation and global integration [43]. In other words, the cerebral networks of these patients exhibited a loss of small-world features in comparison to a group of healthy control subjects. There also was an association between intellectual decline and a disturbed local segregation in the patient group. In an editorial

comment on this paper, a number of questions on the significance of neural network research are formulated [44]. One of these questions is as follows: "do certain network alterations predispose to the occurrence of seizures?" And another question is as follows: "can network measures be useful for the diagnosis or monitoring of therapies?"

Both epilepsy and brain tumors give rise to changes in connectivity that are most prominent in the theta frequency band, and epilepsy patients and brain tumor patients have neural networks that are characterized by a loss of small-world features. We have studied the relation between functional connectivity, network topology, and epilepsy in a group of glioma patients [45]. We were particularly interested in the correlations between network topology in the theta band and epilepsy characteristics. The majority of the 17 patients that participated in this study suffered from a high-grade glioma. It turned out that increased theta band phase lag index (PLI) was related to a larger number of seizures. This association was especially present within the temporal lobe and between the temporal lobe and other lobes. Assessments took place at two time points: (1) directly after surgery and (2) 6 months later. There were no changes in network topology or connectivity over time. For this study the "edge weight correlation" was used. This is a measure for the correlation between weights of neighboring edges, and neighboring edges are defined as edges that connect to the same vertex. A positive weight correlation indicates that transport over the network is increased. This could be beneficial for the functioning of the network, but it also increases the vulnerability of the brain to seizures, due to an abnormally high synchronizability.

In a review on the combination of EEG source imaging and EEG-correlated fMRI, the potential and the limitations of these two techniques are discussed [46]. The combination of both techniques may have clinical relevance for epilepsy surgery planning.

Future Research

When surveying the literature on neural network research, it becomes immediately clear that immense progress has been made during the past two decades, but at the same time one realizes that probably only a minor part of the query of brain functioning has been elucidated.

MEG has proven to be a method that enables us to generate numerous relevant data on functional connectivity and network characteristics like path length, synchronization likelihood, phase lag index, clustering coefficient, and small-world features. MEG has its limitations, such as the problem of volume conduction and a limited spatial resolution, but offers the unique possibility of studying neural network functioning in a noninvasive way. It is, therefore, the designated tool for the longitudinal study of network dynamics. In the specific case of the application of MEG for the study of LGG and LGG treatment, a number of questions are relevant.

First of all, further studies on the relation between network characteristics, as obtained with MEG, on the one hand, and cognition and epileptic seizures as outcome parameters, on the other hand, are warranted. The selection of the most sensitive parameters for specific questions could result from such studies.

In the second place, it would be important to explore whether the combination of MEG with other modalities, such as fMRI, adds specific opportunities.

In the third place, the value of preoperative MEG studies for the planning of surgical strategy in the treatment of LGG needs further attention. In the study of Guggisberg et al. [34] and Martino et al. [5], only the connectivity in the alpha frequency band was used as a parameter for local connectivity and, subsequently, as an indicator for resectability. It has to be seen if the alpha frequency band is the most relevant and if, for example, the theta or lower gamma frequency ranges are more relevant for certain specific purposes.

In the fourth place, the longitudinal monitoring of brain function with the help of MEG could provide us with valuable information on the dynamics of network architecture in an individual patient. The term "natural plasticity" refers to the ability of the brain to redistribute functional maps. This implies that the brain is capable to reorganize itself which is crucial to the process of recovery after brain injury or stroke [47]. But

also slowly progressive lesions, such as LGGs, may give rise to significant functional reshaping [48]. Longitudinal use of MEG in this patient category may reveal important findings on the plasticity of the cerebral network. A preoperative registration could serve as a "baseline measurement." During the postoperative period, follow-up MEG registrations may provide important information on the evolution of various network parameters, directly after the operation and, subsequently, during the process of recovery. The decision to reoperate an LGG in an individual patient, with the aim to further reduce tumor mass after initial recovery, might be supported by postoperative MEG results that indicate favorable reorganization of the network. Also the influence of other treatment modalities, such as chemotherapy and radiotherapy, on functional connectivity of the brain could be monitored by repeated MEG registrations.

The data thus obtained could be matched with the results of cognitive tests, and this would lead to a better understanding of the potential plasticity of the cerebral network. Such understanding could be deepened further with computational models of brain lesions and plasticity [49]. The identification of pivotal "hubs" in the network architecture of individual patients and the significance of these hubs for cognitive performance and for the capacity of restructuring the network might prove to become crucial for the planning of treatment strategies in LGG patients and would eventually lead to a longer survival with optimal preservation of cerebral functions.

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Interactions Between Diffuse Low-Grade Glioma (DLGG) and Brain Plasticity

22

Hugues Duffau

Abstract

The traditional view in neurooncology is to see first the tumor, with very few considerations concerning the brain. However, to select the best treatment for each patient with a DLGG, that is, to optimize the "onco-functional balance," the understanding of the natural history of this chronic disease is not sufficient. One should also study the reaction of the central nervous system elicited by the growth and migration of the glioma. Indeed, due to strong interactions between DLGG and the brain, cerebral adaptive phenomena often occur in order to maintain neurological and cognitive functions, namely, to compensate the spreading of this diffuse tumor. Here, the purpose is to investigate mechanisms underlying such brain plasticity, with the goal to tailor the optimal management according to the dynamic relationships between DLGG course and cerebral functional reorganization at the individual level. Beyond the fundamental interest, it is crucial for the (surgical) neurooncologist to improve his knowledge of brain hodotopy to elaborate new therapeutic strategies, such as multistage surgical approach, made possible thanks to cerebral remapping over years. Therefore, cognitive neurosciences seem to represent a precious help to neurooncology by opening new avenues to improve both quality of life and median survival in DLGG patients, that is, to move toward "functional neurooncology."

Keywords

DLGG • Brain plasticity • Brain hodotopy • Subcortical connectivity • Surgery • Functional neurooncology • Quality of life

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Introduction

The traditional view in neurooncology is to see first the tumor, with very few considerations concerning the host, namely, the brain. Yet, it is crucial to take into account the "onco-functional balance" when selecting the best therapeutic strategy for each patient harboring a diffuse low-grade glioma (DLGG). To this end, although the

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understanding of the natural history of the disease is of course mandatory, it is nonetheless not sufficient. One should also study the reaction of the central nervous system elicited by the growth and migration of the glioma [1]. In other words, due to strong interactions between DLGG and the brain, cerebral adaptive phenomena often occur in order to maintain neurological and cognitive functions, that is, to compensate the spreading of this diffuse tumor.

In this chapter, the purpose is to investigate mechanisms underlying such brain plasticity, with the goal to tailor the optimal management according to the dynamic relationships between DLGG course and cerebral functional reorganization at the individual level.

The Concept of Cerebral Plasticity

History

As early as the beginning of the nineteenth century, two opposite conceptions of the functioning of the central nervous system were suggested. Firstly, the theory of "equipotentiality" hypothesized that the whole brain, or at least one complete hemisphere, was involved in the practice of a functional task. By contrast, the theory of "localizationism," in which each part of the brain was supposed to correspond to a specific function, was built following the seminal description of the "phrenology." Progressively, the frequent reports of lesional studies led into an intermediate view, namely, a brain organized (1) in highly specialized functional areas, called "eloquent" regions (such as the central, Broca's, and Wernicke's areas, early identified), for which any lesion gives rise to major permanent neurological deficits, and (2) in "non-eloquent" regions, with no functional consequences when damaged. Based on these first anatomo-functional correlations, and despite the description by some pioneers of several observations of post-lesional recovery, the dogma of a static functional organization of the brain was settled for a long time, that is, with the unability to compensate any injury involving the so-called eloquent areas. However, through regular reports of improvement of the functional status following damages of cortical and/or subcortical structures considered as "critical," this view of a "fixed" central nervous system was called in question in the past decades. Consequently, many investigations were performed, initially in vitro and in animals and then more recently in humans in order to study the mechanisms underlying these compensatory phenomena: the concept of cerebral plasticity was born (for a review, see [2]). Indeed, current developments in functional mapping and neuroimaging techniques have radically changed the classical modular model for a new dynamic and distributed perspective of brain organization, that is, its capability to reorganize itself both during everyday life (learning) and after a pathological event (e.g., stroke or glioma) [3].

Definition and Mechanisms

Cerebral plasticity can be defined as the continuous processing allowing short-, middle-, and long-term remodeling of the neuron-synaptic organization in order to optimize the functioning of the networks of the brain—during phylogenesis, ontogeny, physiological learning, and following lesions involving the peripheral as well as the central nervous system. Several hypotheses about the pathophysiological mechanisms underlying plasticity have been considered. At a microscopic scale, these mechanisms seem to be essentially represented by synaptic efficacy modulations, unmasking of latent connections, phenotypic modifications, synchrony changes, and neurogenesis. At a macroscopic scale, diaschisis, functional redundancies, cross-modal plasticity with sensory substitution, and morphological changes are suggested to be involved. Moreover, the behavioral consequences of such cerebral phenomena have been analyzed in humans in the past decade, both in physiology—ontogeny and learning—and in pathology. In particular, the ability to recover after an injury of the nervous system—post-lesional plasticity—and the patterns of functional reorganization within eloquent area and/or within distributed networks allowing such compensation have been extensively studied [4].

In other words, cerebral plasticity is conceivable only in a dynamic and not rigid view of the brain. Indeed, according to new theories, the brain is an ensemble of complex networks that form, reshape, and flush information dynamically [5, 6]. Thus, reorganization could occur, based on the existence of multiple and overlapping redundancies hierarchically organized [7–11]. These findings have testified that neuronal aggregates, beside or outlying a lesion, can increasingly adopt the function of the damaged area and switch their own activation pattern to substitute the lesioned area while facilitating functional recovery following brain damage [4].

In this context, the concept of the brain *connectome* has recently emerged. Its goal is to capture the characteristics of spatially distributed dynamical neural processes at multiple spatial and temporal scales [12]. The new science of brain "connectomics" is contributing both to theoretical and computational models of the brain as a complex system [13] and, experimentally, to new indices and metrics (e.g., nodes, hubs, efficiency, modularity) in order to characterize and scale the functional organization of the healthy and diseased nervous system [14]. In pathology, brain plasticity is nonetheless possible only on the condition that the subcortical connectivity is preserved [15] to allow spatial communication and temporal synchronization among large interconnected networks—according to the principle of hodotopy (see below). Indeed, although different patterns of subcortical plasticity have recently been identified, namely, unmasking of perilesional latent networks, recruitment of accessory pathways, introduction of additional relays within neuron-synaptic circuits, and involvement of parallel long-distance association pathways, the real capacity to build a new structural connectivity ("rewiring") leading to functional recovery was not yet demonstrated in humans [16].

The Time Course of DLGG and Brain Reorganization

As already mentioned in previous chapters, DLGG is a slow-growing tumor which progressively invades the brain over years. This slow time course of the disease explains why numerous patients with DLGG have usually only mild or even no functional deficit, despite the frequent involvement of the so-called eloquent structures [17, 18]. This means that these lesions have induced progressive functional brain reshaping, as suggested by preoperative functional neuroimaging. Indeed, it was recently shown that brain plasticity cannot be fully understood and fruitfully studied without considering the temporal pattern of the injury inflicted to the brain [2]. Therefore, in acute lesion such as stroke, even if many patients improved within the months following the damage, only around 25 % of patients totally recovered [19], while more than 90 % of patients with a DLGG (same location than stroke) had a normal neurological examination (independently of the slight neurocognitive deficits often diagnosed thanks to an extensive neuropsychological assessment—see Chap. 19). Of note, the concept of "recovery" should be more clearly defined in the literature. Although this terminology should be reserved for a complete normalization of the neurological status, numerous authors speak about "recovery" in cases of partial functional improvement after brain damage. A standardization of the nomenclature is crucial to compare the results reported in the different series.

Interestingly, using a neurocomputational model based on a training of a series of parallel distributed processing neural network models, a recent work simulated acute versus slow-growing injuries [20]. The results showed a very different pattern emerging in the simulation of DLGG in comparison with the simulation of stroke, with slow decay of the links within the same subnetwork leading to minimal performance decline, in agreement with the patient literature. Moreover, at the end of the decay regime, the entire affected hidden layer could be "removed" on the simulation with no effect on performance—which closely matches the lack of major impairment from DLGG resection (see Chaps. 23 and 24). It is likely due to the fact that abrupt stroke occasions rapid neuronal death, while DLGG initially spares neuronal tissue and thus gives time for cerebral remapping. As a consequence, it could be suggested that the functional status at the time

of diagnosis might be a good reflect of the natural history of the disease, for example, a relevant insight into the behavior of the glioma—which represents a crucial issue since these tumors are very heterogeneous [21].

Concerning the neural basis of such functional compensation in DLGG before any treatment, the patterns of reorganization may differ between patients, a very important notion to know by the neurosurgeon with the goal to optimize both indication of surgery and surgical planning [22, 23]. Indeed, preoperative functional neuroimaging has shown that four kinds of preoperative functional redistribution are possible, in patients without any deficit [1, 2]. In the first one, due to the infiltrative feature of gliomas, function still persists within the tumor, thus with a very limited chance to perform a fair resection without inducing postoperative sequelae. In the second one, eloquent areas are redistributed around the tumor, thus with a reasonable chance to perform at least a near-total resection despite a likely immediate transient deficit—but with secondary recovery within a few weeks to months. In the third one, there is already a preoperative compensation by remote areas within the lesional hemisphere. Fourthly, a network of areas can be recruited in the controlateral hemisphere: consequently, the chances to perform a real total resection (or even a "supra-complete resection," see Chap. 23) of this kind of gliomas are very high, with only a slight and very transient deficit. Finally, these different patterns of reshaping can be associated. Therefore, in cases of brain lesions involving eloquent areas, plasticity mechanisms seem to be based on a hierarchically organized model, that is, first with intrinsic reorganization within injured areas (index of favorable outcome); second, when this reshaping is not sufficient, other regions implicated in the functional network are recruited, in the ipsilateral hemisphere (close and even remote to the damaged area) and then in the controlateral hemisphere if necessary [3].

In summary, as recently supported by magnetoencephalography study, a focal DLGG disturbs the functional and effective connectivity within the whole brain and not only in the restricted area around the tumor [24] (see Chap. 21). Interestingly, these network dysfunctions are related to cognitive processing in DLGG patients [25]. In addition, because surgical treatment itself may induce changes in large-scale functional connectivity [26], such knowledge of individual pattern of remapping should be taken into account in order to elaborate personalized therapeutic management in patients bearing this slow-growing tumor.

Subcortical Connectivity as a Limitation for Cerebral Remapping: Toward Brain Hodotopy

Axonal Connectivity and the Minimal Common Brain

Although plastic potential is high at the cortical level, subcortical plasticity is low, implying that axonal connectivity should be preserved to allow post-lesional compensation. Indeed, lessons from stoke studies have taught that a damage of the white matter pathways generated a more severe neurological worsening than lesions of the cortex. By combining cortical function and axonal connectivity, an updated model of cerebral processing has recently been proposed, moving from a traditional "localizationist" view to a "hodotopical" framework [27]. In pathology, according to this new concept, a topological mechanism (from the Greek *topos* = place) refers to a dysfunction of the cortex (deficit, hyperfunction, or a combination of the two), whereas a hodological mechanism (from the Greek *hodos* = road or path) refers to dysfunction related to connecting pathways (disconnection, hyperconnection, or a combination of the two) [28]. In other words, it is mandatory to take into account the complex functioning of a large-scale distributed cortico-subcortical network to understand both its physiology and the functional consequences of a lesion in this circuit—with possible different deficits depending on the location and the extent of the damage (e.g., purely cortical or purely subcortical, or both).

Recently, it was proposed the elaboration of a probabilistic postsurgical residue atlas computed on a series of patients who underwent incomplete resection for a DLGG on the basis of intraoperative

electrical brain mapping [15, 29]. The anatomo-functional correlations obtained by combining the intrasurgical functional data with postoperative anatomical MRI findings provided both a greater understanding of the functional limits of surgical removal and new insights into the potentials and limitations of brain plasticity. Especially, this probabilistic atlas highlighted the crucial role of the axonal pathways in the reorganization of the brain after a lesion. It provided a general framework to establish anatomo-functional correlations by computing for each brain voxel its probability to be left—due to its functional role—on the postoperative MRI. Its overlap with the cortical MNI template and a DTI atlas offered a unique tool to analyze the potentialities and the limitations of interindividual variability and plasticity, both for cortical areas and axonal pathways. It was observed as a rule a low probability of residual tumors on the cortical surface, whereas most of the regions with high probability of residual tumor were located in the deep white matter. Thus, projection and association axonal pathways seem to play a critical role in the proper functioning of the brain. In other words, the functions subserved by long-range axonal pathways seem to be less subjected to interindividual variability and reorganization than cortical sites [15]. Consequently, these pathways define the surgical deep limits [30], and since DLGG infiltrates these tracts [31, 32], they constitute the main obstacle to radical surgical resection. Two questions arise on why there is no interindividual variability for these areas and why their resection cannot be efficiently compensated by plasticity phenomena. For some of these areas, the explanation could be that they act as input or output areas: input sites convey or are the first relay of information entering the brain, whereas output sites are the last relay or the fiber tracts sending information outside the brain. These areas include the primary motor and somatosensory areas, the corticospinal and thalamocortical tracts, and the optic radiations, that is, the projection fibers. These areas are mainly unimodal and probably organized serially. The absence of parallel alternative pathway explains the impossibility to restore their function after any damage [16].

For all other areas, their non-resectability should be analyzed within a network perspective. High-order cognitive processes are mediated by short- and long-range networks, with cortical epicenters connected by U-shaped fibers and associative and commissural pathways, and a particular network topology (like the "small word" one) is required to allow proper synchronization between several distant areas [33]. The link between the function and the anatomy is not as simple as for input–output areas: in fact, a local lesion can disturb a whole network topology, which in turn could ultimately hamper the function sustained by this network. It has been hypothesized that, beyond the posterior part of the posterior temporal gyrus in the left dominant hemisphere, subcortical structures like the inferior fronto-occipital fascicle and the arcuate fascicle are non-resectable because their lesion would cause so major changes in the network topology that the dynamical plasticity potential would be overwhelmed. Interestingly, these areas are considered as "hubs" in revisited models of cognition — for example, the posterior part of the left dominant superior temporal gyrus and its junction with the inferior parietal lobule [34]. Indeed, these functional epicenters allow a plurimodal integration of multiple data coming from the unimodal areas. In a step forward, this integration may lead to the conceptualization, performed at the level of a wide network which includes the hubs. As a consequence, these hubs are interconnected by subcortical pathways, themselves crucial for brain function, such as the arcuate fascicle or the inferior fronto-occipital fascicle which enables a direct communication between the posterior temporal and frontal plurimodal regions. The reproducibility of these results, despite the interindividual anatomo-functional variability and plastic mechanisms, may lead to suggest the existence of a "minimal common brain," necessary for the basic cognitive functions—even if likely not sufficient for more complex functions such as multiprocessing. This hypothesis is in good agreement with recent biomathematical models, analyzing the effect of a simulated focal lesion on the whole brain network topology [35]. Note that for these areas,

even biological plasticity—which has been shown to offer an axonal rewiring in animal models [36, 37]—would fail on the long term to repair the connectivity required to rebuild an effective network topology, hence a functional network [16].

In practice, it means that neurooncologists (especially neurosurgeons) should improve their knowledge concerning white matter circuitry. Therefore, beyond the well-known corticospinal (pyramidal), thalamocortical (somatosensory), and visual (optic radiations) pathways, subcortical connectivity subserving language and cognitive functions must be more extensively studied for each patient. As mentioned, DLGG is a tumor which migrates along the main projection, commissural and long-distance association bundles [31, 32, 38, 39]. As a consequence, it seems difficult to define the optimal therapeutic strategy against invasive glioma without understanding organization of the neural networks. Thus, cognitive neurosciences are closely related with neurooncology. In addition, the use of brain mapping technique during DLGG surgery (see Chaps. 23 and 24) also provides new insights into the circuits underlying cognitive and emotional functions.

Anatomo-functional Connectivity of Language: Naming Process Investigated by Intraoperative Mapping (Fig. 22.1) [40]

One of the best examples of large-scale circuit which should be better understood by neurologists and neurosurgeons is the network subserving picture naming—a task considered as a cornerstone in intraoperative mapping of language since several decades [41]. In the naming process, the first step is visual perception and recognition. Electrical stimulation of the optic pathway may elicit phosphenes (flashes) and/or reversible visual loss in the contralateral visual field reported by the awake patients, demonstrating an inhibition of visual perception [42]. Visual formal paraphasia has been generated by electrical interferences with a second stage of visual processing, that is, visual recognition [43]. These disturbances were induced by axonal stimulation of a subpart of the posterior inferior longitudinal fascicle, which links the visual cortex with the "visual object form area" [43]. This visual object form area, involved in object recognition, is near the visual word form area, which receives another subpart of the inferior longitudinal fascicle as afference, a sub-pathway involved in reading and generating alexia when damaged [44].

Recently, on the basis of functional disturbances induced by electrostimulation during picture naming, an original dual model for visual language processing in humans (after the first step of visual recognition) was suggested: a ventral stream is involved in mapping visual information to meaning (the "what" pathway) and a dorsal stream in mapping visual information to articulation through visuo-phonological conversion. In this model, because double dissociation between phonemic and semantic processing has been elicited by stimulation [45], it was suggested that both processes are performed in parallel and not serially. Regarding the ventral semantic stream, cortically, semantic paraphasias have been observed during intraoperative stimulation along the posterior part of superior temporal sulcus as well as in the frontal lobe, in the dorsolateral prefrontal cortex, and in the pars orbitalis of the inferior frontal gyrus [46, 47]. Axonally, such errors were elicited by stimulation of the left inferior fronto-occipital fascicle [30, 47, 48], a pathway which connects the posterior occipital lobe and visual object form area to anterior cortical areas comprising the inferior frontal gyrus and the dorsolateral prefrontal cortex [49, 50]. These regions are known to be involved in language semantics, as demonstrated using fMRI studies (for a meta-analysis, see [51]) as well as using cortical stimulation [47]. These sites are able to make the link with higher cognitive function such as plurimodal integration and judgment [52]. Thus, pretreated information by the visual recognition system is subsequently processed by the semantic system (in parallel to the dorsal phonological stream, see below) before being processed by the executive system. In addition to this direct ventral route subserved by the inferior fronto-occipital fascicle, an indirect ventral semantic pathway seems to exist, with a relay at the level of the temporal pole. Indeed, the temporal pole is

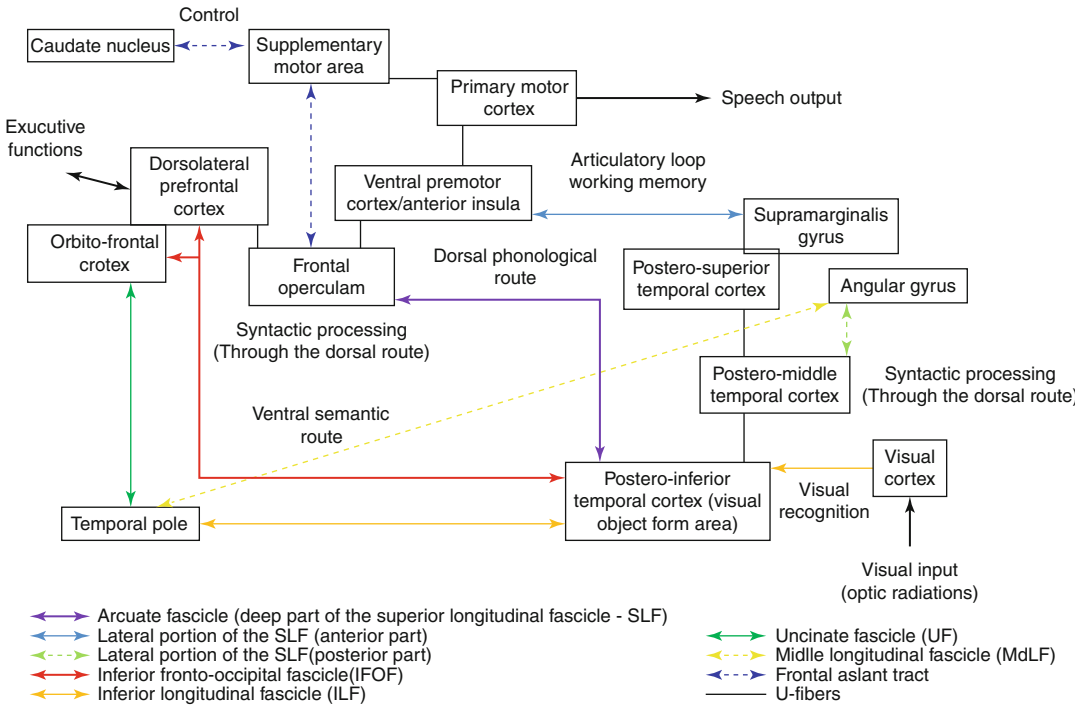

Fig. 22.1 Proposal of a hodotopical model of language, with incorporation of anatomic constraints, elaborated on the basis of structural–functional correlations provided by intraoperative direct cortico-subcortical electrostimulation (Adapted from [40])

a "hub," that is, a functional epicenter allowing a plurimodal integration of the multiple data coming from the unimodal systems—explaining its role in semantics [53]. This indirect ventral stream is constituted by the anterior part of the inferior longitudinal fascicle, connecting the visual object form area with the temporal pole [54], and then relayed by the uncinate fascicle which links the temporal pole with the pars orbitalis of the inferior frontal gyrus [55]. It is nonetheless worth noting that this indirect pathway can be functionally compensated when (unilaterally) damaged, as extensively demonstrated following anterior temporal lobectomy in epilepsy surgery [56]. Even if very mild and selective deficit may persist, as proper name retrieval [57], this is a good illustration of "subcortical plasticity," in which a subnetwork (direct pathway) is able to bypass another subnetwork (indirect pathway) and to functionally compensate it [55].

Regarding the dorsal phonological stream, cortically, phonemic paraphasia can be elicited by stimulating the inferior parietal lobule and the inferior frontal gyrus [48, 58, 59]. Axonally speaking, phonemic paraphasias were elicited when stimulating the arcuate fascicle, which is a fiber tract stemming from the caudal part of the temporal lobe, mainly the inferior and middle temporal gyri, that arches around the insula and advances forward to end within the frontal lobe, essentially within the ventral premotor cortex and the pars opercularis of the inferior frontal gyrus [30, 45, 48, 58–61]. On the basis of lesion studies, Geschwind postulated that lesions of this tract would produce conduction aphasia, including phonemic paraphasia [62], supporting the role of the subpart of the dorsal stream mediated by the arcuate fascicle in phonological processing. Interestingly, the posterior cortical origin of this tract within the posterior part of the inferior temporal gyrus [61] seems to correspond to the visual object form area. Indeed, this region represents a functional hub, involved both in semantic (see above) and phonological processing, dedicated to visual material [51]. Therefore, phonological process subserved by the arcuate fascicle seems to

be performed in parallel to the semantic process underlain by the ventral route [43]. Of note, in addition to this direct dorsal route, recent tractography studies evidenced the existence of an indirect dorsal stream, running more superficially, and underlain by the lateral superior longitudinal fascicle [63]. This pathway is implied in articulation and phonological working memory, as demonstrated by electrostimulation. Cortical areas eliciting articulatory disorders are located in the rolandic operculum, especially the ventral premotor cortex, in the ventral part of the supramarginal gyrus as well as in the posterior part of the superior temporal gyrus [48, 58, 64]. Axonally, stimulation of the white matter in the frontoparietal operculum, as well as under the supramarginal gyrus, laterally and ventrally to the arcuate fascicle, induces anarthria as well [45, 59, 64]. Interestingly, the existence of an operculo-opercular component of the superior longitudinal fascicle, named "SLF III" by some authors [65] or anterior segment by others [63], was recently evidenced. These sub-pathways connect the supramarginal gyrus as well as the posterior portion of the superior temporal gyrus with the frontal operculum [61]. Articulatory codes are stored in this frontoparietal loop. Posteriorly, the ventral supramarginal gyrus receives feedback information from somatosensory and auditory areas (in the parietal lobe and superior temporal gyrus, respectively). That would explain why stimulation induces dysarthria/anarthria when applied over these posterior regions [59, 64]. Anteriorly, the ventral premotor cortex receives afferences bringing the phonological and/or phonetic information to be translated into articulatory motor programs and efferences toward the primary motor area [66].

In summary, the vision of the neural basis of cognition begins to shift. For a long time, cognitive functions such as language were conceived in associationist terms of centers and pathways, the general assumption being that visual and auditory linguistic information were processed in localized cortical regions with the serial passage of information between regions through white matter tracts. Currently, an alternative hodotopical account is proposed, in which language is conceived as resulting from parallel distributed processing performed by distributed groups of connected neurons rather than individual centers [67, 68]. Conversely to serial model of language in which one processing must be finished before the information accedes to another level of processing, these new models of "independent networks" state that different processing can be performed simultaneously with interactive feedbacks [40] (Fig. 22.1). The next step to progress in the understanding of the brain connectivity might be a more accurate analysis of the interactions between the language circuit and the networks underlying the other cognitive functions, in particular the visuospatial component in which the role of the superior longitudinal fasciculus has been emphasized [69], as well as the emotional and behavioral aspects. Such a multimodality approach seems to represent a unique opportunity to move toward an integrative model of the various functions. In this way, the recent advances in biomathematical modeling of the electrophysiological and hemodynamic signal, which allow a reliable study of the activity time course within the neuronal networks via the analysis of the synchrony (the so-called chrono-architecture), may open a new door to the effective connectivity, that is, the influence that one neural system exerts over another.

Cerebral Reshaping: Its Implications for (Surgical) Treatment of DLGG

Maximal surgical resection is the first option in DLGG (see Chap. 23). Consequently, it is important to be reliable in the preoperative estimation of the extent of resection. Interestingly, such prediction will directly depend on the involvement (or not) of subcortical pathway which cannot be functionally compensated—and it will lead to the selection of surgery as a first treatment or in contrast to give neoadjuvant chemotherapy (see Chap. 27). Results of the neuropsychological assessment performed before any treatment will also participate in the investigation of the individual plastic potential. In other words, if the patient already experienced significant cognitive disorders and a fortiori neurological deficit, it means

that limits of brain plasticity have been reached—preventing functional compensation. Such parameters, in addition to the data provided by presurgical functional neuroimaging previously detailed (i.e., what about the pattern of reorganization elicited by DLGG?), should be incorporated in the surgical strategy in DLGG with the goal (1) to extent the indications of resection in eloquent structures so far considered as "inoperable" (2) to maximize the extent of glioma removal, by performing the resection according to (not fixed) functional boundaries with no margin (3) while minimizing the risk of postoperative permanent neurological worsening or even by improving the quality of life [23, 70–73].

Intraoperative stimulation mapping before any tumor resection has allowed the confirmation of the existence of a functional reshaping induced by DLGG, notably with a possible remapping of the sensorimotor homunculus as well as a reorganization of the language sites [1]. Moreover, acute reorganization of functional maps was equally observed throughout the resection, likely due to the surgical act itself which can generate a locoregional hyperexcitability—as already demonstrated in brain injury. Indeed, in several patients harboring a frontal lesion, although stimulation of the precentral gyrus induced motor responses only at the level of a limited number of cortical sites before resection, an acute unmasking of redundant motor sites located within the same precentral gyrus and eliciting the same movements than the previous adjacent sites when stimulated was observed immediately following lesion removal [9]. Acute unmasking of redundant somatosensory sites was also regularly observed within the retrocentral gyrus in patients operated on for a parietal glioma. Furthermore, it was equally possible to detect a redistribution within a more larger network involving the whole rolandic region, that is, with unmasking of functional homologous areas located in the precentral gyrus for the first cortical representation and in the retrocentral gyrus for its redundancy (or vice versa) [8]. Finally, intraoperative mapping has also a prognostic value concerning the postoperative recovery for movement: a positive motor response elicited by cortical stimulation of the primary motor area at the end of the resection means that the patient will recover—even if the patient had a presurgical hemiplegia [74, 75].

Thanks to these phenomena of preoperative and intraoperative plasticity, several surgical series showed that it was possible to remove DLGG invading the following "eloquent" brain structures with a minimal morbidity (Fig. 22.2) [76]:

- Broca's area resection in the left dominant hemisphere: The possibility of surgical resection of DLGG within the pars opercularis and triangularis of the left inferior frontal gyrus without generating permanent language deficits has already been reported [48, 77, 78]. Language compensation may be underlain by the recruitment of adjacent regions, in particular the ventral premotor cortex, the pars orbitalis of the inferior frontal gyrus, the dorsolateral prefrontal cortex, and the insula [48, 68, 77]. Given the fact that Broca's area is located just in front of the non-resectable ventral premotor cortex [15], one can hypothesize that the real motor area for speech output is the vPMC rather than Broca's area, the latter being probably involved in other components of language (like syntactic and phonological processes) which can be compensated [48, 79]. Indeed, recent study with extensive neuropsychological examination following resection of Broca's area confirmed the complete functional recovery [80]. Surgical approach through Broca's area, even though not invaded by the tumor, was recently reported in insular DLGG to decrease the risk of vascular injuries at the level of the Sylvian fissure [76].
- Wernicke's area resection: Language compensation following left dominant temporal resection could be explained by the fact that this complex function is organized in multiple parallel networks. Consequently, beyond the recruitment of areas adjacent to the surgical cavity, the long-term reshaping could be related to progressive involvement of remote regions within the left dominant hemisphere—such as the supramarginal gyrus, the pars triangularis of inferior frontal gyrus, or other left frontolateral regions—as well as the contralateral right nondominant hemisphere due to a transcallosal disinhibition phenomenon [81].

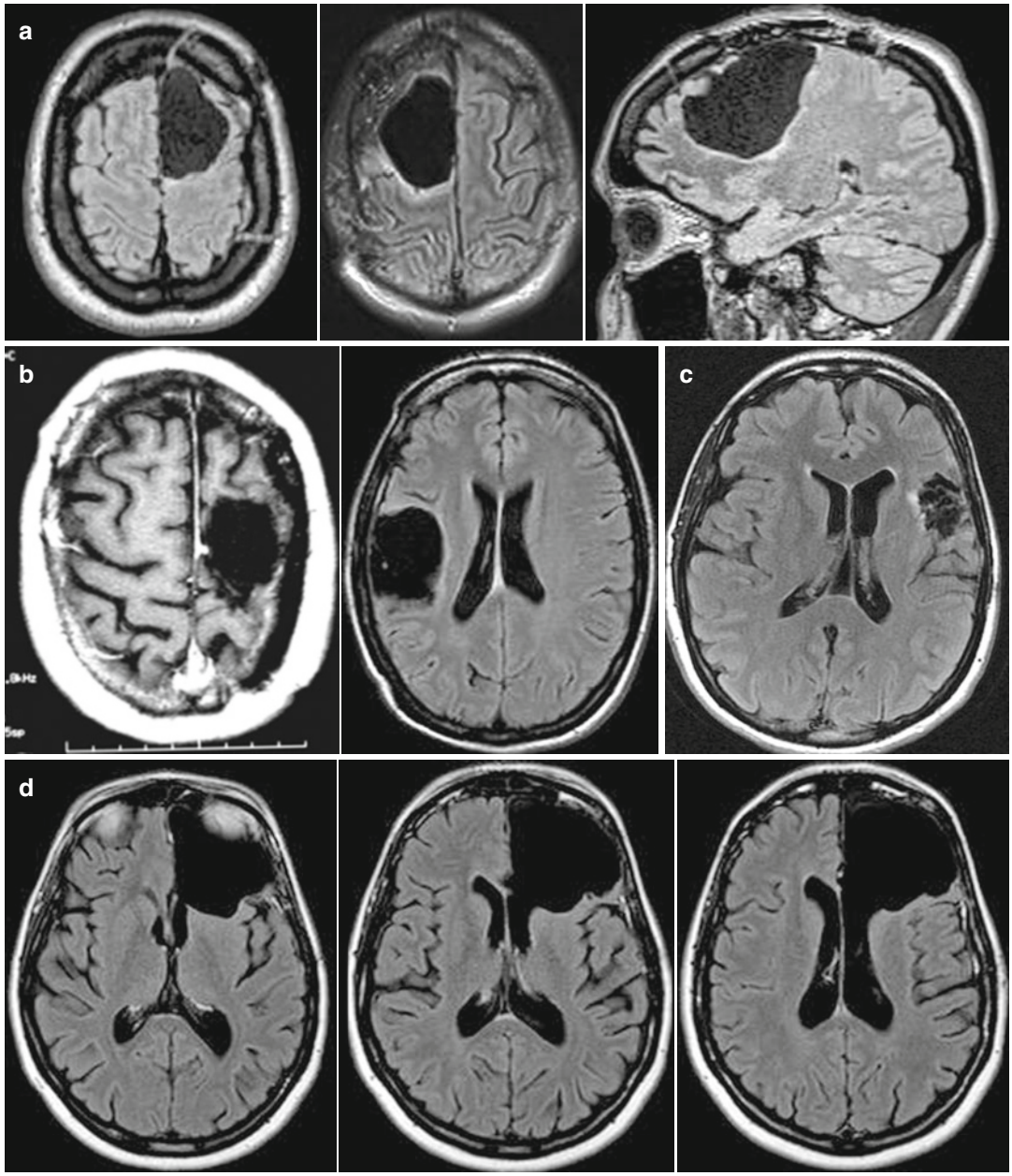

Fig. 22.2 Examples of extensive glioma resections performed within the so-called "eloquent" areas using intraoperative electrical mapping, with preservation the quality of life thanks to brain plasticity. (**a**) Right and left SMA; (**b**) left primary motor area of the hand ("knob of the hand") and right primary sensorimotor area of the face; (**c**) "Broca's area" in the left dominant hemisphere; (**d**) entire left frontal lobe including "Broca's area"; (**e**) right paralimbic system and left insula; (**f**) corpus callosum, anterior or posterior part (splenium); (**g**) anterior/mid and posterior left dominant temporal lobe, including "Wernicke's area"; (**h**) parietal lobe in right and left hemispheres, including the primary somatosensory area (Adapted from [3])

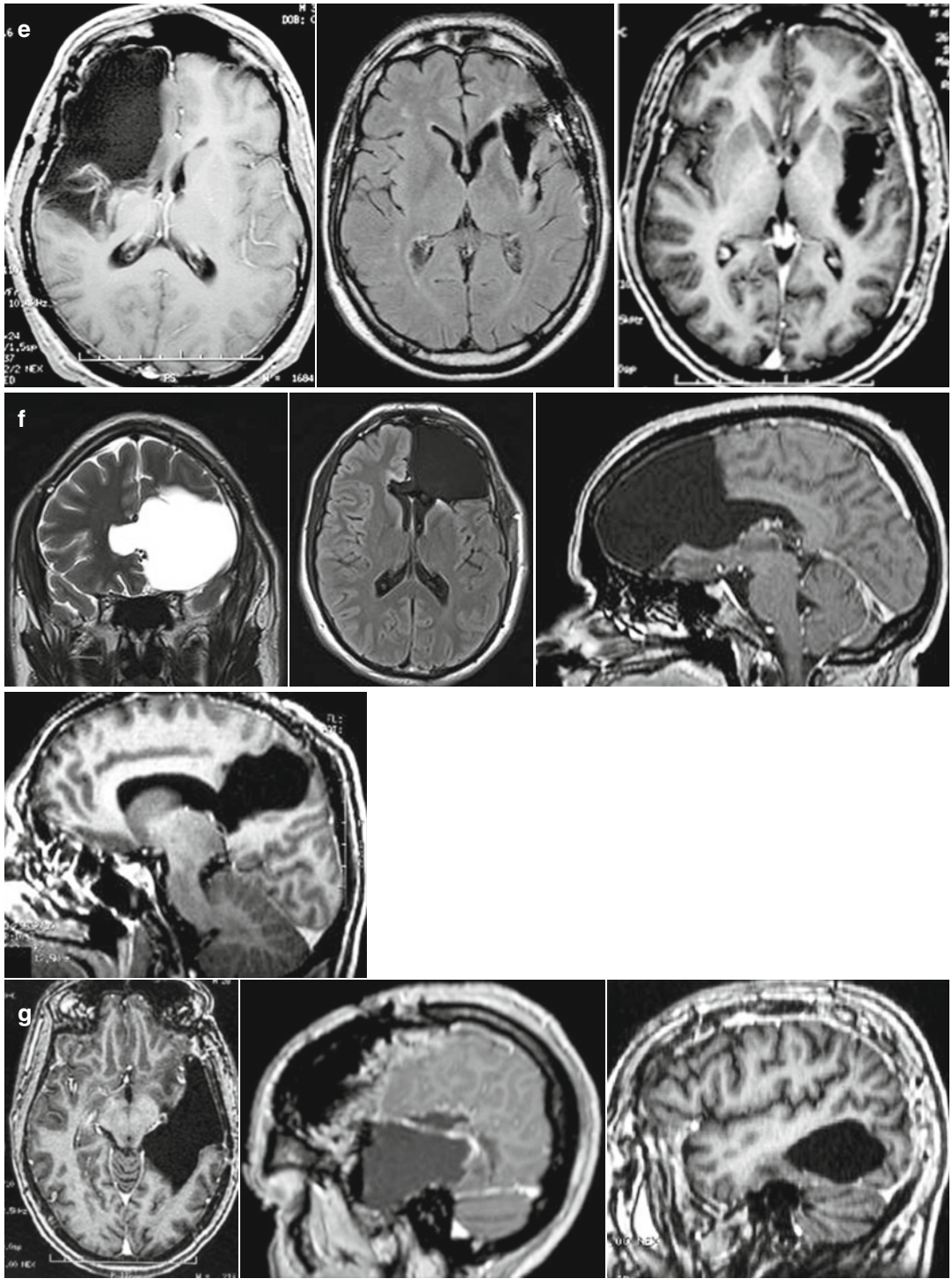

Fig. 22.2 (continued)

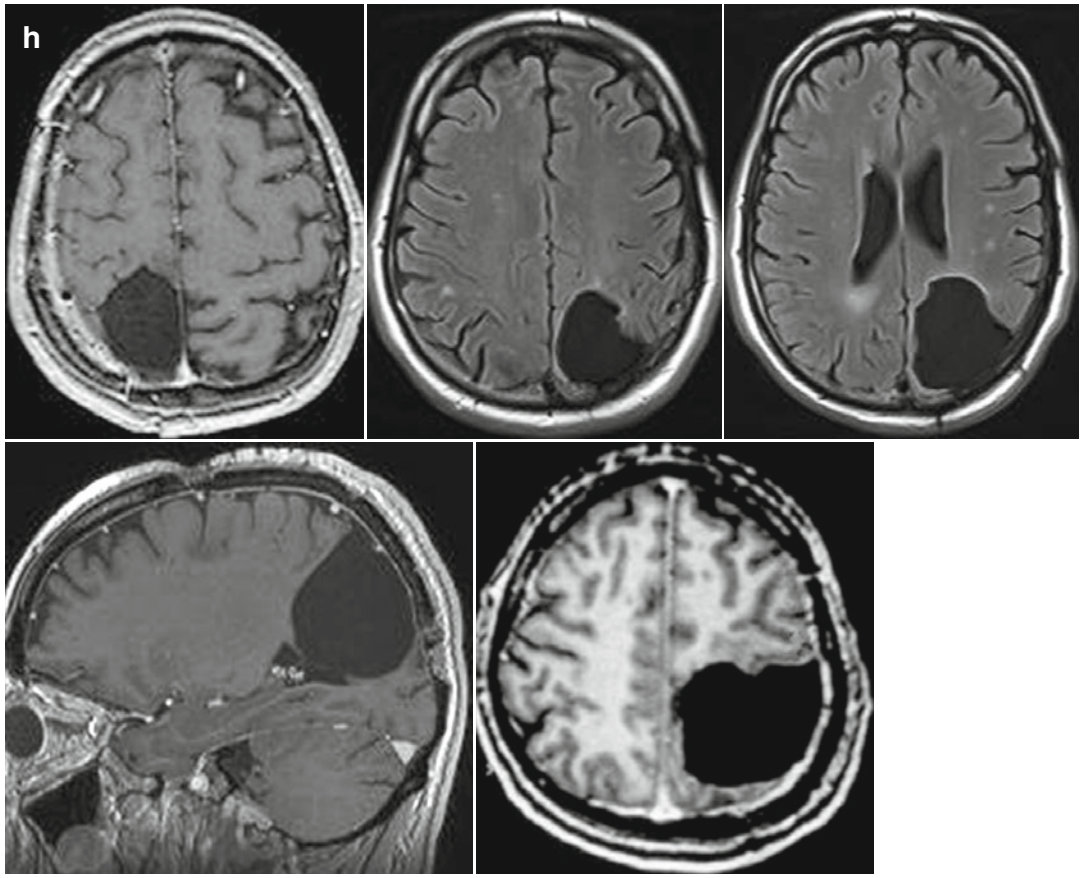

Fig. 22.2 (continued)

- Insular resection: Despite a hemiparesis after right insula removal, likely because this region is a non-primary motor area, and transient speech disturbances following left dominant insula resection, all patients recovered in a personal experience—except in rare cases (2 %) of deep stroke due to a damage of the lenticulostriate arteries [82–86]. Moreover, it was possible in right nondominant fronto-temporo-insular DLGG involving the deep grey nuclei to remove the claustrum without any cognitive disorders (despite its role suggested in consciousness) [87] and also to remove the invaded striatum without inducing neither motor deficit nor movement disorders. This compensation can be explained by a recruitment of parallel subcortical circuits such as pallido-luyso-pallidal, strio-nigro-striate, cortico-strio-nigro-thalamo-cortical, and cortico-luysal networks [88].
- Resection of primary sensorimotor area of the face: The recovery of the usual transient central facial palsy, with a potential transitory Foix–Chavany–Marie syndrome when the insula is also involved [89], is likely explained by the disinhibition of the controlateral homologous sites, via transcallosal pathways [90].
- Resection of primary motor area of the upper limb: On the basis of the existence of multiple cortical motor representations evidenced in humans using functional neuroimaging and intraoperative stimulation mapping, the compensation of the motor function could be explained by the recruitment of parallel networks within the primary motor cortex—allowing the removal of the upper limb area,

eventually using two consecutive surgeries in order to induce durable remapping following the first one [9, 91] (see below).

- Resection of the primary somatosensory area: The first results using pre- and postoperative functional neuroimaging have suggested the possible recruitment of "redundant" eloquent sites around the cavity, within the postcentral gyrus [92]. It is in accordance with the intraoperative electrical mapping data, showing unmasking of redundant somatosensory sites during resection, likely explained by the decrease of the cortico-cortical inhibition. The recruitment of the second somatosensory area or posterior parietal cortex, primary motor area (due to strong anatomo-functional connections between the pre- and retrocentral gyri), and controlateral primary somatosensory area is also possible to explain the recovery [93].
- Supplementary motor area resection: It induces the occurrence of a syndrome, with a transient akinesia, potentially associated with a transient mutism in the dominant hemisphere [94, 95]. However, all patients recovered, probably due to recruitment of the contralateral hemisphere [96, 97]. Of note, deficit in bimanual coordination may nonetheless persist [95], raising the question of the preservation of networks subserving motor control [98].
- Resection of the (dominant) parietal posterior lobe can be performed without inducing any sequelae and even with a possible improvement in comparison to the preoperative status, especially using pointing task [2].
- Interestingly, some white matter pathways can be resected with no permanent functional deficit, as for instance the anterior part of the left inferior longitudinal fascicle and the left uncinate fascicle, because they can be compensated by the "direct ventral semantic pathways" subserved by the inferior fronto-occipital fascicle (see above) [56]. The corpus callosum may also be removed with no morbidity [99]. However, as previously mentioned, in spite of these rare exceptions, the subcortical connectivity should be preserved in the vast majority of cases.

Postoperative Plasticity Evidenced by Serial Mapping: Toward a Multistage Surgical Approach

Beyond preoperative and intraoperative reshaping of brain networks, postoperative plasticity also accounts for the resectability of areas for a long time considered as "inoperable." Again, these areas should be considered as nodes within a wide network: after their removal, the whole functional network will self-reorganize by dynamical and biological plasticity, and the function will finally be preserved. Indeed, the good clinical status 3 months after surgery (as evidenced by extensive neuropsychological testing) as well as the return to a normal life (including the return to work) argue for efficient plasticity mechanisms for these areas [100, 101]. Such mechanisms induced by surgical resection within eloquent areas were also studied by performing postoperative functional neuroimaging once the patient has recovered his preoperative functional status. In particular, several patients were examined following the resection of gliomas involving the supplementary motor area, which elicited a transient postsurgical syndrome. Functional MRI showed, in comparison to the preoperative imaging, the occurrence of activations of the supplementary motor area and premotor cortex contralateral to the lesion: the contrahemispheric homologous thus participated to the postsurgical functional compensation [97].

The price to pay to obtain such favorable functional results is sometimes to perform incomplete resection of the glioma, when the tumor invaded areas still crucial for the function. A new concept recently proposed is to use more systematically such postoperative functional neuroimaging when the patient has totally recovered, since neuroimaging can be easily repeated due to its noninvasive feature, in order to compare the new maps to those obtained before surgery. Indeed, even if this method has some methodological limitations, subtraction between a pre- and postoperative acquisition may nonetheless show a possible additional functional reshaping due to (1) the resection itself, (2) the postsurgical rehabilitation, and (3) the regrowth of the residual DLGG

(as before surgery). Such findings have led to propose a new strategy based on multistage surgical approach [72]. A better understanding of mechanisms underlying this postsurgical plasticity was made possible thanks to experimentations in animals.

Experimental Observations in Animals

Firstly, the possibility that functional recovery is modulated by kinetic factors has been addressed in a series of animal studies. The main idea behind these studies was to mimic the development of slow-growing lesions by performing successive partial surgical ablations within a cerebral structure. These partial excisions were then compared to acute resections. In most experiments, a control group was included. In this case several surgeries were performed but no cerebral tissue was removed ("sham" operation). Beyond some marginal disparities, the take-home message of all these studies is quite clear: the negative functional impact of large cerebral lesions is much smaller in progressive than acute lesions. For instance, in rats, it was shown that major deficits were still present 36 days after an acute ablation of the entire somatosensory cortex. These deficits were absent when the same area was removed in two stages. In this case, the experimental rats could not be differentiated from a non-operated control group [102]. Another similar and even more spectacular report was provided by Adametz in cats [103]. The animals were submitted to a progressive (up to eight surgeries) or acute resection of the midbrain reticular formation. In this latter case, the cats fell into deep coma and died within a few days after the surgery. In the former case, by contrast, complete recovery was found. The same type of dissociation was observed in monkeys. Acute ablations of the prefrontal cortex were found to induce functional deficits that were much more severe than those produced by serial lesions [104].

Probably, the most direct demonstration that functional recovery is directly influenced by the kinetic of the lesion inflicted to the brain has been provided by Patrissi and Stein [105]. These authors trained a group of rats to retrieve water alternatively located in the right or the left branch of a conventional T-maze. Following a period of training, the rats were divided in several subgroups: (1) one-stage bilateral resection of the frontal cortex, (2) two-stage bilateral resection of the frontal cortex (one hemisphere per operation), and (3) one or two-stage sham operations (control group). For the two-stage groups, three interlesion intervals were considered: 10, 20, or 30 days. The rats given sequential (two-stage) frontal lesions with either a 20- or 30-day interoperative interval could not be differentiated from the sham-operated controls. Animals with two-stage lesions produced 10 days apart exhibited substantial deficits when contrasted with the sham-operated, the 20-day or the 30-day two-stage groups. However, the two-stage 10-day animals performed significantly better than the one-stage rats. Similar results were found in other studies involving resections of the frontal cortex [106] and the superior temporal gyri [107]. In all these studies, the animals were reported to exhibit a complete recovery when the different surgeries were spaced by a sufficient interval. This interval varied from study to study, but it was never smaller than 6 days. Whatever the interlesion interval, the level of recovery was always better for the multistage surgeries than for the one-stage operations.

Of course, the positive effect of sequential lesions on functional recovery depends strongly on the amount of tissue resected at each surgical stage. This was clearly shown by Stein and colleagues in a monkey study involving the resection of the sulcus principalis. In this study, the total amount of tissue resected was kept constant. It was reported that four partial lesions performed 3 weeks apart produced a greater level of recovery than two partial lesions performed 10 weeks apart [108]. This result pleads directly for the idea that the progressiveness of neural destruction is a key predictor of functional recuperation.

Application to Patients with DLGG

Interestingly, recent series demonstrated that such remapping was not a theoretical concept, but a concrete reality in humans [1]. Postoperative functional neuroimaging performed some months or years

following the surgery for DLGG in patients with a complete recovery clearly showed a new recruitment of perilesional areas and/or remote regions within the ipsilesional hemisphere and/or a recruitment of controlateral structures [81, 97]. On the basis of these data, a second surgery was proposed in patients who continued to enjoy a normal life, before the occurrence of new symptoms (except possible seizures), only because of an increase of the volume of the glioma [72]. The second surgery was also conducted using intraoperative cortical and subcortical mapping, in order to validate the mechanisms of brain reshaping supposed but not proven by preoperative functional neuroimaging, before to perform the additional resection [71, 109]. The preliminary results have supported the efficacy and the safety of such reoperation for DLGG not totally removed during a first surgery due to their location within eloquent areas. Indeed, in a recent series, 74 % resections were complete or subtotal (less than 10 ml of residue) following the second operation, despite no additional serious neurological deficit—on the contrary, with an improvement of the neurological status in 16 % of cases. Again, the seizures were reduced or disappeared in 82 % of patients with epilepsy before the second operation. The median time between the two operations was 4.1 years, and all patients were still alive with a median follow-up of 6.6 years despite an initial incomplete resection. Therefore, these original data demonstrated that, thanks to mechanisms of cerebral plasticity, it is possible to reoperate patients with DLGG involving eloquent areas with a minimal morbidity and an increase of the extent of resection. Therefore, it was suggested to "overindicate" an early re-intervention, in order to anticipate the second surgery before anaplastic transformation [108]. Such concept of multistage surgical approach was particularly useful to optimize the extent of resection in traditional "critical" areas [76], such as the left premotor region [72], rolandic area [9], or Wernicke's area [81] (Fig. 22.2).

In addition, one can currently consider to perform postoperative functional neuroimaging after rehabilitation, demonstrated to induce a significant improvement in patients with brain tumors [3, 110], and after recovery following a second surgery in order to open the door to a possible third or even fourth resection several years after the previous operations. The goal is both to allow the patient to enjoy a normal life as well as to increase the overall survival. It is also possible to integrate surgeries within a dynamic therapeutic strategy including chemotherapy and radiotherapy, especially when a wide removal is not possible for functional reasons [111]. To this end, neoadjuvant chemotherapy was recently advocated in DLGG, with the goal to induce shrinkage of the tumor before an operation or a reoperation [112]—and also possibly to facilitate functional brain reshaping [113] (see Chap. 27).

Conclusions and Perspectives

Combination of online intraoperative anatomo-functional correlations (transient virtual lesion) with data provided by tractography (subcortical anatomical information), magnetoencephalography (temporal data), and serial fMRI (perioperative functional data) could enable one to elaborate individual and predictive models of functioning of neuron-synaptic circuits. Such models may lead to a better knowledge of the dynamic potential of spatiotemporal reorganization of the parallel and interactive networks, namely, the mechanisms of brain plasticity, thought to play a major role of functional compensation in slow-growing lesions such as DLGG and in their surgical resection. In practice, in order to evolve toward a multistage surgical approach (i.e., second or third surgery more extensive than the first one in cases of initial incomplete resection within eloquent areas), a dynamic strategy has to be envisaged for functional neuroimaging. The goal is to switch from a "static" use of a unique preoperative functional neuroimaging assessment (limited technique with lack of reliability) to longitudinal studies based on the repetition of neuroimaging before and after surgical resection(s), with the goal to analyze a possible brain reshaping at the individual scale and to select the candidates to reoperation(s). The next step is to now use biomathematical models able to examine brain functional interaction through effective connectivity in order to attempt to predict *before surgery* the patterns of postsurgical remapping at the individual scale on the basis of

the data provided by the preoperative functional neuroimaging. The new theory of graphs may probably help to tend toward such kind of individual prediction [114, 115] (see Chap. 21). Nonetheless, it is worth noting that predicting for each patient on noninvasive preoperative imaging and mathematical model if a brain area could be resected or not remains a neuroscientific challenge. This underlines, once again, the inescapable importance of the individual intraoperative study with direct electrostimulation mapping in awake patients.

From the point of view of perspectives, it could be suggested to "canalize" brain plasticity, using notably pharmacologic drugs, functional rehabilitation [116], or even transcranial magnetic stimulation, to promote functional recovery not only following surgery but also before surgery [4]. One could hypothesize that such preoperative remapping might enable to increase the extent of the resection (and possibly to take a margin around the lesion) while avoiding postsurgical worsening, even in the classically so-called "eloquent" areas according to anatomic criteria [74, 117]. Furthermore, the use of plasticity could lead to propose surgery in asymptomatic patients. Indeed, thanks to the current development of neuroimaging, incidental discovery of tumors will progressively increase in the next future. Interestingly, concerning DLGG, it was recently demonstrated that their natural history was the same in the presymptomatic period than after the first symptom (usually seizures) [118]. Therefore, on the basis of the new neuroscientific concept of a "hodotopic and plastic brain," the next surgical goal could be to evolve toward a "prophylactic functional neurooncology" (see Chap. 31) [110, 119, 120].

Consequently, beyond the fundamental interest, it is also crucial for the (surgical) neurooncologist to improve his knowledge of dynamic brain processing and its interaction with the natural course of DLGG at the individual level. In conclusion, cognitive neurosciences seem to represent a precious help to neurooncology by opening new therapeutic strategies to improve both quality of life and median survival [121].

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Part VI

New Insights into the Therapeutic Strategies for DLGG

Surgery for Diffuse Low-Grade Gliomas (DLGG) Oncological Considerations

23

Hugues Duffau

Abstract

For a long time, surgery for DLGG was a matter of debate. The main problem explaining discrepancies in the classical literature is related to the fact that, in the vast majority of series, extent of resection (EOR) was not objectively assessed on postoperative MRI. It was based on the sole subjectivity of the surgeons or on a single computed tomography scan, with no volume measurement of the residue. In the modern series which used a systematic postoperative T2-/FLAIR-weighted MRI, all authors have demonstrated that a more aggressive resection predicted significant improvement in overall survival (OS) compared with a simple debulking. In addition, it was shown that an extended removal of a margin beyond these MR imaging-defined abnormalities, that is, a "supra-total" resection, significantly increased OS by delaying malignant transformation. This means that biopsy in DLGG should be considered only in very diffuse lesions, such as gliomatosis, or when a subtotal resection is not *a priori* possible. Collectively, despite the lack of phase III study, these data strongly argue in favor of achieving a maximal resection of DLGG as the first therapeutic option. Thus, surgeons should change their mind in order to operate the brain involved by a chronic tumoral disease – and no more by operating a tumor mass within the brain. The goal is not to content with a single "tumorectomy" (i.e., to remove only the "top of the iceberg" visible on imaging) but

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to perform the most extensive resection of the brain invaded by DLGG on the condition that this part of the brain is not crucial for cerebral functions. In other words, neurosurgeons should take the habit to perform early and maximal resection according to functional (and not purely oncological or anatomical) boundaries in awake patients. This perspective seems to represent the best way to build a modern and personalized "functional surgical neurooncology."

Keywords
DLGG • Surgery • Supratotal resection • Overall survival • Malignant transformation • Extent of resection • Functional mapping

Introduction

For a long time, surgery for diffuse low-grade gliomas (DLGG) was a matter of debate. First of all, the controversy was underlain by the functional risk of surgical resection because DLGG usually occurs in young patients with no or only slight symptoms and who enjoy an (almost) normal life. However, thanks to the technical advances in mapping methods and above all thanks to a better understanding of dynamic neurobiology subserving brain processing, the surgical risk has dramatically been reduced. This issue will be extensively discussed in the next chapter about functional outcomes following surgery for DLGG.

However, before to detail "how to operate?" it is first mandatory to solve the main question "why to operate?" Here, the aim is to review the recent literature about the impact of surgery on the course of DLGG on the basis of an improved knowledge of its natural history.

New Insights into the Natural History of DLGG

While described in previous chapters, it seems important to remind some crucial points with regard to the behavior of this complex disease.

Growth

Contrary to what was claimed in the traditional literature, there is no stable DLGG. Using biomathematical model allowing the calculation of growth rate (on the basis of at least two MRIs spaced by 3 months before any treatment), it was demonstrated that all the DLGGs had a constant growth during their premalignant phase, with a linear increase of the mean diameter (extracted from the volume) around 4 mm a year [1–3]. This growth was observed not only in symptomatic patients but also in incidental DLGG discovered for independent reasons (e.g., mild head injury) in asymptomatic patients [4]. In essence, it means that the concept of "progression-free survival" does not mean nothing – at least before chemotherapy or radiotherapy – because the growth rate is similar before and after surgery in cases of incomplete resection [5]. Moreover, there is an inverse correlation between growth rates and survival. Indeed, among a series of 143 consecutive cases, Pallud et al. showed that a median velocity of diametric expansion of 8 mm/year or more was associated with a median survival of 5.16 years while a median survival of more than 15 years was seen with a growth rate of less than 8 mm/year [6].

Migration

In addition, these tumors are migrating along the white matter tracts (U fibers, association, projection, and commissural pathways) [7–9]. Therefore, DLGG is not a "tumor mass," as regularly reported in the classical literature, but it is in fact an infiltrating chronic disease invading the central nervous system, especially the subcortical connectivity known to be critical for brain functions

[10, 11] (see Chap. 22 by Duffau). This is an important issue because such a diffusion of glioma cells may induce cognitive disorders, probably due (at least partly) to a "disconnection syndrome" [12, 13]. Indeed, mounting evidence now highlights the existence of disturbances of high-order functions, such as working memory, attention, or executive functions [14, 15]. These deficits are frequently observed when objective neuropsychological assessments are performed immediately after the diagnosis, challenging the traditional view of "DLGG patients with a normal examination" [16]. Furthermore, from a therapeutic point of view, glioma migration along fibers can also limit the extent of surgical resection to preserve the quality of life [17].

Malignant Transformation and Survival

Last but not the least, DLGG will ineluctably become malignant. Such anaplastic transformation will lead to neurological deficit with a worsening of quality of life and ultimately to death. In two EORTC (European Organization for Research and Treatment of Cancer) randomized multicenter trials involving more than 600 patients, in the subgroup of patients with a favorable prognostic score, the median survival was 7.7 years – whereas in the subgroup of patients with a poor prognostic score, the median survival was only 3.2 years [18, 19]. Interestingly, accurate determination of growth rate allows the identification of patients whose gliomas are at high risk for early transformation [20]. Other clinical (age, neurologic and cognitive status, Karnofsky Performance Status (KPS)), radiological (tumor volume, location and kinetics, metabolic parameters), pathological, and molecular factors are also correlated with the risk of degeneration and median survival [21–27], as already described in previous chapters.

Taken together, these results show that DLGG cannot be considered any more as a "benign" tumor but as a precancerous disease. As a consequence, surgical and medical neurooncologists should definitely switch from a traditional "wait and see" attitude to an early therapeutic strategy, with the aim of delaying malignant transformation and increasing overall survival, while preserving or even improving quality of life. In this state of mind, recent surgical series demonstrated that DLGG resection played a crucial role on the course of this chronic brain disease.

The Impact of Surgical Resection in DLGG

The Classical Literature

Despite controversies since many decades regarding the value of surgery in DLGG, recent comprehensive reviews of the literature have suggested that a more extensive resection of this tumor was correlated with a more favorable life expectancy [28–30]. It was demonstrated that the rate of surgical series observing a benefit of resection increased over time [28]. In an analysis of ten studies since 1990 which have applied statistical analysis to examine the role of extent of resection (EOR) in improving survival and delaying tumor progression among patients with DLGG, Sanai and Berger showed that the mean survival changed from 61.1 to 90.5 months with a greater EOR (i.e., gross-total versus subtotal resection) [29, 31].

Advances in Neuroimaging and Objective Assessment of Extent of Resection

However, the main problem explaining discrepancies in the classical literature is related to the fact that, in the vast majority of series, EOR was not objectively assessed on postoperative MRI but was based on the sole subjectivity of the surgeons into the operative theater or on a single computed tomography scan, with no volume measurement of the residue. Due to the invasive feature of DLGG, the residual tumor was doubtlessly underestimated in numerous studies, resulting in erroneous conclusions about the benefit of surgery. Currently, it is well admitted that T2/fluid attenuation inversion recovery (FLAIR)-weighted MRI is the only way to actually calculate the postsurgical volume of (possible) residual tumor in routine practice. It is nonetheless important to underline that isolated glioma cells cannot been detected by

structural imaging and that the emergence of physiological/metabolic imaging techniques (diffusion-weighted MRI, perfusion-weighted MRI, MR spectroscopy, PET) will likely continue to improve the sensitivity of detection of residual disease [32].

The Modern Literature

In the modern neurosurgical series which used a systematic postoperative T2-/FLAIR-weighted MRI, it is remarkable to see that all series since 2005 have demonstrated that a more aggressive resection predicted significant improvement in overall survival (OS) compared with a simple debulking [33]. These studies agreed with the fact that when so signal abnormality was visible on control MRI (i.e., the so-called complete resection), patients had a significantly longer OS compared with patients having any residual abnormality. In the series by the University of California San Francisco (UCSF) group, including 216 DLGGs, after adjusting for the effects of age, KPS, tumor location, and tumor subtype, EOR remained a significant predictor of OS (HR = 0.972; 95 % CI, 0.960–0.983; $p < 0.001$), with 8-year OS of 98 % of patients with complete resection [34]. In 156 DLGGs, Claus et al. reported that patients who underwent incomplete resection were at 4.9 times the risk of death relative to patients with total resection [35]. McGirt et al. found that gross-total resection versus subtotal resection was independently associated with increased OS (hazard ratio, 0.36; 95 % confidence interval, 0.16–0.84; $p = 0.017$) [36]. In an analysis on 130 DLGG performed by Ahmadi et al., extended surgery was shown to significantly prolong OS [37]. Yeh et al. also demonstrated that, in multivariate analysis performed in a consecutive series with 93 DLGGs, EOR and postoperative KPS showed independent prognostic significance for OS rates [38]. Furthermore, in 222 DLGGs with objective assessment of postoperative residue on MRI, Duffau et al. found a significant correlation between complete resection and OS [39]. Recently, the French Glioma Network published the largest surgical series of DLGG ever reported and demonstrated with an experience of 1,097 patients that EOR and the postsurgical residual volume were independent prognostic factors significantly associated with a longer OS (Fig. 23.1) [40].

Moreover, even in cases of incomplete tumor removal, patients with a greater percentage of resection had a significantly longer OS. For instance, the survival was significantly better with at least 90 % EOR compared with less than 90 % EOR, whereas EOR of at least 80 % remained a significant predictor of OS [34]. In addition to the percentage of resection, the postoperative tumor volume is also a predictor of survival, with a significantly longer OS when the residue is less than 10 ml (the so-called subtotal resection) compared with more than 10 ml ("partial resection") [41]. In a subgroup of 122 patients who underwent surgery with intraoperative functional mapping for a DLGG, Duffau et al. showed that, with a median follow-up of 4 years, 20.6 % of patients with more than 10 ml of residue died, while only 8 % of patients with less than 10 ml of residue died (and that no patients with complete resection on postoperative MRI died) ($p = 0.02$) [39]. Interestingly, the value of EOR was evident not only within the general hemispheric DLGG population but also for specific DLGG limited to certain subregions, such as insular DLGG [42, 43].

Such an impact on OS is due to the fact that surgery delayed histological upgrading. It was demonstrated that the volume of residual tumor served as a predictor of anaplastic transformation [41]. In the recent series by the UCSF group 216 DLGGs, after adjusting for the effects of age, KPS, tumor location, and tumor subtype, EOR remained a significant predictor of malignant progression-free survival (HR = 0.983; 95 % CI, 0.972–0.995; $p = 0.005$) [34]. In an experience including 191 consecutive patients with DLGGs, Chaichana et al. also showed that gross-total resection was an independent factor associated with malignant degeneration (relative risk (RR) 0.526; 95 % CI, 0.221–1.007; $p = 0.05$) [44]. Interestingly, as for OS, the value of EOR on malignant transformation was significant not only for the general DLGG population but also for specific areas. For instance, the UCSF group showed that, within the insula, the interval to malignant progression of

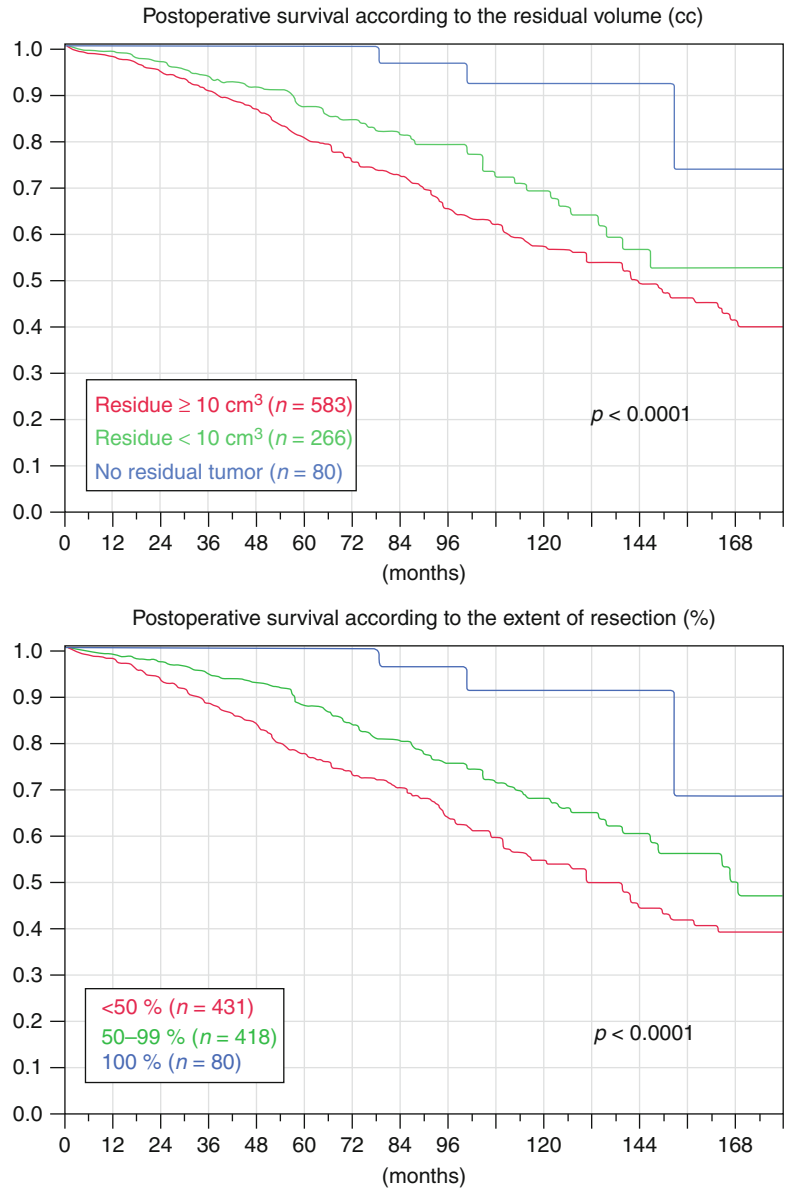

Fig. 23.1 The largest surgical series of DLGG even reported by the French Glioma Network, which demonstrated with an experience of 1,097 patients that both the postsurgical residual volume (*upper*) and the extent of resection (*lower*) were independent prognostic factors significantly associated with a longer overall survival (Modified from [40])

DLGG was longer in patients who had undergone greater resections [42].

Toward a Supratotal Resection of DLGG

Despite this value of gross-total resection (MRI based) on malignant transformation and survival, a recent study using biopsy samples within and beyond MRI-defined abnormalities showed that conventional MRI underestimated the actual spatial extent of DLGGs since tumor cells were present beyond the area of MRI signal abnormalities up to 20 mm – even when gliomas were well defined on MR images [45]. Interestingly, it was suggested that an extended resection of a margin beyond these MR imaging-defined abnormalities might improve the outcome of DLGG. Indeed, a recent series reported that a "supratotal" resection – that is, resection extending beyond the area of MR imaging signal abnormalities (Fig. 23.2) – performed in 15 patients bearing a DLGG within

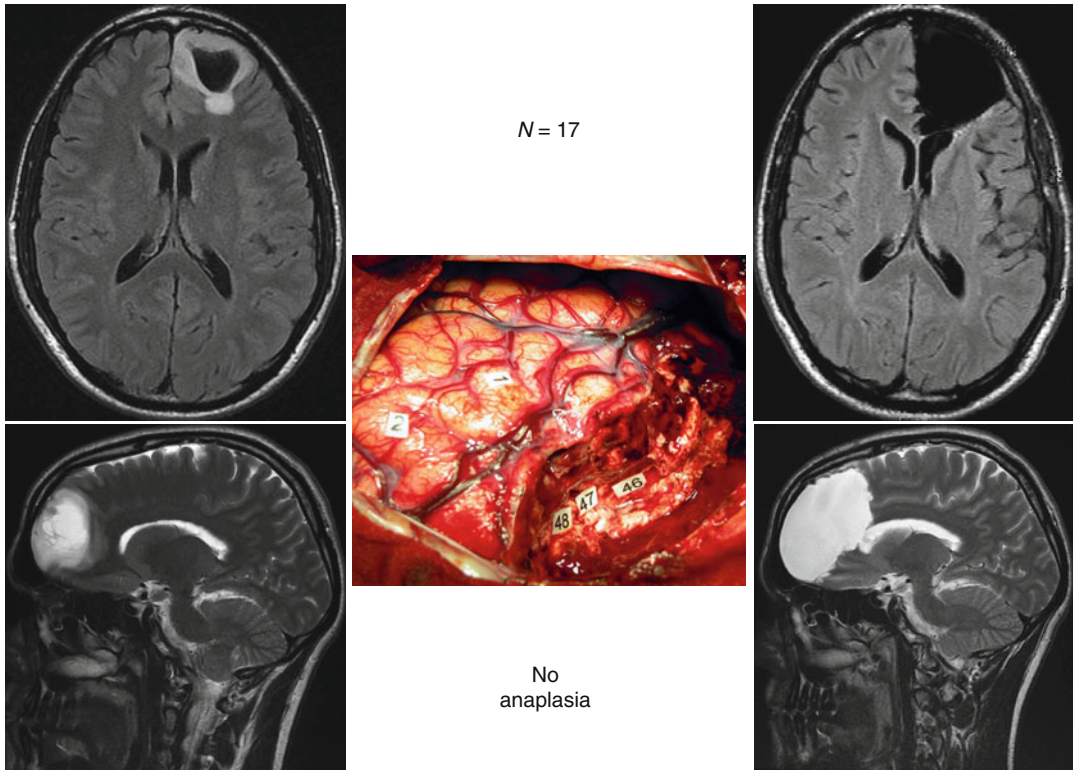

Fig. 23.2 Supratotal resection performed according to functional boundaries detected using intraoperative cortico-subcortical electrical mapping. *Left*: preoperative MRI showing a left prefrontal DLGG; *middle*: intrasurgical photograph; *right*: postoperative MRI demonstrating that the resection was achieved beyond the FLAIR/T2-weighted MRI abnormalities (Modified from [46])

"non-eloquent" brain regions avoided malignant transformation in a mean follow-up of 35.7 months (range 6–135) [46]. This series was compared with a control group of 29 patients who had "only" complete resection for a DLGG: anaplastic transformation was observed in seven cases in the control group but in no cases in the series of patients who underwent supratotal resection ($p = 0.037$). Furthermore, adjuvant treatment was administrated in ten patients in the control group compared with one patient who underwent supracomplete resection ($p = 0.043$). On the other hand, 4 of 15 patients with supracomplete resection experienced recurrence. This is probably due to the fact that it is not possible to take at least 20 mm of margin all around the tumor in all patients due to the functional structures. It is nonetheless worth noting that some patients in this series had a relapse after 18 months while other had no recurrence with 135 months of follow-up after the sole surgery. This could be explained by the fact that some DLGGs are more "invasive" whereas other DLGGs are more "proliferative." It is likely that surgery, in particular supratotal resection, in essence has a better chance of controlling the latter than the former. In the future, advances in physiological/metabolic imaging, closer to the neuropathological tumor infiltration [32, 47], might allow for better selection with respect to indications for supracomplete resection. Another useful method could be the new biomathematical models of proliferation and diffusion, based on atleast two sets of MRI acquired 3–6 months apart before any treatment [3, 48] (see Chapters 17 and 28 by Mandonnet). Therefore, the goal of supratotal resection is currently to delay anaplastic transformation by reducing the number of peripheral tumoral cells and to delay the use of adjuvant therapy, without claiming to cure patients with DLGG [46].

The Value of Reoperation(s)

Due to the invasive nature of DLGG, relapse is possible after total or even supratotal resection, and continuous growth of the residual tumor is ineluctable after incomplete resection. Interestingly, in this setting, some authors provided argumentations in favor of the oncological impact of a second surgery. Schmidt et al. analyzed the surgical results in a series of 40 patients reoperated for recurrent DLGG without other intervening therapy between surgeries. They provided evidence supporting that a gross-total resection was associated with an increased time to repeated surgery [49]. More recently, in a series of 130 DLGGs, Ahmadi et al. showed that extended surgical resection for nonmalignant relapse (a total resection could be achieved in 53.1 % of recurrent tumors) prolonged the OS significantly [37]. In the series reported by the French Glioma Network, subsequent surgical resection was an independent prognostic factor significantly associated with a longer OS [40]. Martino et al. also reported a consecutive series of 19 patients who underwent a second surgery for recurrent DLGG in eloquent areas [50]. A total or subtotal resection was achieved in 73.7 % of patients during the reoperation, despite an involvement of functional areas. Such "multistage surgical approach," with an initial maximal function-guided resection, followed by a period of several years, and then a second surgery with optimization of EOR while preserving the quality of life, is possible, thanks to mechanisms of brain plasticity induced both by the tumor (re)growth (see Chap. 22 by Duffau) and by the first resection itself [51, 52]. In this series, the median time between surgeries was 4.1 years (range 1–7.8 years), and the median follow-up from initial diagnosis was 6.6 years (range 2.3–14.3 years) with no death during this follow-up period. As a consequence, due to a favorable benefit-to-risk ratio of such strategy, the authors suggested to consider reoperation(s) in all recurrent DLGGs. Nonetheless, due to a high rate of anaplastic transformation histologically proven of 57.9 % at reoperation, while the main goal of surgery in (recurrent) DLGG is to prevent malignant transformation, it was suggested to "over indicate" an early re-intervention rather than to perform a late surgery when histological upgrading already occurred [50].

The Limited Role of Biopsy in DLGG

In this state of mind, the indications of biopsy are currently very limited in DLGG. First of all, this is due to the fact that by combining clinical and radiological data, the diagnosis of glioma is typical in the vast majority of cases. Thus, the main goal of neuropathological examination is to give the best reflect with regard to the actual grade of the glioma. However, if stereotactic needle biopsy can obtain tissue for diagnostic purposes, there is a high risk of sampling error. Indeed, Muragaki recently demonstrated that overgrading of WHO grade I gliomas occurred in 11 % of cases and undergrading of WHO grade III gliomas in 28 % [53]. Therefore, neuropathological diagnosis of DLGG with only stereotactic biopsy is associated with a substantial risk of inaccuracy, particularly for tumors with low proliferative activity of for mixed gliomas. On the contrary, maximal DLGG resection provides a more extensive amount of tumoral tissue and thus increases the reliability of the histological diagnosis and grading [30].

Furthermore, in essence, biopsy has only a (unreliable) diagnostic value but no therapeutic impact, contrary to the surgical resection, as detailed above. As a consequence, because the surgical risk of MR-guided stereotactic biopsies is still around 2 % of permanent deficits [54], their indications for presumed DLGG are essentially contraindications of surgery. In practice, it means that beyond patients who do not want or who are not able to undergo surgical resection for medical reasons, biopsy can be mainly considered in diffuse lesions, such as gliomatosis, or when a subtotal resection is not *a priori* possible [30]. To this end, in addition to the experience of the neurosurgeon, such a prediction can be optimized by the use of a probabilistic map of postoperative residue. Such atlas, based on the computation of residual gliomas resected according to functional boundaries in a series of 65 DLGGs, allows a preoperative estimation of the

expected EOR with a success rate of 82 % [17]. This rationale, directly related to a distinct potential of brain reorganization according to the spatial location of the glioma – in particular with a lesser index of plasticity at the level of the white matter pathways [11] – may assist in decisions regarding surgical resection versus biopsy.

Of note, in the selected cases of biopsy, incorporation of new imaging modalities (PET, MR spectroscopy) could improve the sensitivity and specificity of this approach in correctly identifying and grading DLGG [55, 56].

Technical Considerations for Maximal Surgical Resection of DLGG

The Conceptual Shift from an Image-Guided Surgery Toward a Functional Mapping-Guided Resection

On the basis of these strong oncological results, brain surgeons should change their mind in order to operate the nervous system involved by a chronic tumoral disease – and no more by operating a tumor mass within the brain [57]. The goal is not to content with a single "tumorectomy," that is, removal of the part of the tumor visible on imaging, but to perform the most extensive resection of the brain invaded by DLGG on the condition that this part of the brain is not crucial for cerebral functions. In other words, the neurosurgeon should see first the brain, and not the glioma, to adapt his surgical procedure to the three-dimensional anatomo-functional organization of each patient. It implies that brain surgeon must change his technique within the central nervous system, which has to be different from the surgical technique outside the brain [58]. Indeed, the first principle in glioma surgery should be to tailor the resection according to functional boundaries, with no margin, to maximize the tumor removal while preserving eloquent structures [59].

In this setting, the methods of intrasurgical imaging (neuronavigation, intraoperative MRI), despite their increased use, may suffer from serious limitations. First, from an oncological point of view, as already mentioned, it should be kept in mind that conventional MRI, including T2-/FLAIR-weighted MRI, does not show the whole tumoral disease but only the top of the iceberg. Indeed, it is usually admitted that tumors appear on MRI only for cell densities above 500 cells/mm^3 [60]. Thus, MRI underestimates the actual spatial extension of a DLGG, and tumor cells extend far beyond the MRI-defined abnormalities. In this state of mind, when DLGG is distant from eloquent structures, image-guided resection is by definition a nonsense. It could be possible in these specific cases to remove more tumoral cells while preserving the function, that is, to perform a "supratotal" resection, with the impact on malignant transformation detailed above, on the condition nevertheless to not constraint the resection according to the T2-/FLAIR-weighted MRI [46]. It means that the integration of preoperative MRI into neuronavigation or the use of intraoperative MRI is based on a reductionist concept, that is, the exclusive removal of the signal abnormality, with no attempt to increase the resection beyond these landmarks – even if they do not reflect the whole glioma disease. As a consequence, image-guided resection may represent a loss of chance for the patient bearing a DLGG outside critical brain regions [57, 58]. Interestingly, recent advances in the intrasurgical visualization of tumoral cells using 5-aminolevulinic acid fluorescence with confocal microscopy have recently been reported in DLGG and could be useful to increase EOR [61]. On the other hand, in eloquent areas, this new technique as well as intraoperative imaging should be use with cautious because the intention to maximize the resection of the diffuse disease on the basis of the sole oncological criteria may lead to permanent neurological deficit, as already reported in fluorescence-guided resection in high-grade gliomas [62].

From a functional point of view, neurosurgeons have presently a tendency to believe that the data provided by functional MRI (fMRI) and diffusion tensor imaging (DTI) is the "absolute truth" with regard to the individual functional anatomy of the brain. Indeed, a large amount of recent experiences is based on the exclusive use of functional imaging for the surgical indications and planning, as well as on the exclusive use of

functional imaging directly into the operating theater (preoperative data incorporated in a neuronavigational system or intraoperative fMRI/DTI) (for a recent review, see [63]). Yet, functional neuroimaging is based on biomathematical reconstruction, with results which may change according to the model [64], explaining its lack of reliability *at the individual level* regarding cognitive functions such as language – as reported for both fMRI [65] and DTI [66]. Especially, neuroimaging is not yet able to differentiate areas crucial for brain functions from regions which could be functionally compensated. Consequently, there is a risk to not select a patient for DLGG surgery because fMRI activations are visible very near or within the tumor, while it was in fact possible to remove it with no permanent deficit – thus with a loose of chance from an oncological point of view. Furthermore, into the operative theater, beyond the risk to damage crucial structures not identified by fMRI/DTI (due to their actual lack of sensitivity) and/or due to the brain shift increasing throughout the resection of voluminous gliomas (thus decreasing the reliability of the data provided by DTI), the dogmatic rule which emerged because of the poor accuracy of these techniques is to take 5–10 mm of margin around the presumed functional regions according to neuroimaging [67]. Again, such strategy is against the oncological goal, that is, to optimize EOR, whereas it was shown on more than 100 consecutive patients with LGG in language areas that the resection could be pursued with no margin without increasing the rate of permanent morbidity (less than 2 %) [59, 68].

Therefore, due to these limitations, even if the neurosurgeon can of course at least partly take into account the data provided by neuroimaging, he should also incorporate additional concepts in his surgical strategy to optimize the benefit-to-risk ratio of the resection, both oncologically and functionally speaking. According to this new philosophy, intraoperative electrostimulation mapping is actually the more reliable method to identify eloquent regions and thus to continue the resection until eloquent areas have been encountered, with a maximal DLGG removal while preserving the quality of life of patients.

Cortico-Subcortical Mapping: The Value of Function-Guided Surgery to Increase EOR (Fig. 23.3)

Here, the goal is not to accurately detail the methodology for awake surgery using stimulation mapping, which will be extensively described in the next chapter – as well as the functional outcome. However, it is seems important to underline several key issues.

First of all, neurosurgeons should keep in mind that, in physiology, there is a major interindividual anatomo-functional variability, in particular for cognitive functions such as language [69], which can be increased in patients with slow-growing DLGG due to mechanisms of brain plasticity [51, 57]. It means that, even if anatomical landmarks remain essential during cerebral surgery, they are definitely not enough. Therefore, because the aim is to perform a removal of the brain invaded by DLGG according to individual functional boundaries, both at cortical and subcortical levels, it is crucial to benefit from a positive mapping before to begin the resection in order to avoid a possible false negative (especially for methodological reasons) [58]. Indeed, since eloquent structures can be located within the glioma, the standard surgical principle of debulking tumor from inside to outside is not always safe in DLGG [70]. In the UCSF experience, 4 of 243 (1.6 %) patients had a persistent new language deficit: all these four patients had no positive sites detected prior to their resections [71]. In another series with 309 patients who underwent surgeries with intraoperative electrostimulation, mapping was found to be negative in 109 cases, and 9 % of these patients developed a long-term deficit [72]. Taylor and Berstein previously reported negative mapping in 70 % of patients, with 3.6 % of them who experienced a permanent neurological worsening [73]. Thus, negative mapping cannot guarantee the absence of eloquent sites and it cannot prevent persistent postsurgical deficit in all cases. These are the reasons why other authors continue to advocate a wider boneflap in order to obtain systematic functional responses before the resection [58, 74]. In other words, “minimal

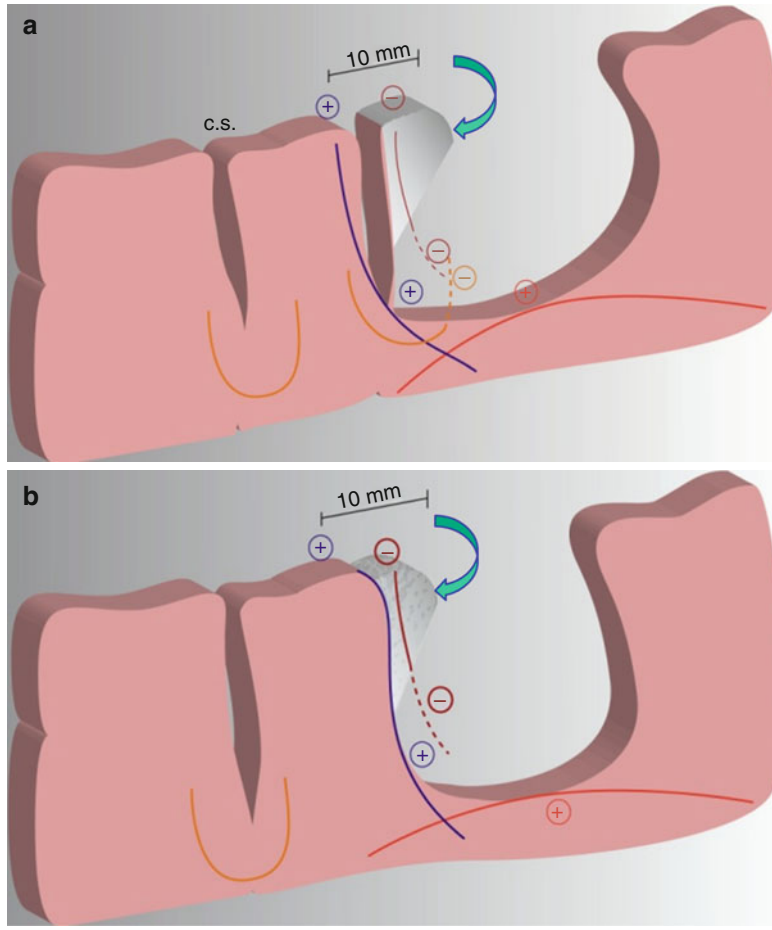

Fig. 23.3 Schematic drawing showing two examples of a resection with margin. (**a**) First, a tumor coming into the contact of a sulcus removed by leaving a 10 mm security margin from the positive cortical stimulation site (*blue* +) (i.e., with no subpial dissection). If subcortical stimulation is performed, the projection fibers "vertical connectivity" will be identified at the bottom of the sulcus (*blue* +, e.g., pyramidal tracts). The U fibers coming from the other side of the sulcus will also be tested throughout the resection (*orange* −). At the bottom of the cavity, the "horizontal connectivity" or long-distance association fibers (e.g., arcuate fasciculus) will represent the deep functional subcortical boundary (*red* +). Interestingly, the cortex invaded by the tumor (in *gray*, shown by the *blue arrow*) is functionally useless since the resection has been pursued until the subcortical pathways have been encountered (*blue* and *red* +). Thus, the fibers arising from this cortex have been disrupted, as they do not respond to stimulation (*brown* −). As a consequence, this cortex was disconnected and can therefore be removed with no functional risk. (**b**) The second illustration shows the same example in a case of an "intragyral dissection" (Modified from [59])

invasive neurosurgery" means "minimal morbidity" and not "minimal boneflap size."

Another important issue is the preservation of the subcortical connectivity. Indeed, since DLGGs are migrating along main white matter pathways and because these tracts which subserve the functional connectivity are crucial, as demonstrated using probabilistic atlas of cerebral plasticity [11], it is mandatory to identify and to preserve such pathways using subcortical stimulation throughout the glioma removal [57, 58, 68, 74]. As a consequence, beyond electrical mapping, on-line cognitive monitoring should be performed in awake patients (especially but not exclusively in the dominant hemisphere, see Chap. 24 by Duffau) to check whether no neurological deficits are generated by the resection. To this end, strong real-time relationships are essential between the patient, the

neurosurgeon, and the speech therapist/neuropsychologist/neurologist directly into the operating theater. On the other hand, due to the fact that the patient can be tired following 1–2 h of continuous task allowing a tailored resection, it is recommended to begin glioma removal directly into the contact of the eloquent structures detected using cortico-subcortical stimulation mapping to win time by disconnecting the part of the brain involved by the DLGG – rather than to "debulk" the tumor from inside and then to come closer to the functional regions only at the end of the resection when the patient is less cooperant. Indeed, once the invaded brain is disconnected according to the functional boundaries provided by the individual mapping, it is possible to remove it under general anesthesia since the on-line feedback of the patient is not necessary anymore [58].

Interestingly, a recent meta-analysis studying more than 8,000 patients who underwent surgical resection for a brain glioma demonstrated that the use of intrasurgical mapping allowed a statistically significant reduction of permanent deficit, despite an increased rate of resection within eloquent areas [75]. In addition, the EOR was increased [75]. These results are in agreement with previous series which compared both functional and oncological outcomes in two consecutive series of DLGGs removed without and then with intraoperative electrical mapping in the same institution [39]. Again, although functional mapping enabled to significantly increase the rate of surgeries within classical inoperable areas, the rate of persistent worsening significantly decreased and the rate of total or subtotal resection significantly improved. Recently, de Benedictis et al. described a series of nine patients who underwent two consecutive surgeries for a DLGG [76]. The first resection was performed in a traditional way, that is, under general anesthesia and without mapping, whereas the subsequent surgery was done in a maximal way, in awake patients using intrasurgical cortico-subcortical electrostimulation mapping defining the boundaries of the resection. The first resection was subtotal in three cases and partial in six cases, with a postoperative worsening in three cases. Following the subsequent awake surgery, postoperative MRI showed that the resection was complete in five cases and subtotal in four cases (no partial removal) and that it was improved in all cases compared with the first surgery ($p = 0.04$). There was no permanent neurological worsening. Three patients improved compared with the presurgical status. All patients returned to normal professional and social lives [76]. These original results demonstrate that awake surgery, known to preserve the quality of life in patients with DLGG, is also able to significantly improve the EOR for lesions located in functional regions. Furthermore, the group of UCSF demonstrated in a series of 281 patients that the use of functional mapping-guided resection of DLGG in presumed eloquent areas, thanks to a reliable delineation of true functional and nonfunctional regions, allowed not only a maximization of tumor resection but also a significant improvement of long-term survival [77]. Finally, as mentioned, in a recent study, awake surgery was used for DLGG located in non-eloquent areas, with the goal to extend the resection beyond the visible part of the glioma on MRI. Because these "supratotal" resections prevented any malignant transformation in a mean follow-up of 35.7 months (range 6–135), it means that the concept of functional mapping resection can also be applied to non-eloquent regions with a significant value on the behavior of DLGG [46].

In other words, DLGG should be resected according to functional boundaries provided by intraoperative mapping *in all patients*, especially when the glioma does not directly involve critical brain structures, because the rate of supracomplete resection and the oncological impact is greater in these cases.

Subpial Dissection and Vasculature Preservation

Once functional areas have been mapped, another cornerstone in DLGG surgery is to preserve the whole vascularization, that is, both arteries and veins, and thus to minimize the use of coagulation. Surprisingly, while this technical point was extensively detailed for extra-axial neurosurgery, it received less attention for glioma resection.

First of all, at the cortical level, the *in passing* vessels running at the surface of the tumor but not vascularizing it, which is the most frequent in DLGG, should be spared. The corticectomy must be performed on both sides of each vessel, with no vascular sacrifice. It is worth noting that in reoperations for glioma recurrence, sometimes several years following the first surgery, the cortical vessels preserved during the initial procedure were systematically found as still permeable – even in cases of bridging veins left within the surgical cavity [58].

In a second stage, the removal of the brain invaded by the glioma has to be continued using a subpial dissection [58]. In other words, when the sulci which will represent the boundaries of the resection have been identified by electrostimulation cortical mapping, these sulci should not be opened in order to preserve the vessels. Indeed, because the vessels are running within the sulci, if one tries to open them, the risk to damage the vessels is higher. As previously mentioned, since the resection is performed with no margin around the eloquent structures, it means in essence that the buried cortex covering the other side of the sulcus is crucial for the function. Therefore, the consequence of possible arteries and/or veins coagulation would be an ischemia of this buried cortex, which will generate neurological deficit. On the contrary, subpial dissection with aspiration of the glioma but without coagulation allows the preservation of the vessels within the sulcus and thus avoids any injury of the other side of the sulcus. This technique can be used for all sulci within the brain, including the Sylvian fissure. This is the reason why in paralimbic DLGGs, it was proposed by several authors to remove the (frontal and/or temporal) operculae – even if not invaded by the tumor – to have an optimal access to the insula, rather than to open the Sylvian fissure, in order to minimize the risk of vascular damage [42, 78, 79]. Preservation of the pia mater also avoids spasm, since there is no direct manipulation of the arteries. Indeed, it is important to underline that long perforator arteries which supply the corona radiata may arise from the M2 segment of the middle cerebral artery. As a consequence, a deep stroke may also be due to a damage of the vessels in the Sylvian fissure [80]. Interestingly, the rate of permanent deficits in series with trans-opercular approach [42, 78, 79] is lower in comparison with series with trans-sylvian approach [80]. Such strategy can also be applied for deep brain DLGGs in non-insular locations. Indeed, when a tumor is located near the depth of a sulcus, especially in presumed functional areas, it could seem logical to open the sulcus to reach the lesion. However, beyond the risk to damage the intra-sulcal vessels, thus with an increased risk of stroke, it should be kept in mind that the deep cortex which has to be crossed to reach the tumor might be eloquent, while not detected by the initial mapping performed at the level of the cortical surface. Conversely, it is safer to pass through the gyrus after a corticotomy performed according to the results of the cortical mapping on the surface of the brain, with no vascular risk – at the condition nevertheless to continue to perform subcortical mapping throughout the trajectory within the white matter to reach the deep lesion [58, 74].

In practice, it is possible to perform subpial dissection by using the aspirator or the CUSA, even if the former is safer, because the risk of lesion of the pia mater and thus of vascular injury may be higher with the CUSA – which is nonetheless a best tool for the resection within the white matter despite the fact that it can induce a transient inhibition of axonal conduction [81]. When the pia is damaged due to the surgical dissection or due to the tumor itself (especially in cases of high-grade glioma or in cases or reoperation), it is recommended to identify first the normal pia all around the portion which is damaged and to converge on this point at the end of the resection. The last but not the least, the pia mater also represents a very good anatomic landmark. When the deep part of a sulcus is identified at the end of a subpial dissection, it means that the resection arrives into the contact of the subcortical connectivity and that it is time to begin to perform electrical mapping of the long-distance association white matter pathways (see Chap. 24 by Duffau). Moreover, in the specific cases of insular/paralimbic DLGG, the deep part of the pia mater buried within the anterior insular sulcus

is very close to the lateral part of the anterior perforating substance and may represent the end of glioma resection after the removal of the limen insulae – to avoid injury of the lenticulo-striate arteries [78].

Conclusions and Future Prospects

Collectively, despite the lack of phase III study, these data strongly argue in favor of achieving a maximal resection of DLGG [30]. As a consequence, such a radical resection, when possible, is currently the first therapeutic option to consider in DLGG, as recommended by the European Guidelines [82]. In front of this amount of evidence, a prospective randomized study seems now unethical, while retrospective matched studies or prospective observational trials may be considered.

This resection should be functional mapping guided, with no margin around the crucial structures, that is, with a resection pursued until functional areas have been encountered *and not before*, even for DLGG located within non-eloquent areas, in order to optimize EOR. Indeed, Gil Robles and Duffau showed that it was not logical to leave a small amount of tumor involving the cortex when the resection was already performed at the subcortical level (into the contact of eloquent white matter pathways according to the results of subcortical stimulation), because it means that the cortical area not removed was in fact already disconnected and thus not functional anymore (Fig. 23.3) [59].

In order to help to better select the surgical strategy and to improve the quality of patient counseling, probabilistic atlas allowing preoperative estimation of residual volume for DLGG resection with intrasurgical functional mapping can been used [11, 17]. Such prediction of the postoperative residue according to the location of the glioma may be useful to take a decision of surgical resection versus single biopsy followed by "neoadjuvant chemotherapy" – that is, inducing shrinkage of the DLGG and opening the door to a subsequent surgery with a real chance to perform at least a subtotal removal [83]. To this end, biomathematical models of proliferation and diffusion, based on the acquisition of at least two MRIs spaced by 3–6 months, can also play a major role to predict the growth as well as the migration of the glioma [3, 48, 84]. Such anticipation could be helpful to plan a possible reoperation with the goal to improve EOR while preserving brain functions, thanks to the mechanisms of plasticity induced by DLGG evolution (see Chap. 22 by Duffau).

In summary, because DLGGs frequently invade eloquent regions [85, 86], neurosurgeons should take the habit to see the brain first, and not the tumor [57], by performing early and maximal resection according to functional (and not purely oncological or anatomical) boundaries in awake (asymptomatic) patients [87, 88]. This perspective seems to represent the best way to build a modern and personalized "functional surgical neurooncology."

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Surgery for Diffuse Low-Grade Gliomas (DLGG) Functional Considerations

24

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Abstract

After many decades of controversies, guidelines now recommend maximal surgical resection as the first therapeutic option in DLGG. This paradigmatic shift should lead neurosurgeons to switch toward an early and extensive removal of this chronic and diffuse tumoral disease of the brain. Yet, preservation of the quality of life (QoL) is also a priority in surgery for DLGG. Interestingly, because of the frequent location of DLGG within "eloquent" areas and due to their infiltrative feature, it was considered for a long time that the chances to perform an extensive glioma removal were low, whereas the risk to generate postoperative sequelae was high. To solve this dilemma, the brain surgeon should change his philosophy and his technique on the basis of a new concept, that is, to perform resection according to cortico-subcortical functional limits (with no margin) and not according to oncological boundaries. In other words, the neurosurgeon should see first the brain, and not the glioma, to adapt his surgical procedure to the individual cerebral anatomo-functional organization, which can be highly variable from patient to patient or even in the same patient over time due to brain reorganization induced by the slow growth of DLGG. The ultimate aim is to remove a part of the brain invaded by tumoral cells, on the condition nonetheless that it can be functionally compensated – thus, with no consequences on the QoL. In this setting, neurosurgeons need to take advantage of mapping methods to create individualized maps and management plans. These technical and conceptual advances, which consist in performing early (and possible repeated) resection(s) for DLGG, based on functional boundaries provided by pre-, intra-, and postoperative methods of mapping both at cortical and subcortical levels, in a hodotopical and plastic framework of cerebral processing,

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have allowed a dramatic improvement of the benefit-to-risk ratio of surgery. This chapter reviews how, in addition to functional neuroimaging, the method of intraoperative stimulation mapping, especially in awake patients, has enabled (1) an increase of the surgical indications for tumor located within eloquent areas classically considered as "inoperable," (2) a significant optimization of the extent of resection, and (3) a preservation or even an improvement of the QoL. Therefore, stronger interactions between cognitive/behavioral neurosciences and oncological neurosurgery begin to solve the classical dilemma – survival versus brain functions – by giving the possibility to become more ambitious, namely, to increase both survival and QoL in DLGG patients. To this end, awake mapping should be more systematically considered, even in presumed "non-eloquent areas."

Keywords

DLGG • Surgery • Multistage surgical approach • Awake mapping • Direct electrical stimulation • Anatomo-functional connectivity • Quality of life • Functional neuroimaging

Introduction

In the previous chapter, it was demonstrated that the better understanding of the natural course of diffuse low-grade gliomas (DLGG) has participated in a renewed interest in surgery in the past decade. Indeed, rigorous studies based on objective evaluation of the extent of resection on postoperative MRI showed that surgery had a significant impact on both malignant transformation and overall survival in patients with DLGG [1–4]. As a consequence, after many years of controversies, guidelines now recommend maximal surgical resection as the first therapeutic option [5]. This paradigmatic shift should lead neurosurgeons to not content with a single "tumorectomy" (i.e., to remove only the "top of the iceberg" visible on imaging) but to switch toward an extensive resection of a chronic and diffuse tumoral disease [6]. In parallel, due to advances in neuroimaging allowing an earlier diagnosis, most of patients with DLGG experience no or only slight neurological deficit at time of first MRI, usually performed because of inaugural seizures or even for independent reason with incidental discovery (see Chap. 31 by Duffau) [7]. Therefore, in addition to the optimization of extent of resection, preservation of the quality of life (QoL) is currently a priority in (precocious) surgery for DLGG [8, 9] – even if for a long time, the vast majority of studies focused on overall survival but did not accurately analyze the QoL. It is worth noting that, due to the frequent location of DLGG near or within the so-called eloquent areas [10, 11] and due to their infiltrative feature (poorly demarcated), it was considered during several decades that the chances to perform an extensive glioma removal were low, whereas the risk to generate postoperative sequelae was high. Indeed, many surgical series have reported a postoperative rate of permanent and severe deficit between 13 and 27.5 % (for a review, see [2]).

To solve this dilemma, namely, to optimize the onco-functional balance of surgery, brain surgeon should change his philosophy as well as his technique on the basis of a new concept, that is, to perform surgical resection according to cortico-subcortical functional and not to oncological boundaries. In other words, the principle is first to understand the cerebral anatomo-functional organization at the individual level, because a major interindividual variability has previously been demonstrated in healthy volunteers [12] as well as in epileptic patients [13]. Furthermore, this variability is increased

in cases of DLGG, due to phenomena of brain reorganization induced by the slow growth of the tumor [14–17]. Therefore, many arguments support the unpredictability of functional eloquence based on anatomical features alone and the fact that patients should not be considered ineligible for surgical intervention based on anatomical considerations alone (for a review, see [18]). Rather, neurosurgeons need to take advantage of modern technology and mapping methods to create individualized maps and management plans. The ultimate aim is to remove a part of the brain invaded by DLGG, on the condition nonetheless that this part of the central nervous system can be functionally compensated – that is, with no consequences on the QoL.

This chapter reviews how, in addition to functional neuroimaging, the method of intraoperative stimulation mapping, especially in awake patients, has enabled a significant improvement of the results of DLGG surgery, with (1) an increase of the surgical indications for tumor located within eloquent areas classically considered as "inoperable," (2) an optimization of the extent of resection, and (3) a preservation or even an improvement of the QoL.

Preoperative Study of the Individual Functional Anatomy: Conceptual and Technical Considerations

It is amazing to observe a low rate of neurological deficits in patients with diagnosis of DLGG, even if these tumors are frequently located within the so-called eloquent areas. This is due to mechanisms of cerebral plasticity, explained by the fact that DLGG is a slow-growing tumor (increase of mean diameter around 4 mm/year) [19], giving many years to the brain for functional remapping with a recruitment of perilesional or remote areas within the ipsilesional hemisphere and/or recruitment of contra-hemispheric homologous areas (see the Chap. 22 by Duffau) [14–17]. The recent integration of these concepts into the therapeutic strategy has resulted in dramatic changes in the surgical management of DLGG patients, with an increase of surgical indications in functional areas classically considered as "unresectable" [6, 8, 20, 21].

Nevertheless, one must be aware of a high rate of cognitive deficits at time of diagnosis, despite a normal social and professional life in most of DLGG patients.

Neurocognitive Assessment: Not a Luxury, but a Crucial Parameter Before and After Treatment

An extensive neuropsychological examination should be performed in all cases, because many DLGG patients experienced disorders of high-order functions, such as executive functions, attention, concentration, working memory, or emotion [22, 23]. This is the reason why a systematic preoperative assessment of higher functions and health-related QoL is now recommended (see the Chap. 19 by Moritz-Gasser and Herbet) (1) to search the possible neuropsychological deficit not identified by a classical neurological examination, (2) to adapt the strategy according to these individual results (e.g., decision of surgery first versus neoadjuvant chemotherapy in cases of very diffuse DLGG inducing important cognitive deficits), (3) to adapt the surgical methodology itself to the results of this assessment (e.g., to perform functional mapping under local anesthesia even in the right hemisphere in right-handers in cases of preoperative – slight – language deficits or to select intrasurgical tasks during awake surgery), (4) to benefit from a presurgical baseline allowing a comparison with the postsurgical evaluation, and (5) to plan a specific functional rehabilitation following the resection, which can induce a transient neurological worsening. Indeed, when objective neuropsychological and health-related QoL assessment have been performed after surgery, postoperative visuospatial, memory, attention, planning, learning, emotional, motivational, and behavioral deficits have regularly been observed (for a recent review, see [24]). Interestingly, a recent study showed that increased reaction time during naming task performed immediately after resection was significantly correlated to return

to work [25]. However, in spite of these reports, it is likely that more patients experienced this kind of disorders than described in the literature. This underestimation by neurosurgeons is due to the fact that the identification of such "subtle" deficits is not possible using a single "standard clinical examination." Unfortunately, extensive neurocognitive evaluation was very rarely performed after DLGG surgery, especially when the lesion was located outside the so-called language areas. As a consequence, a standardized examination of neurocognitive outcome has recently been proposed [24].

In the same state of mind and beyond the objective neuropsychological scores, it is essential to define what exactly means "QoL" for each patient, on the basis of his(her) job, habits, hobby, and projects. The goal is to prioritize the brain functions which should be preserved throughout the resection and to adapt the intraoperative tasks according to this preparatory discussion with the patient and his(her) family. For instance, it can be important to test different languages as well as language switching intrasurgically in multilingual patients or to map calculation in school teacher, spatial cognition in dancer, working memory in manager, syntax in writer, judgment in lawyer, etc. [26]. It means that intraoperative mapping should be personalized for each patient (in addition to standard tasks such as picture naming for lesions located within the "dominant hemisphere").

Preoperative Neuroimaging: Advances and Limitations

Although progress in neuroimaging has allowed a better knowledge of the natural history of gliomas (growth, invasion, as well as malignant transformation) [7, 27–29] and has participated in (re)opening the door to more extensive glioma surgery, paradoxically, it also led to several conceptual limitations.

From an oncological point of view, one should be aware about the fact that conventional MRI, including T2/FLAIR-weighted MRI, does not show the whole disease. Indeed, DLGG invades the brain beyond the abnormalities visible on imaging, with tumor cells present at a distance of 10–20 mm of the tumor boundaries defined by MRI [30]. This led to perform larger glioma removal, at least in nonfunctional areas: such so-called supra-complete resection dramatically changed the natural history of this tumor (even if it cannot cure it) by delaying the recurrence and above all by avoiding anaplastic transformation [4]. As a consequence, when DLGG is distant from eloquent structures, in essence, image-guided resection is a nonsense, because it could be possible in these cases to remove more tumoral cells while preserving the function, on the condition nevertheless to not constraint the resection according to the T2/FLAIR-weighted MRI but according to functional boundaries. Unfortunately, the integration of preoperative MRI into neuronavigation or more and more the use of intraoperative MRI is based on a reductionist concept, that is, the exclusive removal of the signal – abnormality, with no try to increase the resection beyond these landmarks – even if they do not reflect the whole glioma disease. In other words, image-guided resection may represent a loss of chance concerning patients with a DLGG outside eloquent brain areas (see the Chap. 23 by Duffau).

From a functional point of view, advances in functional neuroimaging, for example, functional MRI (fMRI), magnetoencephalography, diffusion tensor imaging (DTI), and more recently transcranial magnetic stimulation [31–34], have enabled to perform a noninvasive mapping of the whole brain and are currently considered as a standard before resection of DLGG. Functional imaging gives an estimation of the location of the eloquent areas (e.g., regions involved in sensorimotor, language, visual, and even higher cognitive function – see the Chap. 20 by Bizzi) in relation to the glioma and provides information with regard to the hemispheric language lateralization. Thus, these methods may be useful for (1) the surgical indications, partly depending on the location of the tumor and its relationships with eloquent areas detected by functional imaging (allowing an estimation of the tumor resectability); (2) the surgical planning, namely, the

selection of the surgical approach and the delineation of the limits of resection; and (3) the selection of the surgical technique, especially the decision to wake up the patient intraoperatively if the glioma is close to somatosensory, language, or cognitive areas – even in the right hemisphere, on the basis of the laterality index on fMRI for language in addition to the handedness of the patient provided by the neuropsychological examination [35]. Indeed, although language areas are the most often identified in the left hemisphere, language sites can also be detected in the right hemisphere not only in left-handed or ambidextrous patients [36] but also in right-handers, thus with a risk of crossed aphasia [35].

However, despite such contribution of functional imaging, neurosurgeons seem to believe that the data provided by fMRI and DTI is a direct reflect of the neural foundations of the brain. Indeed, numerous recent studies are based on the exclusive use of fMRI/DTI for the surgical indications and planning, as well as on the exclusive use of functional imaging directly into the operating room (preoperative data incorporated in a neuronavigational system or intraoperative fMRI/DTI) (for a recent review, see [37]). Yet, it is crucial to underline that functional neuroimaging methods are not yet enough reliable *at the individual scale,* despite constant efforts for their improvement, mainly because they are based on biomathematical reconstruction, and that their results may change according to the model used [38]. Concerning fMRI, correlations with intraoperative electrophysiology have recently demonstrated that the sensitivity of fMRI was currently only around 71 % for movement [39], and from 59 to 100 % for language (specificity from 0 to 97 %) [40, 41]. Such discrepancies can be explained by a neurovascular decoupling in cases of glioma (bold response in the vicinity of gliomas does not reflect the neuronal signal as accurately as it does in healthy tissue), by inadequate tasks (not adapted to the location of the glioma and/or to the neurological status of the patient), or by methodological problems (e.g., selection of the threshold). As a consequence, there is a risk of false negative and then to operate a patient without intraoperative mapping although the glioma is actually located in crucial areas for the function – but not detected by preoperative fMRI – thus, with a high risk to induce a permanent deficit. According to this principle, the calculation of the lateralization index may be dangerous in patients with atypical distribution of language. Indeed, in patient with right tumor, even if the lateralization index shows a majority of language activations in the left hemisphere, it does not mean that the minority of language sites activated in the right hemisphere does not correspond to crucial epicenters. Therefore, in the doubt, awake language mapping should be performed intraoperatively, because *fMRI is not able to differentiate essential regions from areas which can be functionally compensated (and thus removed)* [6, 8]. Moreover, an erroneous interpretation of brain reshaping ("pseudo-reorganization") can be made [42].

Concerning DTI, which allows the identification of the main bundles, that is, their tractography, as well as their location in relation to the glioma, this new method needs nonetheless to be validated, especially by intraoperative electrophysiological techniques, before it can be used routinely for surgical planning. Indeed, comparison between distinct fiber tracking software tools found different results, showing that neurosurgeons have to be cautious about applying tractography results intraoperatively, especially when dealing with an abnormal or distorted fiber tract anatomy [38]. Furthermore, correlations between DTI and intrasurgical subcortical stimulation demonstrated that, despite a good correspondence in 82 % of cases, DTI is not yet optimal to map language tracts in patients. Negative tractography does not rule out the persistence of a fiber tract, especially when invaded by a glioma [43]. Moreover, DTI enables the study of the sole anatomy of the subcortical pathways, but not their function.

As a consequence, several negative impacts of functional imaging have to be underlined. The main limitation is to consider that DLGG surgery should not be proposed because fMRI activations are visible very near of within the tumor, while it was in fact possible to remove it with no permanent deficit – thus, with a loose of chance from an

oncological point of view. This point was especially described for DLGG invading the supplementary motor area, the insula, or even the so-called Broca's area and Wernicke's area, which have been removed with favorable outcome although these regions were a priori thought to be eloquent on the basis of functional neuroimaging [44–48]. In addition, intraoperatively, beyond the risk to damage eloquent structures not detected by fMRI/DTI (due to their actual lack of sensitivity) and/or due to the brain shift increasing throughout the resection of voluminous glioma (thus decreasing the reliability of the data provided by DTI), the dogmatic rule which emerged because of the poor accuracy of these techniques is to take 5–10 mm of margin around the presumed functional regions according to neuroimaging (for a review, see [49]). As already mentioned in the previous chapter by Duffau, such strategy is against the oncological goal, that is, to optimize extent of resection, whereas it was shown on more than one hundred consecutive patients with DLGG in language areas that the resection could be pursued with no margin without increasing the permanent morbidity [49, 50]. Finally, a recent study comparing which aimed to assess the utility of DTI in the surgical treatment of motor eloquent tumors demonstrated that tractography of pyramidal pathways did not influence the surgical planning or the intraoperative course [51]. In summary, there is a double risk (1) to not select a patient for surgery while the tumor was operable or (2) to stop prematurely the resection with a lower impact on the natural history of the DLGG.

With the aim to overcome these pitfalls, one can currently consider to perform longitudinal studies based on pre-, intra-, and postoperative mapping rather than to content itself with static information based on a unique presurgical functional neuroimaging analysis [6, 8, 52]. Nevertheless, due to current limitations of fMRI/DTI, even if the neurosurgeon can of course at least partly take into account the data provided by neuroimaging, he should also integrate invasive electrophysiological investigations in his surgical strategy, especially (but not exclusively) for surgery in eloquent structures, in order to optimize the benefit-to-risk ratio of the resection, functionally as oncologically speaking.

Intraoperative Monitoring and Mapping: The Major Contribution of Direct Electrostimulation

As mentioned, intraoperatively, the integration of multimodal imaging into frameless stereotactic surgery was extensively used in the past decade and referred to as "functional neuronavigation." However, the sole randomized trial failed to demonstrate the significant impact of navigation on postoperative results [53]. It can be explained by the limitations of fMRI and DTI detailed above, as well as by the high risk of intraoperative brain shift, due to surgical retraction, mass effect, gravity, extent of resection (especially for voluminous DLGG), and cerebrospinal fluid leakage. Several technical improvements have been proposed to reduce the effects of this shift, but their reliability has still to be optimized: combination with intraoperative ultrasound, producing real-time imaging; use of mathematical models based on data from ultrasonography or digital images that track cortical displacement; and intraoperative MRI [37]. Nonetheless, their actual value on the improvement of extent of resection and preservation of QoL remains to demonstrate in DLGG.

As a consequence, *invasive electrophysiological investigations currently remain the* "gold standard" *when operating in eloquent brain structures.*

Intraoperative Monitoring

First, the technique of somatosensory and motor evoked potentials was extensively used in the past decades for intraoperative identification of the central region [54]. However, its reliability regarding the localization of the rolandic sulcus is not optimal, with accurate localization of the central sulcus reported only between 91 and 94 %. Estimation of the overall sensitivity and negative predictive value of intrasurgical somatosensory evoked potential was evaluated around

79 and 96 %, respectively [55]. Moreover, phase reversal recording identifies only the central sulcus itself but offers no direct information on the particular distribution of sensory-motor function on the adjacent exposed cerebral structures. This limitation can be dangerous in cases of brain reorganization, since evoked potential may be an inappropriate guidance to estimate true eloquent areas [56]. Also, whereas the method of motor evoked potentials was improved, when recording compound muscle action potentials, only the monitored muscles can be controlled, that is, there is an inability to detect and possibly avoid motor deficits in nonmonitored muscles. In addition, monitoring of muscle action potentials does not mean monitoring of complex movements, action adapted to the environment, and intention to act, which is nonetheless the ultimate goal for the patient [57–60]. Above all, intraoperative evoked potentials cannot currently be used to map language, memory, or other higher functions crucial for the quality of life of the patients (for a review, see [61]).

Numerous authors have also proned the use of extraoperative electrophysiological recordings (electrocorticography) and stimulations via the implantation of subdural grids [62]. Using this method, the patient is in optimal conditions, in his room, to perform the tasks: this point is particularly important for children. Moreover, recent advances in the interpretation of the electrophysiological signal, such as electrocorticographic spectral analysis evaluating the event-related synchronization in specific bands of frequency, have allowed a better understanding of the organization of the functional cortex, and a study of the connectivity, in particular via the recording of "cortico-cortical evoked potential" [63]. However, extraoperative electrophysiological mapping usually used grids with 1-cm-spaced electrodes, thus, with a limited accuracy. Also, it is necessary to perform two surgical procedures, one to implant grids and a second to remove the lesion. In addition, there is still a risk of infectious complications due to the presence of subdural grids during several days. Above all, although this method was extensively advocated in epilepsy surgery, because it also allows detection of the seizure foci, only the cortex can be mapped: it provides no information about the axonal connectivity, that is, it is not possible to map the subcortical structures. Thus, this technique is not adapted to neuro-oncology, since it is well known that gliomas migrate along the white matter bundles [28].

Intraoperative Cortical Mapping Using Direct Electrostimulation (DES)

Taking into account the limitations of the different mapping techniques described above, DES is the goal standard during surgery in eloquent areas, more and more frequently under local anesthesia [2, 4, 6, 20, 26, 40, 50, 64–67]. Indeed, except for tumors located within the motor structures, DES mapping is performed in awake patients. However, as previously mentioned, because movements and action are more complex than single muscle contractions, it is also currently recommended to map the motor function under local anesthesia with an active participation of the patient [26]. Indeed, in a recent study, Schucht et al. demonstrated the existence of a large frontoparietal network involved in motor control. Its stimulation in awake patients, especially at the subcortical level, may induce involuntary arrest or acceleration of the movement, impossible to detect under general anesthesia with a single electrophysiological monitoring [58].

From a technical point of view, DES has extensively been demonstrated to represent an easy, reliable, reproducible, safe, and not expensive method in numerous previous reports [2, 50, 64–67]. The main goal is to perform on-line anatomo-functional correlations, thanks to active interactions between the anesthesiologist, speech therapist/neuropsychologist/neurologist, neurosurgeon, and the patient him(her)self. The principle is to use DES to mimic a focal and transitory virtual lesion, to obtain an individual functional mapping both at cortical and subcortical levels, and to test if a structure involved by a lesion is still crucial for the function, what is observed in 15–20 % of cases in DLGG. It is thus possible to

decide whether the brain area tested can be removed (or not), according to the induction of transitory functional disturbances (or not) during its stimulation. Indeed, DES of an essential area generates a transient disruption of the task performed by the patient, and this area should be preserved. An individual cortical mapping is thus obtained before the resection, which can be tailored according to the results of this functional map. In practice, a bipolar electrode tips spaced 5 mm apart and delivering a biphasic current (pulse frequency 60 Hz, single-pulse phase duration 1 ms) is applied to the brain. The current intensity adapted to each patient is determined by progressively increasing the amplitude in 1 mA increments from a baseline of 2 mA until a functional response is elicited, with 5 mA as the upper limit under local anesthesia – with the goal of avoiding the generation of seizures. The patient is never informed when the brain is stimulated. No site is stimulated twice in succession, to avoid seizures. Each cortical site of the entire cortex exposed by the bone flap is tested three times. Indeed, it is admitted nowadays that three trials are sufficient to assure if an area is crucial for brain function, by generating disturbances during its three stimulations, and with normalization of the function as soon as the stimulation is stopped. This limitation of trials and tasks is required by the timing of the surgical procedure, because the patient is awake and can be tired at the end of the resection.

Interestingly, recent series showed that the surgical procedure could be simplified by avoiding the use of intraoperative electrocorticography despite an equivalent reliability of the electrical mapping and without increasing the rate of seizures [2, 50, 68, 69]. However, in cases of stimulation-induced seizures, the use of cold Ringer's lactate is recommended to abrogate the seizure activity [70]. Moreover, some authors emphasized the value of "negative mapping" (no identification of eloquent sites) in the setting of a tailored cortical exposure [65, 66]. Although such recommendation could be acceptable for high-grade gliomas, since the surgical goal is mainly to remove the enhanced part of the tumor, a negative mapping is very dangerous in surgery of DLGG, especially in nonexpert hands. Indeed, due to the fact that DLGG is poorly delineated, the limit of the resection will be essentially guided according to functional criteria. Because negative mapping can be due to false negative for methodological reasons, it does not guarantee the absence of eloquent sites. Thus, negative mapping cannot prevent persistent postsurgical worsening in all patients: 1.6–9 % of permanent postoperative deficits have been reported in cases of negative mapping [64–66]. Therefore, other authors continue to advocate a wider bone flap in order to obtain systematic functional responses before the resection [2, 68, 69]. *Moreover, a positive mapping might also allow an optimization of the extent of resection, since the tumor removal can be pursued until eloquent areas are encountered, that is, with no margin around the functional structures* [6, 8]. A recent study demonstrated that, in a consecutive and homogeneous series of 115 DLGG in the left dominant hemisphere, the rate of permanent deficit remained lower than 2 % despite the absence of margin around the language sites [50]. Indeed, Gil Robles and Duffau [49] showed that the extent of resection could be dramatically increased by avoiding the preservation of 5–10 mm around the functional areas, as usually proposed in the classical literature. Interestingly, Gil Robles et al. also showed that it was not logical to leave of a small amount of tumor involving the cortex when the resection was performed at the subcortical level into the contact of white matter pathways (see below), since it means that the cortical area not removed was in fact disconnected and then no more functional [49].

One of the major advantages of DES for brain mapping in adult patients is that they *intrinsically* do not cause any false negatives – if nevertheless the methodology is rigorously applied, as detailed above. Indeed, DES is highly sensitive for detecting the cortical and axonal eloquent structures, and it also provides a unique opportunity to study brain connectivity, since each area responsive to stimulation is in fact an input gate into large-scale network – rather than an isolated discrete functional site. DES, however, has also a limitation: its specificity is suboptimal. Indeed, DES may

lead to interpretation that a structure is crucial, due to the induction of a transient functional response when stimulated, whereas (1) this effect is caused by the backward spreading of the electrostimulation along the network to an essential area and/or (2) the stimulated region can be functionally compensated thanks to long-term brain plasticity mechanisms. In brief, although DES is still the gold standard for brain mapping, due to the risk of "false positives," its combination with new methods such as perioperative functional neuroimaging and biomathematical modeling is now mandatory, to clearly differentiate those networks that are actually indispensable to function from those that can be compensated [71].

Intrasurgical Cognitive Monitoring and Test Selection

The selection of the tasks administrated to the patient intrasurgically is crucial to preserve a normal life [12]. Intraoperatively, this means that, after asking to all patients to count, in order to detect the ventral premotor which systematically induces speech arrest or anarthria when stimulated (whatever the hemisphere) – with the aim of identifying the optimal intensity threshold for the rest of the mapping [35, 36, 50, 68, 69, 72] – the intraoperative testing should be adapted to each patient. Such a task selection should be made according to several individual parameters: job, hobby, handedness, results of the preoperative neuropsychological examination, location of the tumor, and results of the preoperative functional neuroimaging [12, 73]. For instance, language mapping can be performed in order to detect possible language epicenters in the right "nondominant" hemisphere in left-handers or ambidextrous (and even in some cases in right-handers), according to the results of the presurgical cognitive assessment, that is, if language disturbances have been identified even in cases of left-lateralization on functional MRI [35, 36, 73]. The goal is to map the networks underlying the different but interactive subfunctions which have to be preserved intraoperatively – and which will serve as boundaries of the resection.

Indeed, DES allows the mapping of numerous brain functions:

- Movement, which is not only the ability to contract a muscle but which is a real cognitive function enabling to plan, execute, and monitor a complex action (see above) [57–60].
- Somatosensory function: stimulation may generate dysesthesia/tingling described by the patient him(her)self intraoperatively, as well as movement disorders due to a deficit of haptic feedback [58, 61].
- Visual function: stimulation may elicit phosphenes, visual illusion, visual hemiagnosia, and/or visual field deficit described by the patient, with a feedback made possible thanks to the presentation of two objects situated diagonally on a screen divided into four quadrants [74–77].
- Auditory-vestibular function: In particular, stimulation may induce vertigo [78].
- Spatial awareness: This complex function, which integrates the previous sensorimotor, visual, and auditory-vestibular functions to allow a consciousness of the interactions between human body and the environment, can be mapped intraoperatively by using line bisection in order to avoid permanent hemineglect [79, 80].
- Language: Beyond spontaneous speech and counting, object naming, comprehension, writing, reading, syntax, bilingualism, and language switching from one language to another can be tested throughout the resection [50, 65, 72, 81–87].
- Higher-order functions such as calculation, memory, attention, cognitive control, crossmodal judgment, or even emotional processing may also be mapped using intrasurgical DES [88–93].

As a consequence, due to the limitation of time during surgery, the goal is to prioritize the intrasurgical tasks according to the wishes of the patient, in order to preserve his(her) real QoL: in other words, the individual mapping must be personalized.

To this end, it is crucial that a speech therapist/neuropsychologist/neurologist be present in the operative room, in order to interpret accurately

the kind of disorders induced by DES, for instance, speech arrest, anarthria, speech apraxia, phonological disturbances, semantic paraphasia, perseveration, anomia, syntactic errors, and so on (see Chap. 19 by Moritz-Gasser and Herbet) [50, 81]. *In summary, DES is able to identify in real time the cortical sites essential for the function before the beginning of the resection, in order to both select the best surgical approach and to define the cortical limits of the lesion removal.*

Intraoperative Subcortical Mapping Using DES: Detection and Preservation of the Neural Connectivity

Another major issue is the use of subcortical mapping throughout the resection, in addition to the cortical mapping before the lesion removal [50, 58, 59, 61]. Indeed, conversely to the major potential of brain reorganization at the cortical level, subcortical brain plasticity is very limited [94, 95]. Recently, a probabilistic atlas of functional resectability of LGG was built, on the basis of residual tumor left due to involvement of critical areas – as determined by intraoperative functional mapping. Interestingly, a "minimal common brain" was evidenced, that is, a common core among patients which could not be removed, mainly including white matter connectivity and supporting the essential role of these subcortical networks in cerebral processing [95]. Indeed, injury to restricted regions of white matter can cause the dysfunction of large-scale cognitive networks. Thus, the neurosurgeon should know the fibers which cannot be resected and should be able to identify and to preserve them in all case, that is, (1) the input and output networks, that is, the pyramidal, thalamocortical, and optic tracts subserving motor, somatosensory, and visual functions, respectively; (2) the oral and written language networks in the "dominant hemisphere"; and (3) the spatial network, generating disturbances of spatial cognition (e.g., hemineglect or vestibular responses) during stimulation – see below for more details.

In other words, these pathways must be detected during the lesion removal, in order to preserve the anatomo-functional connectivity while optimizing the EOR, that is, to pursue the resection until eloquent pathways are detected. Interestingly, according to the same principle as that described at the cortical level, DES can also identify eloquent subcortical structures. It allows the study of the anatomo-functional connectivity by directly and regularly stimulating the white matter tracts and deep gray nuclei throughout the resection and by eliciting transitory functional disruption when into the contact with deep crucial areas.

As for vascularization preservation (see Chap. 23 by Duffau), it is puzzling to note that intrasurgical subcortical mapping was very scarcely reported in glioma surgery and that neurosurgeons began to demonstrate some interest for the white matter pathways essentially since the recent development of DTI – which is nevertheless not reliable enough, as already discussed. Consequently, it seems important to extensively detail the different tracts participating in distinct neural functions, because it is crucial for a surgeon to have a good three-dimensional representation of the subcortical bundles in his mental imaging before to operate the brain (with or without intraoperative functional neuroimaging). To this end, even if DTI may represent an excellent didactic tool to learn this complex architecture, it is essential to go back to the laboratory to perform anatomic dissection on cadavers, especially concerning the white matter tracts using the Klinger's method [96, 97]. Indeed, new anatomic dissections of the white matter pathways can be now performed in the lights of data provided by axonal mapping (see below), especially with regard to the cortical terminations of the subcortical pathways, which are still poorly known [98–101]. Such knowledge concerning relationships between brain structure and function can be successfully applied to a better understanding of the surgical anatomy, as follows (Fig. 24.1):

- Motor pathways
  In precentral DLGG, after detection and preservation of the primary motor cortex using cortical stimulation, the corresponding descending motor pathways and their somatotopy – that is, the fibers in the corona radiata, with, from medial to lateral, the pyramidal tracts of the lower limb, of the upper limb, and

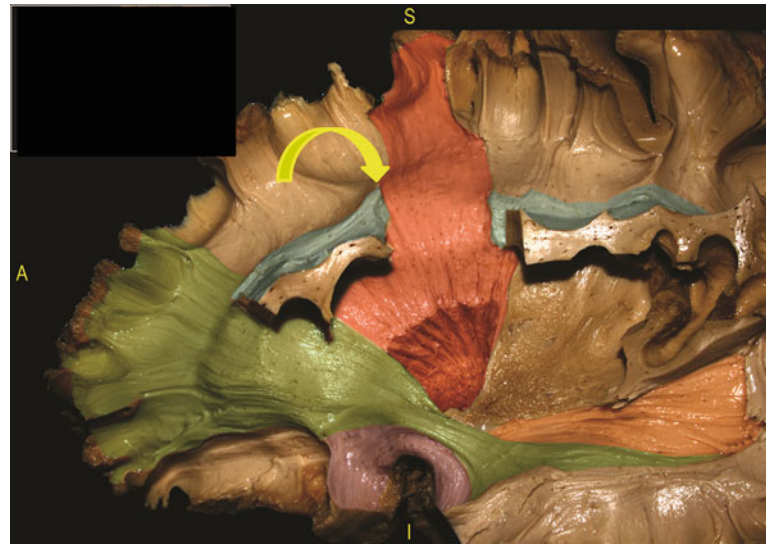

Fig. 24.1 The main association and projection fascicles demonstrated in anatomic dissections were summarized and were colored for an easier recognition. *Green* IFOF, *violet* UF, *blue* SLF, *red* CS tract, *orange* stratum sagittale (Left hemisphere; *A* anterior, *P* posterior, *S* superior, *I* inferior). According to this anatomy, the *yellow arrow* shows one of the major deep functional boundaries during glioma resection within the left frontal lobe, that is, the crossing between the left SLF and the CS tract (Modified from [101])

of the face – should be identified using subcortical stimulation and should be spared. As at the cortical level, subcortical motor fibers constitute the posterior and deep functional limits of the resection, until the opening of the ventricle [61]. The pyramidal pathways may also be detected within the posterior limb of the internal capsule, representing the deep boundaries for temporal or (fronto-)temporo-insular paralimbic gliomas [102]. Interestingly, as mentioned, our team recently evidenced the existence of a "modulatory motor network," eliciting movement arrest when stimulated and involved in motor control [58].

- Somatosensory tracts
  In retrocentral gliomas, the thalamocortical somatosensory pathways and their somatotopy can also be detected by DES, which generate dysesthesias or tingling in awake patients [21, 103]. Of note, stimulation of the white matter under the retrocentral gyrus may also induce disturbances in movement control, likely due to transient inhibition of U fibers within the rolandic region [58].
- Optic radiations
  Visual pathways can be mapped in awake patients operated on for a temporo-occipitoparietal glioma. Their stimulation may generate a "shadow" (negative effect) or phosphenes (positive effect) in the contralateral visual field, sometimes associated with metamorphopsia (i.e., visual illusion) or visual hemiagnosia [74–77]. In all cases, these phenomena lead to a transitory visual deficit in the contralateral hemifield. However, due to the subjectivity of the response, it was recently proposed to use a picture naming task with presentation of two objects situated diagonally on a screen divided into four quadrants. An image was presented in the quadrant to save and another image was presented in the opposite quadrant. Using this specific test, in a consecutive series of 14 patients (including 12 DLGGs), visual symptoms have been elicited in all cases during DES. These disturbances led to stop the tumor resection at this level. Postoperatively, no patient but one had a permanent hemianopia, despite an expected quadrantanopia in 12 cases, with a mean EOR of 93.6 %. These original findings showed that real-time identification of optic radiations is a reliable and effective method to avoid hemianopia in surgery for DLGG involving visual pathways. Patients can be left with only a residual quadrantanopia without consequence on the quality of life, especially for driving [76].
- Language pathways: a surgical point of view
- In precentral DLGG within the dominant hemisphere, after identification of the motor and language cortical sites in the ventral premotor cortex (lateral part of the precentral

gyrus) and in the inferior frontal gyri (the so-called Broca's area), stimulation may allow the detection of the language pathways [47, 50, 72, 104]. Medially, subcortical mapping can identify the fasciculus subcallosal medialis (running from the supplementary motor area and cingulate gyrus to the head of the caudate nucleus) which induces a transient transcortical motor aphasia during its stimulation – because this tract participates in the initiation of language [104]. It seems that this fascicle could correspond to the indirect pathway of the frontal aslant tract connecting the superior frontal gyrus to the inferior frontal gyrus [105, 106]. Posteriorly, the tracts coming from the premotor ventral cortex must be detected and spared, since it is crucial for speech production: its stimulation generates dysarthria or complete anarthria with a high level or reproducibility. More laterally, the operculo-insular connections should also to be identified by eliciting a complete speech arrest during stimulation: these connections are involved in speech planning [47, 50, 72, 104].

- In addition to these loco-regional language pathways, subcortical stimulation also allows the detection of long-distance association pathways, with first of all, the deep part of the superior longitudinal fascicle (i.e., the so-called arcuate fascicle) (AF) (Figs. 24.1 and 24.2) [101, 104]. In patients with a DLGG involving the dominant insula or inferior frontal gyrus, subcortical stimulation can identify the anterior part of AF, running within the anterior floor of the external capsule (under the superior part of the insula) to go to the posterior portion of the inferior and middle frontal gyri [99]. DES generates transitory symptoms observed in conduction aphasia, that is, phonemic paraphasia and repetition disturbances. AF must also be detected at the level of its posterosuperior loop, located under the supramarginal gyrus, in patients operated on for a parietal DLGG involving the dominant hemisphere [103]. The same symptoms combining phonemic paraphasias and repetition disorders are elicited with reproducibility, without any semantic paraphasia [107]. In the same vein, AF can constitute the deep limit of resection in temporal DLGG within dominant side, because the inferior and middle temporal gyri correspond to the posterior cortical terminations of the long segment of AF. Thus, the posterior part of its posterior funiculus should represent the anterior functional limit of resection in posterior temporal DLGG, and the anterior part of the anterior funiculus of AF should be used as the posterior functional boundary in anterior and mid-temporal DLGG [102]. Interestingly, dominant AF seems also to subserve a wide network involved in language switching (from a native language to another language or vice versa). Its stimulation may disrupt such function, important to detect and to spare in bilingual patients [84]. More recently, grammatical gender errors were induced by axonal stimulation of the dominant AF, supporting the possible role of this pathway (connecting the middle temporal gyrus and the inferior frontal gyrus, themselves inducing the same grammatical disturbances when stimulated) in syntactic processing [83].
- In addition to the AF, the lateral part of the superior longitudinal fasciculus is important to preserve. In left retrocentral suprasylvian DLGG, after detection of the language cortical sites over the ventral premotor cortex in front of the tumor and over the supramarginal gyrus and/or angular gyrus behind it, stimulation of the corresponding frontoparietal subcortical network generates speech apraxia [103, 107, 108]. This operculo-opercular loop, called "the lateral part of the superior longitudinal fascicle, part III," constitutes the anterior segment of the indirect pathway of the dorsal phonological route, which runs parallel and lateral to the AF, by connecting "Broca's territory" with the inferior parietal lobe "Geschwind's territory" – as recently showed by DTI as well as by anatomic dissection [99, 109]. This subcomponent of the superior longitudinal fascicle should be preserved during surgery because it seems to represent the neural basis of verbal working memory [107].
- In parallel, subcortical DES also demonstrated the crucial role of the inferior

fronto-occipital fascicle (IFOF) in the "ventral semantic route," by eliciting reproducible semantic paraphasia when stimulated [50, 81]. In frontal DLGG involving the dominant hemisphere, especially in tumors located within the pars orbitalis of the inferior frontal gyrus and the dorsolateral prefrontal area, the anterior part of the IFOF should be detected and should represent the deep boundaries. Indeed, a recent anatomic study combining dissection and DTI showed that IFOF had five anterior cortical terminations: the inferior frontal gyrus, middle frontal gyrus, dorsolateral prefrontal cortex, orbitofrontal cortex, and frontal pole [100]. IFOF must also be identified throughout resection for insular DLGG in the dominant side, by inducing the same symptoms (semantic paraphasias) during DES of its intermediate part located in the anterior floor of the external capsule (in front

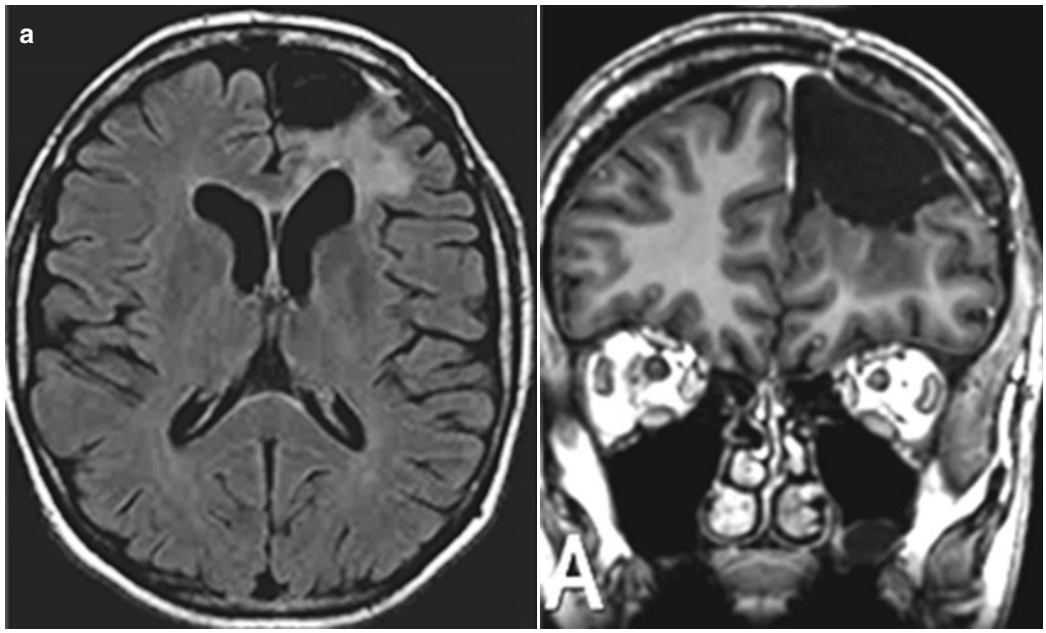

Fig. 24.2 Illustrative case of resection according to functional boundaries, concerning a patient who underwent a reoperation for a left dominant frontal DLGG (**a**). The first surgery was performed in another institution under general anesthesia, achieving a partial resection. A second surgery was performed in our institution under direct brain mapping in awake conditions. The intraoperative picture shows the cortico-subcortical boundaries identified by DES (**b**). Reproducible speech arrests were induced at the level of the ventral premotor cortex (tags 1, 2). No further functional responses were elicited by stimulations of other frontal cortices, including the so-called Broca's area. The resection was extended up to the pars opercularis of the inferior frontal gyrus posteriorly and to the precentral gyrus medially, with preservation of the supplementary motor area. The fronto-orbital and fronto-polar region was removed, reaching the head of the caudate nucleus in the depth. White matter critical components constituted the deep limits of the resection. Perseverations were induced by DES at the level of tag 48, phonemic paraphasias at the tag 49, semantic paraphasias at the tag 50, and language and motor arrest at the tag 45 (**c**–**e**). The anatomical analysis of the subcortical pathways has been provided according to functional results of the DES in awake surgery. Tag 48, corresponding to the head of caudate nucleus (*pink arrow*), represents the deep limit of the lobectomy and is identifiable anterolaterally by means of the tip of frontal horn of the lateral ventricle (**c**); tag 49 refers to the arcuate fascicle stem running in the depth of the inferior frontal sulcus (*blue tags*, *blue arrows*) (**c**–**e**); tag 50 represents the frontal fibers of the inferior fronto-occipital fascicle (*green tags*), crossing the superior longitudinal fascicle at the level of the pars opercularis and triangularis of the inferior frontal gyrus (*green arrow*) (**e**); and tag 45 are the fibers coming from the supplementary motor area (*red arrow*) (**d**). The tumor was radiologically completely removed, as showed by the postoperative MRI (**f**). The patient fully recovered despite some transitory postoperative language disturbances and resumed a normal socio-professional life. (Left hemisphere; *A* anterior, *P* posterior, *B* base, *V* vertex) (Modified from [101])

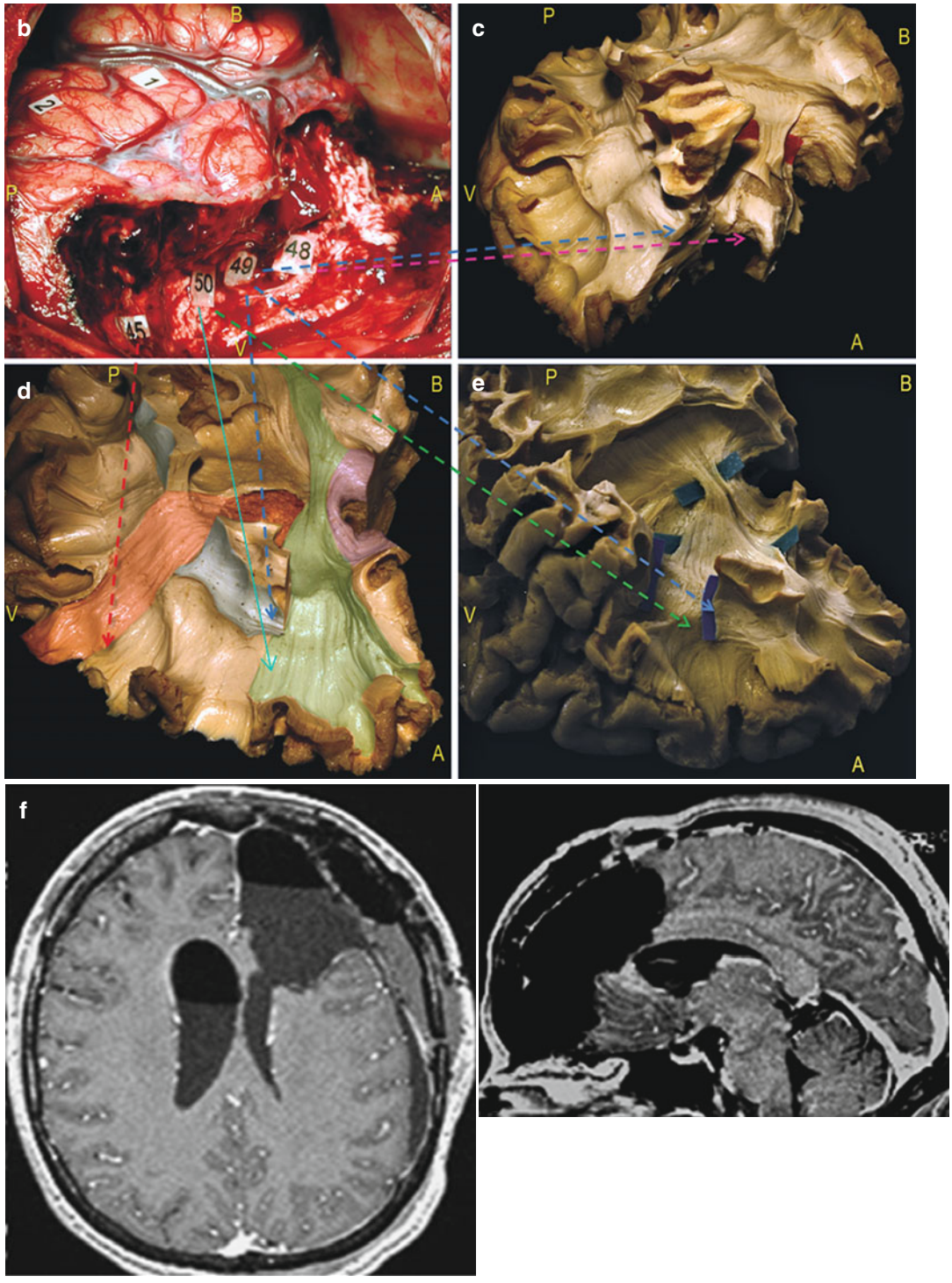

Fig. 24.2 (continued)

and inferiorly to the AF and behind and superiorly to the uncinate fasciculus) [46, 81]. Again, IFOF should be detected in temporal DLGG (semantic disorders when stimulated) because it represents the deep limit of the resection (above the roof of the temporal horn of the ventricle) [50, 81, 98]. Of note, the IFOF could also be involved in visual-verbal incongruence judgment [89].

- A recent series specifically dedicated to the study of occipital DLGG showed that, at least in the dominant hemisphere, both AF and IFOF may represent the deep and anterior boundaries of the resection when an extensive occipital lobectomy has been decided [110].
- Interestingly, DES of the anterior part of the inferior longitudinal fascicle, in front of the visual object form area (i.e., the basal part of the temporo-occipital junction, involved in high-level visual processing such as reading) [75, 111], as well as DES of the uncinate fascicle [112], never generated language disturbances, at least during the picture naming test – even if the uncinate fascicle was recently described as likely involved in proper name retrieval [113]. In the same way, DES of the anterior part of the middle longitudinal fascicle, that is, a pathway connecting the angular gyrus to the temporal pole and running under the superior temporal sulcus [114], seems to not generate language disturbances [115]. In practice, it means that these fasciculi can be removed without aphasia in temporal DLGG involving the dominant hemisphere. This indirect pathway from the temporo-occipital areas to the prefrontal region, with a relay in the temporal pole (temporo-occipital area, inferior longitudinal fasciculus, temporal pole, uncinate fasciculus, orbitofrontal, and prefrontal areas), might be compensated by the direct pathway constituted by the IFOF [111]. However, the posterior part of the inferior longitudinal fasciculus should be preserved in temporo-occipital DLGG, because it plays a crucial role in reading, as demonstrated by DES which elicited reproducible visual paraphasia and dyslexia [75].
- Of note, beyond the stimulation of the white matter, mapping of the deep gray nuclei is important when they are involved by DLGG. Stimulation of the head of the dominant caudate in frontomesial glioma coming into the contact of the striatum in the depth generates perseverations, namely, the repetition of the previous item while the next item is presented to the patient. These data support an inhibitory role of the caudate in the control of cognition [91, 116]. Furthermore, at the end of the resection of insular DLGG in the dominant side, stimulation of the lateral part of the lentiform nucleus induces anarthria [91], supporting the likely role of this structure in the planning of articulation, in association with the insula and ventral premotor cortex [116, 117].
- Finally, it is also crucial to underline the need of intrasurgical language mapping, both at cortical and subcortical levels, for gliomas involving the right hemisphere in left-handed and ambidextrous patients [36] or even in some atypical right-handers [35], due to a possible bilateral distribution of language networks – with in rule a mirror organization of both hemispheres.
- Pathways underlying spatial cognition
- In glioma involving the right ("nondominant") parietotemporal junction, intraoperative DES must also map the white matter tracts implied in spatial awareness, to avoid postoperative left neglect. To this end, it is possible to use a task of line bisection during awake surgery. During the stimulation of the part II of the superior longitudinal fasciculus, a significant rightward deviation is observed [79].
- DES of the right superior longitudinal fascicle may also induce vertigo, by disrupting a large network between the parieto-insular vestibular cortex, the visual, and the sensory-motor areas [78].
- Interestingly, although mapping of the interhemispheric white matter pathways has been performed, no functional responses were elicited by DES of the corpus callosum. Such results have allowed resection of DLGG involving this structure without any consequence on the quality of life, whatever the location of the "callosectomy" [118].

In All Cases, These Language Bundles Should Constitute the Subcortical Functional Limits of the Resection (Fig. 24.2)

Of note, this list of tracts is non-exhaustive. Indeed, the functional connectivity underlying emotional and behavioral processing is currently poorly known. Furthermore, the role of some pathways is still unclear, as for instance the role of the middle longitudinal fascicle [115].

In summary, it is mandatory to map both horizontal cortico-cortical connectivity (long-distance association fibers) as well as vertical cortico-subcortical connectivity (projection fibers), with the goal to preserve the networks underlying the "minimal common core" of the brain [95, 119]. Consequently, from a practical point of view, removal of voluminous glioma infiltrating several subcortical bundles can be performed without the use of microscope. Indeed, the main risk in this kind of surgery, except vascular injury, is to cut the connectivity. Therefore, it is important in the depth of the resection to have a global three-dimensional view of the surgical field and not to focus on a specific point, with the goal to know when the subcortical stimulation must begun (Fig. 24.2). In the same state of mind, it may be recommended to systematically use the same lateral position for each patient, whatever the location of the glioma (since it is possible to operate frontal, parietal, temporal, insular, and even occipital tumor in this position), in order for the neurosurgeon to keep his same mental reconstruction of the different fibers from one patient to another, without introducing any confounding factor such as head rotation [6]. Interestingly, the CUSA may represent an additional mapping tool within the white matter, because it can induce transient language disturbances that were confirmed afterward by stimulation. This interference with language (and also with motor) mapping might be interpreted as a transitory inhibition of axonal conduction [120]. This important point means that when the patient is awake and performs continuously the tasks throughout the tumor removal, it is not mandatory to stimulate a lot, but only when the resection arrives close to the functional pathways (information which is given on-line thanks to the ecological feedback from the patient, the transient disturbances induced by the CUSA, and the knowledge of the white matter anatomy). In other words, it is possible to not loose time (by stimulating only at the end of the procedure to find the eloquent structures), which is precious in awake surgery due to the fact that the patient can be tired after 1–2 h of resection. In the same way, as already detailed in the previous chapter by Duffau, in voluminous gliomas (for instance fronto-temporo-insular tumors), in order to win time, it can be considered to identify the cortical boundaries first, to perform a subpial dissection into the contact of these eloquent structures until the depth of the sulci, to arrive within the white matter pathways, and to remove it until functional fibers have been identified. If this technique is applied all around the glioma, once this part of the brain invaded by the tumor is "disconnected," it is possible to put the patient under general anesthesia and to take all the time to finish the resection (especially into the contact of vessels such as the sylvian fissure, which does not require the participation of the patient). Using this method, the patient is awake only to give the functional boundaries of the resection to the surgeon. However, it implies that the surgeon must change his state of mind, that is, that he should not begin the tumor removal in "non-eloquent" areas (and then with a progressive extension of the resection toward functional regions), but he should begin the resection directly near the critical structures [6].

Finally, it is worth noting that, for deep lesions, the shorter trajectory is not always the safer. In some cases, it can be more adapted to select a more complex surgical approach in order to avoid to cut functional pathways, on the basis of the results provided by intrasurgical cortical and subcortical mapping [119].

Results

In the past decade, brain mapping has led to an impressive improvement of functional and oncological results in DLGG surgery.

Firstly, patients who were classically not selected for surgery for a long time, on the basis of pure anatomical criteria (e.g., gliomas involving the precentral gyrus or the pars opercularis of the left inferior frontal gyrus), can now benefit from resection with no dogmatic a priori against the surgical feasibility due to the tumor location. In particular, it was demonstrated that the use of intrasurgical DES allowed a significant increase of the surgical indications for DLGG involving so-called eloquent areas, when compared with a control group of patients who underwent resection under general anesthesia with no mapping [2]. For example, surgical resection is possible with no permanent neurological worsening for DLGG located within the Broca's area [47, 121], Wernicke's area [48], insula [46, 102], left dominant inferior parietal lobule [103], retrocentral area [61], and even the precentral region [21, 122]. In practice, it means that the contraindication for DLGG resection is essentially represented by very diffuse glioma "gliomatosis-like," especially when invading both hemispheres through the corpus callosum [23].

Secondly, despite an increased number of surgeries in critical regions, the rate of permanent neurological deficits was shown to be significantly lesser thanks to awake mapping [2, 123, 124], that is, less than 2 % in the recent series using intraoperative stimulation. Interestingly, this rate of less than 2 % of permanent deficits is very reproducible among the teams using awake mapping worldwide [2, 65]. In comparison, in series which did not use awake mapping, the rate of sequelae ranged from 13 to 27.5 %, with a mean around 19 % (for a review, see [2]). Interestingly, a recent meta-analysis studying more than 8,000 patients who underwent surgical resection for a brain glioma demonstrated that the use of intrasurgical mapping allowed a statistically significant reduction of permanent deficit, despite an increased rate of resection within eloquent areas [123]. In addition, the extent of resection was increased [123]. In other words, despite a frequent transitory neurological worsening in the immediate postoperative period – due to the attempt to perform a maximal tumor removal according to cortico-subcortical functional limits using intraoperative mapping, leading to a specific functional rehabilitation (see Chap. 30 by Herbet and Moritz-Gasser) – *more than 98 % of patients recovered the same status than before surgery after glioma resection within eloquent brain areas guided by functional mapping and returned to a normal socio-professional life* [2, 25, 65]. Furthermore, beyond the fact that surgery is able to preserve brain functions, a new concept is to emphasize its possibility to increase the QoL by removing a DLGG, as demonstrated by extensive neurocognitive assessment performed after the surgical resection [22–25]. This can be explained by a relief of seizures after resection in at least 80 % of patients who suffered from preoperative intractable epilepsy [125]. In a recent series, Ghareeb et al. demonstrated the significant impact of hippocampectomy in patients with intractable epilepsy generated by a paralimbic DLGG, even if the glioma did not invade the hippocampus. Indeed, hippocampal resection allowed seizure control in all cases, with an improvement in Karnofsky Performance Scale score since all patients resumed their social and professional activities after surgery – while they were not able to work before surgery [126]. In addition, following DLGG resection, it was observed an objective improvement of high-order functions such as working memory in more than 30 % of patients, especially following a personalized cognitive rehabilitation [22].

Thirdly, it could be argued that the use of mapping, even if it enables to preserve or to improve the QoL, might prevent to achieve an optimal removal of DLGG. In fact, DES also allows a significant increase of extent of resection (see previous chapter by Duffau).

Future Directions: The Study of Individual Functional Brain Remapping After Initial Resection

In the past decade, numerous observations of dramatic recovery following massive resection of brain regions invaded by DLGG have been reported [14–17]. Such functional compensation was attributed to cerebral plasticity, namely, the continuous process allowing short-term, middle-term, and long-term remodeling of the

neurosynaptic maps, to optimize the functioning of brain networks [16]. Beyond the evidence of intraoperative acute remapping using repeated electrostimulation throughout the resection (likely due to the unmasking of redundancies) [122], noninvasive functional neuroimaging allows the additional study of mechanisms of reshaping before and after surgical resection [48, 52, 127]. Interestingly, longitudinal studies based on serial functional imaging after surgery showed new degrees of reshaping, as a probable consequence of tumor removal as well as adapted postoperative functional rehabilitation. For example, functional neuroimaging performed following the recovery of a transient postsurgical supplementary motor area syndrome showed the compensatory recruitment of the contralesional supplementary motor area and premotor area [127]. A "jump" of the perilesional activations was also reported following DLGG resection, for instance, from the precentral sulcus to the central sulcus in tumors involving the premotor region (Fig. 24.3) [52].

This better knowledge of plasticity phenomena has led to propose reoperation(s) when the resection was not complete at the end of the first surgery, due to the involvement of eloquent areas by the tumor. Thanks to functional reshaping (verified using intrasurgical awake mapping), it was possible to increase the extent of resection

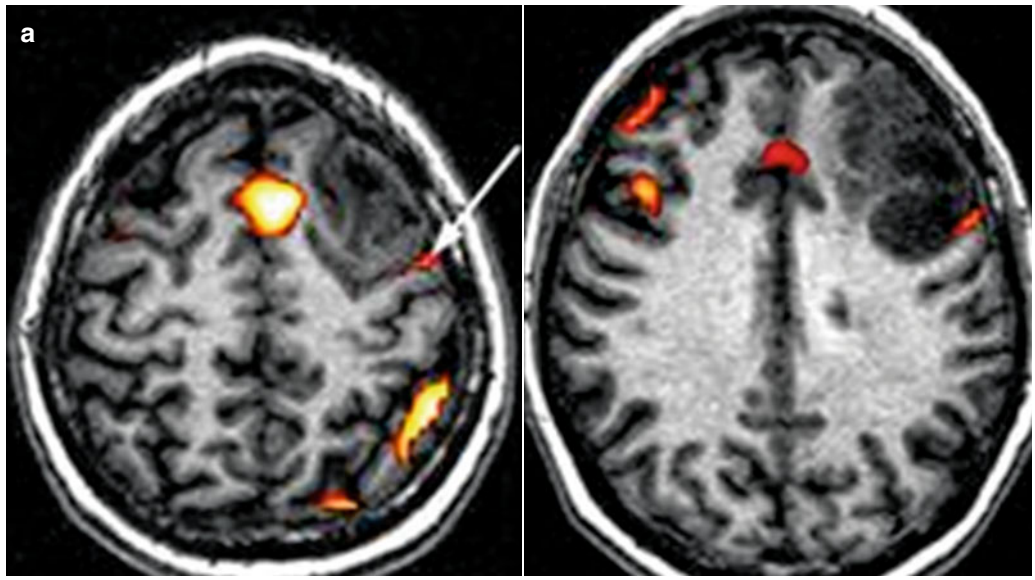

Fig. 24.3 Illustration of the multiple-stage surgical approach. (**a**) Preoperative language fMRI in a patient without deficit, harboring a DLGG involving the left premotor area: language activation was very close to the posterior part of the tumor, within the precentral sulcus (*arrow*). (**b**) Intraoperative views before (*left*) and after (*right*) resection of the glioma, delineated by letter tags. DES shows a reshaping of the eloquent maps, with a recruitment of perilesional language sites, allowing a subtotal resection with nevertheless a posterior residue due to invasion of crucial areas (number tags). The *yellow arrow* shows the precentral sulcus, demonstrating that it was not possible to remove the part of the glioma involving the precentral gyrus. (**c**) Immediate postoperative enhanced T1-weighted MRI showing the residue (*arrow*), in front of the precentral gyrus. (**d**) Postoperative language fMRI 4 years after the first fMRI, demonstrating a recruitment of the contralateral hemisphere and the posterior displacement of activation previously located at the posterior border of the tumor, now within the central sulcus (*arrow*). (**e**) Intraoperative view during the second surgery, confirming the remapping and allowing a more extensive tumor resection posteriorly, with no permanent deficit. Again, the *yellow arrow* shows the precentral sulcus, demonstrating that, this time, it was possible to remove a part of the glioma involving the precentral gyrus. (**f**) Immediate postoperative axial FLAIR-weighted MRI (3 h after surgery) showing the improvement of the extent of resection within the left precentral gyrus, thanks to functional reshaping (the *red arrow* shows the central sulcus). The follow-up is now 11 years since the first surgery, with no recurrence since the second operation, in a patient enjoying a normal social and professional life (Modified from [52])

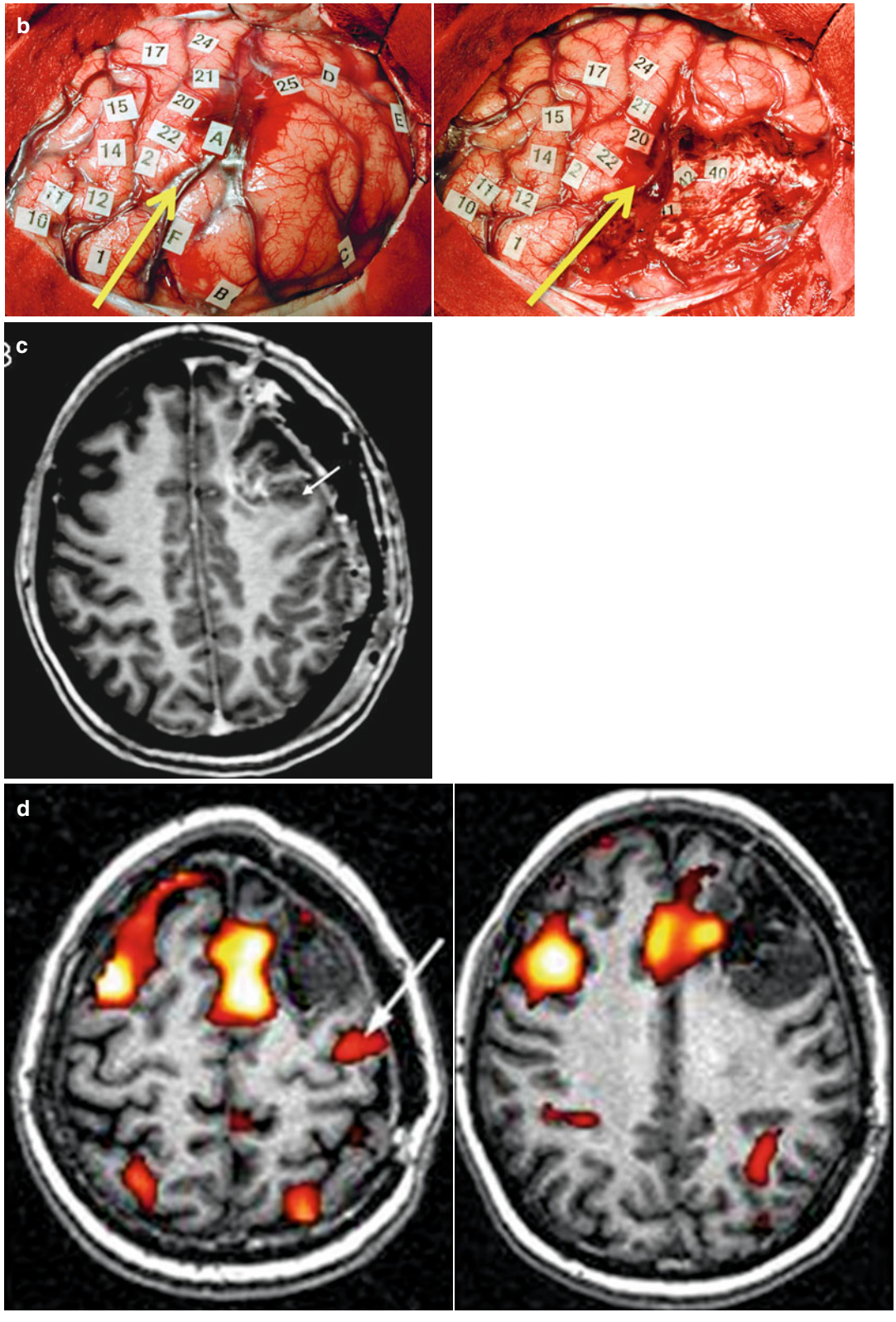

Fig. 24.3 (continued)

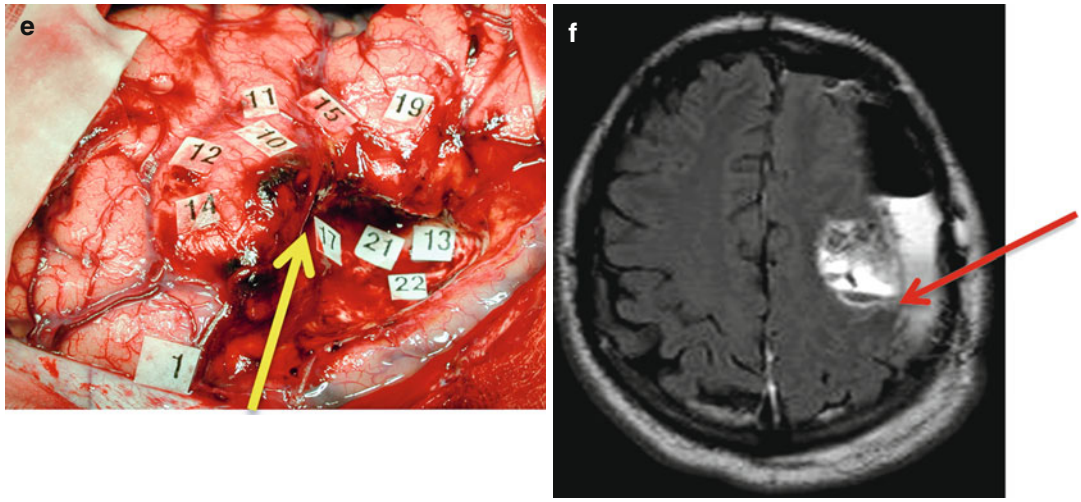

Fig. 24.3 (continued)

during a second and even during a third surgery, while preserving brain functions (Fig. 24.3) [8, 52, 128]. Therefore, this multistage surgical approach made possible DLGG removal in critical regions traditionally considered as unresectable, such as the central area, Broca's area, Wernicke's area, or the insular lobe (even in the left "dominant" hemisphere) [15, 17, 21, 46–48, 61, 103, 122].

However, these extensive resections with no or only slight neurological consequences can be only achieved on the condition that the essential subcortical connectivity is preserved. In other words, it is only possible in a "hodotopical" view of brain organization (i.e., in dynamic and parallel distributed large-scale networks able to compensate themselves) and no more in a "localizationist" framework (one area corresponding to one function) (see the Chap. 22 by Duffau) [117, 119].

Conclusions and Perspectives

These recent technical and conceptual advances, which consist in performing early (and possible repeated) surgical resection(s) for DLGG according to functional (and not oncological) boundaries provided by pre-, intra-, and postoperative methods of individual mapping both at cortical and subcortical levels, in a hodotopical and plastic framework of cerebral processing, have allowed a dramatic improvement of the onco-functional balance of surgery.

Recent stronger interactions between cognitive/behavioral neurosciences (which aim of studying the neural basis of cerebral functions, thanks to a combination between anatomy, functional mapping, and cognitive models) and oncological neurosurgery begin to solve the classical dilemma – survival versus brain functions – by giving the possibility to become more ambitious, that is, to increase the survival as well as to preserve (or even to improve) quality of life of patient with gliomas [129]. However, such evolution means that brain surgeons should change their state of mind, in order to operate the nervous system involved by a chronic tumoral disease (and no more by operating a tumor mass within the brain). To this end, awake mapping should be more systematically considered, even in presumed "non-eloquent areas." This also means that brain functions should be more accurately mapped in asymptomatic patients, since the preoperative cognitive baseline is by definition optimal (see last chapter by Duffau). The "philosophical" question raised is thus to know whether emotional and behavioral tasks should be incorporated intrasurgically, in addition to the sensorimotor, visual, language, and cognitive

tasks, in one hand with a real chance to optimize the QoL but in the other hand with a higher risk to leave more residual tumor. This perspective seems to represent the best way to build a modern and personalized "functional resective surgery of the central nervous system," especially a "functional surgical neuro-oncology."

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Chemotherapy for Diffuse Low-Grade Gliomas

25

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Abstract

Diffuse low-grade gliomas are rare tumors. Therapeutic strategies have dramatically changed in recent years, thanks to observational data, insight of some authors, retrospective studies, and, incidentally, results of few phase III and II trials. Surgery has become the cornerstone of the treatment. Radiotherapy, because of its potential delayed neurotoxicity and the equivalent results in terms of survival whatever the timing of the treatment (early or late), is increasingly offered to patients with unresectable tumors (or tumor that cannot be reoperated) and in case of progression after chemotherapy. Chemotherapy, subject of this chapter, has shown clinical benefits regarding tumor progression for nonsurgical patients, before or after radiotherapy: initial chemosensitivity almost constant, improvement of epilepsy and thus of cognition, and preservation of quality of life (despite a possible transient alteration). Its articulation with surgery has been more recently discussed by allowing, thanks to tumor shrinkage, subtotal or total resection (whose impact on anaplastic transformation and survival has been demonstrated), in addition to potential effects on cerebral plasticity. It remains to show the direct or indirect impact on survival, to refine its risk-benefit ratio, especially in the context of prolonged treatment with temozolomide, and to develop further research from a neurological (impact on plasticity) and oncological (involved molecular pathways, identifying new therapeutic targets) points of view.

Keywords

Diffuse low-grade gliomas • WHO grade II gliomas • Chemotherapy • Epilepsy • Quality of life • Survival

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Introduction

Diffuse low-grade glioma (DLGG) is a rare disease whose therapeutic concepts have profoundly been challenged these recent years [1].

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Thus, surgery, marginally considered until the 1990s, saw his place reinforced, thanks to the development of neuroscience researches through the cortical and subcortical stimulations, awake surgery, and functional imaging progress [2, 3].

Potential neurotoxicity of long-term conventional radiotherapy was stressed. Therefore, this treatment was little used in the initial stages of the disease. Recent technical advances allow nevertheless considering a more focused therapeutic volume. It should so lead to new assessments with a clear evaluation of the ratio between expected benefits/potential risks [4].

Finally, chemotherapy, despite many theoretical limitations (intrinsic chemoresistance, difficult access to the tumoral site, low number of available molecules), gradually developed, first in case of progression after conventional treatments deliverance and then more precociously in the disease history and in close coordination with surgery.

We will develop this point below by considering the conceptual and historical bases while highlighting the unresolved issues.

Current Practices

Conceptual Bases of Chemotherapy

The place of the chemotherapy for DLGG remains difficult to encircle. Many theoretical arguments can be opposed to the principle of prescription: subnormal blood–brain or blood-tumor barrier (low penetration of drugs), spontaneous chemoresistance of gliomas, and very limited number of potentially active molecules.

Nevertheless, there are situations like tumor progression in case of patients with unresectable tumor previously treated by radiotherapy (RT) where chemotherapy (CT), in absence of therapeutic alternative, could be discussed or considered before anaplastic transformation.

The literature on the subject remains relatively poor.

For a long time, only the paper by Eyre et al. from the South Western Oncology Group (SWOG) served as a reference and discouraged all therapeutic inclinations. In fact, the SWOG conducted the first randomized trial concerning CT for DLGG. It has compared RT alone versus RT plus lomustine-based CT after subtotal/partial surgery or biopsy. No benefit was shown and the trial was prematurely terminated [5]. To date, it is difficult to consider this relatively old trial because of the associated methodological bias regarding the selected population (more specifically from a pathological and radiological point of view).

Cairncross and Macdonald have, the first, evoked the possibility of a real objective response for diffuse low-grade tumors within a series of aggressive oligodendrogliomas [6, 7]. Six years later, Mason was able to note 9/9 responders under procarbazine + cecenu + vincristine association (PCV) [8], while Soffietti [9] reported 13/13 stabilized or responding patients also under PCV.

Since then, a little over 30 articles have been published. There are mostly retrospective series that included most often a small number of patients (see below). We will come back to some of these articles in the following chapters.

Available Data and Chronology

Table 25.1 adapted from Ducray [10] summarizes the literature on the subject. As we can appreciate, series are very heterogeneous in term of disease entities, time of illness, treatment modalities, and evaluation of the response.

We can nevertheless confirm that, in recent years, regardless the growing role of surgery, we have seen a real interest in chemotherapy (especially temozolomide) in the management of these tumors [11].

This led to the creation of a dedicated European Task Force and to the establishment of recommendations recently published (and being updated). These recommendations clearly propose chemotherapy in specific situations to which we will return in the course of this chapter: “Chemotherapy can be useful both at recurrence after radiotherapy and as initial treatment after surgery to delay the risk of late neurotoxicity from large-field radiotherapy” [12].

Table 25.1 Key publications about chemotherapy and diffuse low grade gliomas

Year	Authors and journal	*N*	Tumor type	Contrast enhancement (%)	Prior RT (%)	Prior CT (%)	Chemotherapy regimen	Response rate CR+PR/MR	1 year PFS	Median PFS
1996	Mason et al. *Neurology*	9	O	33	11	No	PCV	66 %/NA	NA	35
1998	Soffietti et al. *Neurosurgery*	13+7+M=26	0, 0A	73	42	No	PCV	62 %/NA	80	24
1998	Van den Bent et al.	52	O, OA	100	100	No	PCV	63 %/NA	NA	10
2003	Brada et al. *Ann Oncol*	11O+17A+M=30	O, OA, A	O	No	No	Temozolomide	10 %/48	>90	>36
2003	Buckner et al. *J Clin Oncol*	29	O+OA	46	No	No	PCV	52 %/NA	91	NA
2003	Pace et al. *Ann Oncol*	4O+29A+10 OA	O, OA, A	60	65	37	Temozolomide	47 %/NA	39	10
2003	Quinn et al. *J Clin Oncol*	20 O+16A+5 OA+M=46	O, OA, A	70	15	22	Temozolomide	61 %/NA	76	22
2003	Van den Bent et al. *Ann Oncol*	32	O, OA	100	100	100	Temozolomide	22 %/NA	11	3.7
2003	Van den Bent *J Clin Oncol*	38	O, OA	100	100	No	Temozolomide	52 %/NA	40	10.4
2004	Higuchi et al. *Neurology*	12	O	50	No	No	PAV	58 %/NA	100	>60
2004	Hoang Xuan et al. *J Clin Oncol*	49 O+11 OA	0, 0A	11	No	No	Temozolomide	17/14	73	NA
2005	Stege et al.	16 NG+5 R	O+OA	21	24	No	PCV	19/57	ND	>24
2006	Catenoix et al. *Rev Neurol*	7	O, OA	0	No	No	PCV	42/28	100	>60
2006	Duffau et al. *J Neurooncol*	1	O	0	No	No	Temozolomide	1/1	100	NA
2006	Levin et al. Cancer	28	0	NA	No	28	Temozolomide	36/25	89	31
2006	Ty et al. *Neurology*	7	O	NA	28	No	PCV	71/NA	100	>30
2007	Lebrun et al.	33	O	22	No	No	PCV	27/NA	90	>30
2007	Sunyach et al. *J Neurooncol*	24	O	NA	No	No	PCV Temozolomide	NA	NA	47
2007	Kaloshi et al. *Neurology*	149	O, OA, A	15	No	No	Temozolomide	15/38	79.5	28

(continued)

Table 25.1 (continued)

Year	Authors and journal	*N*	Tumor type	Contrast enhancement (%)	Prior RT (%)	Prior CT (%)	Chemotherapy regimen	Response rate CR + PR/MR	1 year PFS	Median PFS
2007	Pouratian et al. *J Neurooncol*	25	O, OA, A	24	No	No	Temozolomide 75 mg/m^2 – ¾ weeks	24/28	72	> 20
2007	Ricard et al. *Ann Neurol*	107			No	No	Temozolomide	92 % with initial decrease of MTD	NA	NA
2008	Tosoni et al. *J Neurooncol*	30	O, OA, A	0	No	No	Temozolomide P 75 mg/m^2 – ¾ weeks	30/NA	73	22
2009	Kesari et al. *Clin Cancer Res*	44	O, OA, A	NA	27	No	Temozolomide P 75 mg/m^2 7/11 weeks	20/NA	91	38
2009	Kaloshi et al. *Neurology*	62	O, OA, A	0	No	No	Temozolomide	–	–	–
2009	Taillandier et al. *Neurosurgical Focus*	46	O, OA, A	0	No	1	Temozolomide PCV	NA	NA	NA
2010	Peyre et al. *Neurooncol*	21	O, OA, A	14	No	No	PCV	38/42	100	40
2010	Kaloshi et al. *J Neurooncol*	20	O, OA	56	No	100	Nitrosoureas second line	0/10	28	6.5
2010	Houillier et al. *Neurology*	84	O, A, OA	0	No	No	Temozolomide	–	–	–
2011	Blonski et al. *J Neurooncol*	10	O, A, OA	0	No	No	Temozolomide	10/10	–	–
2011	Kaloshi et al. *Neurology*	149	O, A, OA	0	No	No	Temozolomide	77/149 (53 %)	–	*1p19q* LOH associated with longer OS
2011	Taal et al. *Neurooncol*	58	A	100	100	No	Temozolomide	54/NA	25	8

Adapted from [10]

N number of patients, *O* oligodendroglioma, *OA* oligoastrocytoma, *A* astrocytoma, *RT* radiotherapy, *CT* chemotherapy, *CR* complete response, *PR* partial response, *MR* minor response, *temozolomide* 200 mg/m^2 5/28 days, *PCV* procarbazine + cecenu + vincristine

We should nonetheless note that all except eight published series have fewer than 50 patients. This low number of inclusions reflects the relative scarcity of the pathology but also the difficulties to include such patients in therapeutic trials probably because of the specificity of this particular tumoral entity (too heterogeneous for normative constraints of clinical trials) and the conceptual differences between major involved groups.

Types of Chemotherapy

Two main modalities of chemotherapy were used for DLGG: procarbazine + cecenu + vincristine (PCV) association and temozolomide (TMZ), according to different patterns.

There are little variations in the reported dosages concerning the PCV combination used first by Gutin in 1975 [13] and Levin in 1980 and 1985 [14, 15]. Classically, cecenu is administered on day (D) 1 (110 mg/m^2), procarbazine (60 mg/m^2) from D8 to D21 and vincristine (1.4 mg/m^2 – max 2 mg) at D8 and D29. A cycle is administered every 6–8 weeks. Intensified protocols have also been described but not used in DLGG [16].

Temozolomide (TMZ) is, to date, the most widely used treatment. The conventional scheme proposes a daily dose of 150 mg/m^2 for 5 days during the first course. If it is well tolerated, the dose is increased to 200 mg/m^2 per day for 5 days from the second course. Cycles last for 28 days. Other plans, including intensified protocols, have been proposed. Lashkari et al. attempted to assess the impact of these different TMZ regimens on the treatment of DLGG. They performed a systematic review of the literature and identified all the studies published in PubMed, Embase, and Cochrane databases that met the inclusion criteria. Eighteen studies and 736 patients were analyzed. Although there is possibly an indication that metronomic regimens of TMZ result in better *progression-free survival* (PFS) and response rate when compared with the conventional standard 5 day regimen, insufficient available data and study heterogeneity preclude any safe conclusions. Authors offer as conclusion that "well-designed randomized controlled clinical trials are needed to establish the efficacy of metronomic regimens of TMZ in LGGs" [17].

To date, we can consider, mainly because of the good immediate tolerance and the respect for the quality of life (cf. infra), that temozolomide used with conventional doses remains the reference treatment.

Results

Chemotherapy, Volumes, and Growth Rate

The response assessment after chemotherapy for DLGG remains a difficult and nonconsensual issue.

For many years, MacDonald criteria, created to evaluate WHO grade III and IV gliomas [18] and based on 2D enhanced tumor measurements on computed tomography or magnetic resonance imaging (in conjunction with clinical and steroid dosage evaluations), were used for DLGG after adaptation (especially by considering the two largest diameters on T2-weighted or FLAIR slides and not on injected images and by abandoning the reference to steroids). This procedure does not allow to objectively monitor the evolution of a tumor under treatment and underestimates the number of responders. This was clearly the case for many initially reported studies [19–21].

New recommendations were proposed [22]. These latter do not appear optimal by considering that *published studies that have compared calculations based on single, multidimensional, and true volumetric measurements and the strength of their correlations with the outcome (PFS, OS) are absent and thus that evidence-based data for the preferred measurement system are not available*. We disagree with this opinion (see the dedicated chapter), because we consider that the volumetric evaluation is absolutely necessary for monitoring DLGG patients receiving chemotherapy. Otherwise, the risk is to dramatically underestimate responses and thus to be in an absolute inability to properly monitor the treatment duration.

The papers by Hoang-Xuan et al. [23] and Ricard et al. [24] were the firsts most important considering the impact of chemotherapy on

DLGG. In the second one's, authors were, indeed, among the first to report a longitudinal real volumetric assessment in a population of 107 patients treated exclusively with temozolomide. The method of the three diameters was used to obtain volumes and mean tumoral diameters (MTD) [25]. During the treatment, they found that more than 60 % of patients achieved a minor or partial response. At the onset of TMZ treatment, the MTD decreased in 92 % of patients, demonstrating an early initial chemosensitivity (38 of 39 patients who had a pre-, per-, and postevaluation of the MTD slope experienced a breakdown of the MTD growth curves after chemotherapy onset). After the initial phase of MTD decrease and despite continuous administration of TMZ, the tumors of some patients started to resume growth again, whereas others continued to decrease. Tumor regrowth occurred in 16.6 % of 1p19q codeleted tumors and in 60.6 % in non-codeleted tumors ($p<0.0004$). Tumors overexpressing p53 had also a much greater rate of relapse (70.5 % vs. 25 %). The evolution of the MTD was also tested after discontinuation of TMZ. The greater part of the population remains stable or sometimes continues to decrease despite the interruption of treatment. Nevertheless, a majority of tumors starts to grow again: 59 % rate of MTD regrowth after a median follow-up of 200 days after TMZ discontinuation (range 60–630 days).

Our group has also published a retrospective study concerning chemotherapy followed by surgical resection for DLGG. The impact of chemotherapy on the tumor volume was estimated using Volume Viewer® software (General Electric GE Healthcare, Milwaukee, WI, USA). For exams in which only printed images were accessible, a three-diameter technique was used. We also demonstrated that chemotherapy induced a tumor shrinkage (median volume decrease 38.9 %) in 10/10 cases (ipsilateral in six patients and in the contralateral hemisphere in four patients) [26].

Chemotherapy and Epilepsy

Seizures are the most common initial symptom in patients with DLGG. Their occurrence strongly depends on the tumor location including insular and central topography [27]. Some authors have also suggested a link between IDH 1/2 mutation (frequent in DLGG) and the onset of metabolic changes capable of promoting seizures [28].

For a long time, chemotherapy and irradiation were considered having just some minor beneficial effects on the patients' seizure disorder using the argument that overall 60–70 % of patients may experience recurrent epilepsy during long-term follow-up [29].

The progressive development of this therapeutic modality, its conceptual changes (prolongation of treatment time), and more precise analysis of the impact of such therapy on seizures have radically changed the view of many authors. Thus, it is now considered (despite the usual difficulties with seizure quantification in retrospective studies) that (1) the negative course of seizure frequency was mostly correlated to tumor progression, (2) surgery had almost always a favorable effect on epilepsy, and (3) chemotherapy had a mostly favorable effect with acceptable tolerance [2, 30, 31].

The improvement in seizure frequency during treatment with temozolomide seems, moreover, independent of antiepileptic drug adjustment [32].

An extensive experience with insular DLGG (topography considered as the most epileptogenic) was also reported by our group. We confirmed the interest of a surgical removal and supported the role of chemotherapy from an epileptological point of view [33].

We need to address in this chapter, regarding the relationship between chemotherapy and DLGG, the special place of antiepileptic treatments. Recommendations in this area are identical to the recommendations for all brain tumors. Most authors recommend first-line noninducing drugs such as lamotrigine, levetiractam, or lacosamide [34, 35]. The place of valproate remains debated. A clear efficiency is reported [35]. Combined antiproliferative activity through its inhibitory properties of histone deacetylase could improve survival as it was evoked for glioblastomas [36]. Nevertheless there are potential side effects (weight gain, thrombocytopenia) and enzyme inhibition may increase the hematologic toxicity of chemotherapy.

Chemotherapy and Cognition

Cognitive functioning is correlated with quality of life, itself linked with return to work [37]. This point is absolutely crucial in general neuro-oncology and, still more, in the management of patients with DLGG. Approximately one quarter of patients with DLGG reported serious neurocognitive symptoms [38]. Neurocognitive deficits are far more frequent than previously thought and can be caused by the tumor itself, tumor-related epilepsy, treatments, and psychological distress [12]. For some authors, the role of radiotherapy and chemotherapy in the treatment of DLGG remains controversial regarding their effect on survival and the development of neurotoxicity. Forty DLGG patients participated in the study of Correa et al. 16 patients had RT +/− chemotherapy and 24 patients had no treatment. In this series, RT +/− chemotherapy, disease duration, and antiepileptic treatment contributed to mild cognitive difficulties [39]. The same team published a new paper with 25 DLGG patients who underwent neuropsychological evaluations at study entry, 6 and 12 months subsequently. Nine patients had RT +/− chemotherapy prior to enrollment and 16 had no treatment [40]. Longitudinal follow-up showed that both disease duration and treatment with RT +/− chemotherapy contributed to a mild decrement in nonverbal recall and in some aspects of executive functions and quality of life. In these two articles, the widespread use of combined strategies (radiotherapy + chemotherapy) makes difficult to analyze the specific contribution of chemotherapy in the cognition modulation. Our group [26] reported a retrospective work with a neuropsychological assessment (NPA) of ten patients who underwent a strategy with a first chemotherapy followed by functional surgery. Nine patients were right-handed and one left-handed. No one presented with premorbid intelligence deterioration. Three patients did not show any neuropsychological deficit. Seven patients failed at three or less out of the 18 cognitive tests that were applied. The three others failed at least four tests. The main cognitive domains where deficits were observed concern episodic memory, especially verbal modality (five patients), and executive functions (five patients). Interestingly, the patients who did not continue to work were not the same who presented the most severe cognitive impairment. Our conclusion was that this combined strategy is highly likely to preserve cognitive function.

Chemotherapy and Quality of Life

As already mentioned, quality of life is correlated with cognitive functioning with itself linked with return to work [37]. Works on these three fundamental aspects of DLGG patient's evaluation are very rare. We know, generally, that female sex, epilepsy burden, and number of objectively assessed neurocognitive deficits were associated significantly with both generic and condition-specific HRQOL [38]. The major impact of PCV on HRQOL is on nausea/vomiting, loss of appetite, and drowsiness during and shortly after treatment. There are no long-term effects of PCV chemotherapy, since patients recover a "normal" state when they move away from the treatment period [41].

Liu et al. described the quality of life (QOL) of DLGG patients at baseline prior to chemotherapy and through 12 cycles of temozolomide. The Functional Assessment of Cancer Therapy-Brain (FACT-Br) was obtained at baseline (prior to chemotherapy) and at 2-month intervals under chemotherapy. Patients at baseline had higher reported social well-being scores (mean difference = 5.0; $p < 0.01$) but had lower reported emotional well-being scores (mean difference = 2.2; $p < 0.01$) compared with a normal population. Patients with right hemisphere tumors reported higher physical well-being scores ($p = 0.01$): 44 % could not drive, 26 % did not feel independent, and 26 % were afraid of having a seizure. Difficulty with work was noted in 24 %. Mean change scores at each chemotherapy cycle compared with baseline for all QOL subscales showed either no significant change or were significantly positive ($p < 0.01$). Authors concluded that DLGG patients on therapy were able to maintain their QOL in all realms. Patients' QOL may be further improved by addressing their emotional well-being and

their loss of independence in terms of driving or working [42].

In our work concerning patients treated with presurgical chemotherapy [26], the Karnofsky Performance Scale (KPS) scores ranged from 80 to 100 (median 90) and were globally stable during the whole follow-up period. The main domain that presented with significant impairment in the QOL assessment was role functioning (feeling of independence and socio-professional life) with a median score of 66.7 % (range 50–100). The global QOL score was preserved after chemotherapy and surgery for most patients with a median value of 66.7 % (range 33.3–83.3). Cognitive, emotional, physical, and social well-being scores were also relatively preserved (medians 83.3, 79.2, 100, and 100 %, respectively). Among the general symptoms, the main complaints were fatigue (median score 33.3 %, range 11.1–100) and pain (median score = 16.6 %, range 0–66.7) due to different associated diseases like osteoarthritis and arteriopathy. Sleeping troubles (mean score = 20 ± 30.6 %), financial impact (mean score = 23.3 ± 39.6 %), and digestive troubles (mean score = 20 ± 30.6 %) seemed to have a moderate influence on the QOL. No patient reached the cut-off of 15 in the inventory for signs or symptoms of depression (BDI) with a mean score of 8.7 ± 3.6. However, seven subjects showed a tendency for "mild depression," characterized by a score between 8 and 14.

We can therefore consider that TMZ alone or combined with surgery is able to maintain or even to improve the quality of life and that PCV alters transiently the QOL, with a return to the "normal" situation when we once move away from the treatment period.

Chemotherapy and Survival

To date, there is no direct evidence for DLGG patients that confirms the impact of chemotherapy on patients' survival. We know, however, that presumed eloquent location of DLGGs is an important but modifiable risk factor predicting disease progression and death [43] and that the risk of malignant transformation and subsequent survival may be predicted by pretreatment and also by treatment-related factors [44].

We are thus entitled to imagine that indirectly, this treatment modality may have an impact on patient survival.

In a retrospective selected series (personal unpublished data), 17 patients, considered at diagnosis or recurrence as "nonoperable" because of a functional area infiltration or a too large contralateral extension, underwent temozolomide-based chemotherapy inducing tumor volume decrease immediately followed by a radical surgery. The median follow-up since initial radiological diagnosis was 5.9 years (range 1.4–11). Median time to malignant transformation was 99.6 months. We demonstrated that age, volume at diagnosis, 1p19q, IDH, and MGMT promoter status had no impact on time to malignant transformation. Chemotherapy reduced tumor volume (median −33.43 %, range − 61.6 % to −5.1 %) and significantly decreased the imaging tumor growth whatever 1p19q, IDH, and MGMT status. We confirmed that a tumor volume decrease of more than 20 % was significantly correlated with a lower postoperative residual tumor (median = 3.4 cc, $p = 0.003$), a greater extent of resection ($p = 0.03$), and a better prognosis ($p = 0.05$). Postoperative tumor volume less than 10 cc was significantly associated with better outcome ($p = 0.042$). We thus concluded that, regardless of the molecular status, neoadjuvant chemotherapy could optimize surgical resection of DLGGs and could have an impact on their natural history (Blonski et al. submitted).

Tolerance

Hematological Toxicity

The "PCV" association possesses a cumulative acute hematologic toxicity making impossible the administration of more than six courses. Previous papers provide evidence that nitrosoureas are leukemogenic in human beings and confirm observations that adjuvant chemotherapy with alkylating agents may increase the risk

of leukemia [45]. In the paper of Boice et al. concerning adjuvant treatment of gastrointestinal cancer with semustine (methyl-CCNU), the 6-year cumulative mean risk of acquiring a leukemic disorder after treatment with semustine was 4.0 +/– 2.2 % for an incidence rate of 2.3 cases per 1,000 persons per year [46]. In a meta-analysis of five randomized clinical trials for adult patients with brain tumors, Greene et al. identified 2 of 1,628 individuals who experienced acute nonlymphocytic leukemia after carmustine chemotherapy [47]. The risk of developing this complication was 24.6 times higher than expected [45]. Baehring et al. identified well-documented case reports and small case series of patients who developed therapy-induced myelodysplasia (t-MDS) and therapy-induced acute myeloid leukemia (t-AML) during or after treatment with alkylating chemotherapy for primary brain neoplasms. Moreover, they performed a comprehensive review of the literature on the subject and they noted that the overall incidence of primary MDS was estimated at 3–20 cases per 100,000 population with 10–15 % of all MDS cases arising in patients exposed to chemo- or radiation therapy administered for other tumors [48]. It seems that t-MDS/t-AML risk among patients with brain tumors may be lower than in patients with other primary neoplasms [49]. Nevertheless, this observation may be linked to the often-reserved prognosis of the central nervous system tumors, not allowing the late hematological complications emergence. Perry et al. reported two cases of AML following therapy for malignant glioma and found 26 other examples of therapy related leukemia in adult and pediatric brain tumor patients (including 12 patients with malignant glioma). The median interval from treatment to diagnosis of AML was 31 months. Nine adult malignant glioma patients received all nitrosoureas and some of them as the sole chemotherapy. Authors concluded that "if regimens such as PCV continue to prove valuable in neurooncology the risk of leukemia will require integration into the clinical decision process" and recommended a search for "more effective therapy with minimal mutagenicity remains critical" [50].

The risk of late hematological complications with TMZ seems low compared with other alkylating agents like nitrosoureas mentioned above. An Australian team reported the cases of three patients treated with TMZ for a progressive glioma. These patients have continued the treatment respectively for 5, 7, and 8 years! No serious side effects were reported. Thus, it was often considered that most individuals receiving exceptionally large doses of alkylating agents over an extended period did not develop T-MDS/AML. This is true for patients receiving TMZ [51]. In contrast, Natelson et al. published a case report concerning a patient who had received temozolomide as a single agent for treatment of malignant glioma and who developed t-MDS. After a literature review, authors suggested that the cumulative dose threshold (CDT) for temozolomide that could predispose to t-MDS and which may potentially lead to acute myeloid leukemia would be around 18,000–20,000 mg/m^2 [52]. The authors acknowledge, however, that the objective assessment of the real risk appears much difficult for tumors with a worse prognosis such as gliomas than for tumors associated with a long survival like Hodgkin's lymphoma, testicular cancer, or breast cancer. They concluded that all alkylating agents, including TMZ, should be considered potentially leukemogenic when administered long term. Nevertheless, the risk of direct (progression or recurrence, malignant evolution) or indirect tumor complications (permanent deficit, seizures) or short latency adverse reactions to treatment (myelosuppression, opportunistic infection, encephalopathy due to radiation therapy) remains, at this day, much higher than the t-MDS/t-AML risk [48].

We nevertheless have to be careful with our prescription and to demonstrate in well-structured databases that prolonged use of alkylating chemotherapy until tumor progression or unacceptable toxicity is superior to treatment with a defined and limited number of cycles.

Chemotherapy and Gonadotoxicity

Data concerning chemotherapy, DLGG, and gonadotoxicity are almost nonexistent. Alkylating chemotherapy containing procarbazine (and/or

cyclophosphamide) causes prolonged azoospermia in 90–100 % of men and premature ovarian failure in 5–25 % of women under the age of 30 [53]. We are also entitled to fear a marked gonadal toxicity of vincristine [54]. Thus, we can assume, although with no specific published data, that the PCV association is clearly gonadotoxic. We so recommend (1) to warn patients of this possibility, (2) to propose systematically a fertility preservation (easier in men than in women), and (3) to avoid this association in patients wishing to preserve essentially their reproduction capabilities.

Concerning temozolomide, a retrospective study was recently published. It concerns 24 female patients treated for a glioma. Fifteen patients had no fertility preservation and the remaining nine had a cryopreservation of embryos with or without an oocyte cryopreservation. Four patients are or have been pregnant (delivery, spontaneous miscarriage, pregnancy in the group of preserving fertility, and a current pregnancy in the group where no fertility preservation has been achieved). The conclusion of the authors is that temozolomide is not totally gonadotoxic [55]. Paternities have also been reported after temozolomide [56]. We could apply the two previous recommendations (information, fertility preservation) when a TMZ-based chemotherapy is needful in the course of a DLGG and when the patient wishes to preserve its reproduction capabilities while integrating the concept of a likely lower toxicity compared with that seen with nitrosoureas.

Other Toxicities

The peripheral neurological risk of vincristine cannot be neglected. There is currently no way to prevent it [57]. The risk of lung fibrosis with cecenu is also a parameter to be integrated during the establishment of such a combined therapy with cecenu [58]. Otherwise, patients under the PCV association complain frequently about an intense asthenia and/or about a loss of weight [41].

Temozolomide-induced hepatitis can be particularly severe, especially the cholestatic form [59].

Open Questions

How to Evaluate the Benefit of Chemotherapy

For more objective assessment of the impact of chemotherapy, it is conventional in neuro-oncology to use parameters such as overall survival and progression-free survival.

Overall survival is sensitive to all instituted treatments including “salvage” therapies. In this type of disease, treatments are often multiple and repeated. That makes difficult to analyze the specific impact of a given treatment (chemotherapy in our case) on survival. Progression-free survival could be interesting parameters to use if and only if (1) there is longitudinal and rigorous volumetric assessment and (2) this morphological parameters are associated with quality of life data [60]. The same remark can be made for the classical time to malignant transformation.

It was recently pointed out that clinical trials for DLGG “need to consider other measures of patient’s benefit such as cognition, symptom burden, and seizure activity, to establish whether improved survival is reflected in prolonged well-being” [22] should move in this direction also emphasized by Klein and colleagues “the multidimensional scales used to study changes in HRQOL studies in brain tumor patients provide a more comprehensive view of what is important to the patient concerning living with their disease and receiving treatment” [61].

How to Monitor the Treatment (Response Assessment)

To date, most radiologists and physicians analyze the images and decide the direction of treatment for gliomas and especially DLGG via a side-by-side comparison of images. This procedure can be considered as very imperfect and even dangerous. It was indeed clearly demonstrated that automated change detection and image subtraction are superior to side-by-side image comparison for brain tumors in general [62] and more obviously for DLGG [63].

In the same manner, the majority of dedicated centers simply monitored patients with conventional MRI without volumetric assessment and a fortiori without multiparametric examinations able to assess tumor cellularity, hypoxia, disruption of normal tissue architecture, changes in vascular density, and vessel permeability [64]. However, today, these parameters seem absolutely essential [65].

Links Between Chemotherapy and Clinico-radiological Factors

There are several factors clearly related to the prognosis of DLGG. These factors formed the "EORTC scoring system" [66] or the "UCSF LGG prognostic scoring system" [67] by combining different parameters: (1) location of tumor in presumed eloquent cortex (UCSF), (2) tumor crossing the midline (EORTC), (3) presence of neurologic deficit (EORTC), (4) Karnofsky Performance Scale score ≤80 (UCSF), (5) age > 50 years (UCSF)/≥40 years (EORTC), (6) maximum diameter (≥6 cm for EORTC/>4 cm for UCSF), and (7) histology (astrocytoma histology subtype for EORTC). Patients that combine two or more factors are classified in the high-risk group for the EORTC scoring system. For UCSF, the stratification of patients is based on score-generated groups (0–4) with statistically different OS and PFS estimates ($p < 0.0001$, logrank test). It has more recently been shown by a multivariate analysis constructed on the basis of two European Organisation for Research and Treatment of Cancer radiation trials for low-grade gliomas that tumor size and MMSE score were significant predictors of OS whereas tumor size, astrocytoma histology, and MMSE score were significant predictors of "PFS" [68]. It is so far difficult if not impossible to determine whether these factors are only prognostic factors or predictors of treatment response, including chemotherapy response.

Dynamic susceptibility-weighted contrast-enhanced perfusion imaging can identify progression and can also predict treatment failure during follow-up of DLGG with, for some authors, the best diagnostic performance [69].

Concerning spectroscopy, Murphy et al. reported in 2004 that there was interest to evaluate the reduction in the tumor choline/water signal in parallel with tumor volume change and that this marker could reflect the therapeutic effect of temozolomide [70]. In addition and very interestingly, Guillevin et al. demonstrated that the mean relative decrease of metabolic ratio – *Δ(Cho/Cr)(n)/(Cho/Cr)(o)*– 3 months after the start of a TMZ-based chemotherapy was predictive of tumor response over the 14 months of follow-up. The (1) H-MRS profile changes more widely and rapidly than tumor volume and represents an early noninvasive predictive factor of outcome under temozolomide-based chemotherapy [65].

Links Between Chemotherapy and Pathological Phenotype

The diagnostic criteria, in particular for oligoastrocytoma but also for "simple" astrocytomas or oligodendrogliomas, are highly subjective [71]. Most authors now propose to go beyond the pathological (morphological) classification by including other criteria, notably molecular [72, 73], to refine the prognostic significance of the diagnosis according to the WHO classification. Due to these important limitations of the morphological analysis of DLGG, it seems difficult to build clinical trials for chemotherapy or to decide, in the daily practice, the indication of chemotherapy on the sole basis of histology despite the fact that oligodendroglioma differentiation seems to respond better to chemotherapy than astrocytomas [10, 44].

Links Between Chemotherapy and Molecular Biology

Before talking about chemotherapy, the prognostic role of molecular markers after surgery alone could be discussed. Thus, even if the concept of "progression-free survival" (PFS) after partial surgery in the context of a DLGG is highly questionable, Hartmann et al. considered that no molecular marker was prognostic for this

endpoint after surgery alone using multivariate adjustment for histology, age, and extent of resection [74] and all, on their side, screened 360 WHO grade II gliomas for mutations in the IDH1, IDH2, and TP53 genes and for 1p/19q loss and correlated these factors with clinical outcome. TP53 mutation was considered as a significant prognostic marker for shorter survival ($p=0.0005$) and 1p/19q loss for longer survival ($p=0.0002$), while IDH1/2 mutations had no prognostic value ($p=0.8737$). Their conclusion was that "molecular classification on the basis of IDH1/2 mutation, TP53 mutation, and 1p/19q loss has power similar to histological classification and avoids the ambiguity inherent to the diagnosis of oligoastrocytoma" [71].

Data regarding chemotherapy are partly contradictory. Iwadate et al. have treated 36 consecutive low-grade oligodendroglioma patients (postoperative residual tumors or recurrence after total resection) by a modified PCV-based chemotherapy-preceding strategy and without radiotherapy. In this study 1p and 19q status were analyzed by fluorescence in situ hybridization. 1p/19q codeletion was observed in 72 % of cases. There was no significant association between 1p/19q codeletion and chemotherapy response rate. No significant difference has been found as well in terms of survival: median PFS of 121 months for 1p/19q-deleted tumors and 101 months for non-deleted tumors (logrank test: $p=0.894$). Recurrent tumors were also well controlled by chemotherapy irrespective of 1p/19q status [75]. Following the work of the Hoang-Xuan et al. [23] and in contrast, Kaloshi et al. reported a retrospective single center observational study with 149 consecutive patients. The median number of TMZ cycles delivered was 14 (range 2–30). Seventy-seven patients (53 %) experienced an objective response (15 % of partial response, 38 % of minor response, 37 % of stable disease, and 10 % of progression). The median time to maximum tumor response was 12 months (3–30 months). The median "PFS" was 28 months (95 % CI, 23.4–32.6). Material for genotyping was available for 86 patients. Combined 1p/19q LOH was present in 42 % of the cases. Codeletion was significantly associated with (1) a higher response rate ($p<0.02$), (2) a longer objective response to chemotherapy ($p<0.017$), (3) a longer PFS ($p<4.10^5$), and (4) a longer overall survival ($p<0.04$) [76]. The same team, through Houiller et al., reported a series of 271 patients with a DLGG in which 84 patients were treated up front. IDH (1 or 2) mutations were found in 132/189 patients (70 %). IDH mutation and 1p19q codeletion were associated with a prolonged overall survival in multivariate analysis ($p=0.003$ and $p=0.004$). 1p19q codeletion, MGMT promoter methylation, and IDH mutation ($p=0.01$) were also correlated with a higher rate of response to temozolomide. Inside the untreated subgroup, 1p19q codeletion was associated with prolonged "progression-free survival" (this concept is highly questionable in an untreated population) in univariate analysis, whereas IDH mutation was not [77]. Our understanding of the problem may also be informed by the work of Ochsenbein et al. Twenty-two patients with histologically verified DLGG (WHO grade II) were treated with temozolomide (TMZ) for tumor progression. LOH 1p and/or 19q correlated with longer time to progression but not with radiological response to TMZ. The volumetric response to chemotherapy analyzed by MRI and time to progression correlated with the level of MGMT promoter methylation [78]. Data on tumors considered as pure astrocytomas are likewise difficult to interpret. In the study of Taal et al. concerning temozolomide-based chemotherapy, MGMT promoter methylation and IDH1 mutations were not correlated with "PFS," but the interval between the first symptom and the start of the TMZ was significantly ($p=0.02$) longer in the patients with a methylated MGMT promoter and with IDH mutations ($p=0.01$) [79].

The reported results appear thus contradictory. Although, at a population level, there is a quite pronounced correlation between 1p19q deletion (and a smaller correlation between MGMT promoter methylation or IDH1 mutation) and response to chemotherapy, it appears today absolutely impossible to consider the indication of chemotherapy on this sole argument at an individual level. We have previously shown [24] that 1p19q codeletion was primarily a marker of the duration of response and not a marker of response.

In the case of a presurgical chemotherapy continued under a strict volumetric monitoring until obtaining a plateau, depriving patients of such a strategy (which can potentially change the natural history of the disease by allowing to move towards a possible subtotal resection not originally envisaged) seems to us a significant error.

When to Treat

To date only four large randomized trials in patients with low-grade glioma have been published. They allow concluding that early radiotherapy does not improve overall survival and supports alternative approaches like chemotherapy without providing evidence on the timing of chemotherapy [12]. We have previously emphasized the heterogeneity of the various reported series concerning chemotherapy. Often, within the same study, patients could be included before or after the anaplastic transformation of the tumor. To be very convenient, prescriptions at the "low-grade" stage can theoretically be proposed (1) in case of progression after surgery (regardless the quality of debulking) and radiotherapy, (2) for nonoperable progressive tumor and before radiotherapy in order to delay radiation and so radiation induced cognitive impairment, (3) in case of progression after a first-line surgery if reoperation cannot be immediately considered and before either surgery if the volume reduction obtained with chemotherapy allows it, and (4) up front in order to allow, in case of volume reduction, a surgical procedure before radiotherapy.

Only one randomized trial has been achieved. It compared primary temozolomide versus radiotherapy in progressive low-grade gliomas (EORTC/NCIC 22033/26033) regardless of the initial surgical status. The results are not yet published except data concerning dummy run and conformity indices [80].

Chemotherapy After Surgery and Radiation Therapy

Historically, the first prescriptions of chemotherapy [8, 9] were made after standard surgery and radiation therapy, which were unambiguously considered as the reference treatments while chemotherapy was considered as less or not effective. Clinical and radiological responses were clearly observed [8, 9, 21, 81, 82]. Can we nevertheless draw from these publications that chemotherapy is able, at this time of prescription, to modify enough the natural history by delaying, for a given time, the evolution of the disease including the anaplastic evolution? Is the impact of post radiation chemotherapy, considering the duration of response more or less important than before the radiotherapy? More or less important before malignant transformation than after? Does it allow the patient to maintain longer a high quality of life in comparison with earlier (pre radiotherapy) or later (after anaplastic evolution) prescription of chemotherapy?

It is difficult if not impossible to answer these questions. This point will, indeed, depend on many parameters: tumor volume, tumor heterogeneity, time of the disease where radiation therapy was performed, time from the end of radiotherapy, type of chemotherapy or duration of this one…. It is also very difficult to assess the type of response evaluation (both clinically and radiologically) in the various published papers. The majority of reported series does not relate real reproductible parameters. The limited retrospective data (which constitute the majority of available data) on all of these parameters does not help to get a clear vision of the real impact of chemotherapy at this time of the disease.

Nevertheless, a phase III prospective study was conducted by the RTOG group (9,802 trial). This is the only phase III trial that raised the question of the role of adjuvant chemotherapy in DLGG. Inclusion criteria of this trial were related to high-risk patients with a residual tumor after surgery and an age over 40. Patients were stratified by age, histology, Karnofsky performance status, and presence or absence of preoperative contrast enhancement (suggesting that an unspecified proportion of patient had anaplastic transformation!). They were randomized to radiotherapy (RT) alone (54 Gy/30 fractions) versus RT followed by six cycles of standard dose PCV. Finally, results have so far not been published except through an abstract presented at the ASCO meeting in 2008

[83]. We often find in the literature statements such as "adjuvant use of PCV-chemotherapy in high-risk patients failed to improve progression-free and overall survival in comparison with radiotherapy" [84]. Accurate analysis of data in the abstract contradicts significantly this assertion. 251 cases were indeed included in the study between 1998 and 2002. The median follow-up was 5.9 years. The median "PFS" time were not reached for the RT + PCV group and at 7.5 years for the RT group. The 5 years OS were 72 % for the RT + PCV group and 63 % for the RT group ($p = 0.06$, logrank $p = 0.005$). Beyond 2 years, the OS and PFS curves separated significantly favoring PCV + RT patients. For 2 years survivors ($n = 211$), the probability of OS for an additional 3 years was 84 % with RT + PCV versus 72 % with RT ($p = 0.03$) with comparable data for "PFS" (74 % with RT + PCV versus 52 % with RT alone, $p = 0.002$). Finally, The hazard ratio for RT + PCV versus RT was 0.52 for death ($p = 0.02$) and 0.45 for progression ($p = 0.0004$). The conclusion is rather oddly worded. It is indeed initially specified that *PFS but not OS were improved for adult WHO grade II glioma patients receiving RT + PCV* versus *RT alone* and then that *beyond 2 years, the addition of PCV to RT conferred both a significant OS and PFS advantage, and reduced the risk of death by 48 % and progression by 55 %, suggesting a delayed benefit for chemotherapy.* Nonetheless, after analyzing the abstract, we would like to conclude to a positive impact of chemotherapy as for "PFS" and for OS. This point, clearly in favor of an early chemotherapy after radiotherapy, seems to have been insufficiently reported. The absence of the final publication must be an explanation to consider.

We must specify that another phase III study was conducted by the ECOG group as "E3F05 trial" [11]. In the reference arm, patients undergo 3D conformal or intensity-modulated radiotherapy once daily, 5 days a week for 5½ weeks (28 fractions). In the experimental arm, patients undergo radiotherapy as in the previous arm and receive concurrent oral temozolomide once daily for 5½ weeks. Beginning 28 days after completion of chemoradiotherapy, patients receive also oral temozolomide alone once daily on days 1–5. Treatment with temozolomide repeats every 28 days for 12 courses in the absence of disease progression or unacceptable toxicity. The primary objective is to determine whether the addition of temozolomide to fractionated radiotherapy improves the "progression-free survival" of patients with symptomatic or progressive DLGG and to determine whether the addition of temozolomide to fractionated radiotherapy improves the median overall survival (OS) of these patients. The design of this trial may fear an excess of delayed neurotoxicity.

Finally, a randomized phase II tries to analyze the place of temozolomide as an adjuvant treatment (RTOG 04-24). High-risk LGGs (at least 3 risk factors: age ≥ 40, tumor diameter ≥6 cm, tumor crossing midline, astrocytoma subtype, preoperative neurologic deficit) are treated with RT + temozolomide. Comparison will be the historical EORTC patient population. The study is closed, but the results are not yet reported [85].

Chemotherapy Before Radiotherapy

Chemotherapy for Nonsurgical Tumor

Some locations (e.g., primary motor area), multifocal tumors, or "gliomatosis-like" aspects remain forever nonsurgical. These tumors are evolving as much as the other gliomas, clinically and radiologically. A primary chemotherapy course has to be discussed because, especially in the case of multifocal tumors or gliomatosis, volumes to be theoretically irradiated remain at risk of high cognitive toxicity. Data from the literature remain, again, rare and affect mainly the gliomatosis. We know that chemotherapy (temozolomide, "PCV") can be effective in terms of symptoms and volumes [86]. The main question concerns the duration of temozolomide treatment for long responders. Can we continue the treatment for a very long time (even several years) when chemotherapy is (1) well tolerated and (2) able to produce a volume reduction and stability or do we fear the risk of late complications related to it (myelodysplasia, induced leukemia) [87]? There is to date no formal response. The question must be clearly asked by analyzing the risk/benefit ratio with on one side a tumor often with pejorative

prognostic factors and on the other side a low risk at a medium term of complications.

Articulation Between Chemotherapy as First-Line Treatment and Surgery

Our group reported the first case of a complete surgery made possible thanks to an initial chemotherapy. This patient was initially diagnosed because of seizures. He benefited from a first partial conventional resection. The tumor continued logically to grow with an invasion of the contralateral hemisphere via the corpus callosum. A temozolomide-based chemotherapy was then prescribed and allowed a regression of the contralateral extension. Post chemotherapy surgery was performed with intraoperative functional mapping and allowed a complete resection without sequelae [88]. The patient now continues to enjoy a normal life with 10 years of follow-up since the first surgery (without radiotherapy). Spena et al. reported the same strategy for a patient and concluded that this new therapeutic approach of chemotherapy followed by surgery can offer safer and more radical surgical resection while improving the quality of life of the patient [89].

We then published a series of ten patients who benefited from a presurgical chemotherapy. In all cases we observed a tumor shrinkage that made possible the resection of these initially inoperable tumors. All the patients were secondarily evaluated from a cognitive and quality of life points of view. We demonstrated that the combined treatment was (1) feasible, (2) efficient, and (3) well tolerated with few cognitive deficits (mostly related to the tumor location) and with a good quality of life [26]. Martino et al., also within our group, reported a series of 19 patients who benefited from two operations separated by at least 1 year. Nine of these patients received chemotherapy before the second operation that allowed a subtotal or total resection in 14/19 cases. After the second operation, 16/19 patients improved or stabilized their clinical situation, while, in 14/17 cases, seizures were reduced or disappeared. Therefore, the authors concluded that chemotherapy did not prevent or even favored a second operation [90].

We described in chapter II.E our submitted series of selected 17 initially nonoperable patients who underwent temozolomide-based chemotherapy. Here, we just would like to point out that chemotherapy was able to reduce the growth slope in all 17 cases whatever the molecular status and that the quality of surgery was directly related to the magnitude of response to chemotherapy. TMZ appears thus as a way to optimize surgery and an additional way to potentially modify the natural history of this disease.

How Long to Treat (Efficacy Versus Potential Toxicity)

Temozolomide

Duration According to Volumic Evolution

The article that can argue, at best, this question about the duration of chemotherapy is that of Ricard and colleagues [24] we talked about earlier. In this paper, the great majority (92 %) of patients experienced initial decrease of the mean tumoral diameter after initiation of temozolomide. Ricard et al. found a clear correlation between 1p19q codeletion or absence of p53 overexpression and the duration of the response. Under chemotherapy, the volume is better controlled in codeleted patients (while recognizing that the maximum duration of temozolomide was 24 months). Otherwise, a majority of the patients resumed their progressive growth within a year after discontinuation of the chemotherapy. This observation of Ricard et al. raises the question of the validity of an arbitrary interruption of treatment in patients whose mean tumoral diameter (MTD) is still decreasing when the a priori fixed number of chemotherapy courses has been reached. Should treatment be pursued as long as the MTD continues to decrease (or stabilize), knowing that this option should be balanced with the potential long-term toxicity of prolonged treatment? Alternatively, should we abbreviate chemotherapy to four to six cycles to prevent formation of drug resistance? In this case, can we be certain that the tumor will remain sensitive to the treatment resumed after several months of interruption? Outside volume aspects, must we incorporate other parameters in the longitudinal

follow-up of patients? We know that H-MRS imaging 3D volumetric maps of choline (Cho) over creatine (Cr) is more accurate in DLGG for the detection of glioma progression in comparison with conventional magnetic resonance imaging (MRI) and clinical symptoms [65, 91] and that the (1)H-MRS profile represents an early predictive factor of outcome over 14 months of follow-up under temozolomide [65]. It appears so obvious that, in the future, we will focus on a multimodal monitoring.

Duration According Tolerance

We know that prolonged administration of adjuvant temozolomide is safe and can be favorable for patients with anaplastic gliomas [92]. Other authors have nevertheless a more pessimistic view highlighting the fact that 15–20 % of patients (in a high-grade glioma population) treated with TMZ develop clinically significant toxicity, which can leave further treatment unsafe [23]. This point was developed in chapter II.F.

PCV

Peyre et al. [93] reported a series of 21 patients with a DLGG. All the patients presented a decrease of the mean tumoral diameter (MTD) during the chemotherapy. After discontinuation of the PCV, all but one patient presented an ongoing decrease of the MTD. The mean duration of the MTD decrease after PCV onset was 3.4 years (0.8–7.7). The mean duration of the MTD decrease after the end of PCV was 2.7 years (0–7). According to adapted McDonald's criteria, the rates of partial and minor responses were 43 % at the end of PCV but 80 % at the time of maximal mean tumoral diameter decrease, which occurred after a median period of 3.4 years after PCV onset [94]. This prolonged impact of treatment is to be balanced against the increased toxicity of the association mainly during its administration [41] and also sometimes after, via lung and hematological long-term complications (cf. supra).

Retreatment with Chemotherapy

Taking again temozolomide for a patient initially responder with this drug and after an interruption for a given period can possibly be discussed even if the data are very rare [95]. The alternative way may be based, in this configuration (TMZ pretreatment) and in the absence of other possible therapeutic modality, on the prescription of a nitrosourea. The results reported by Kaloshi et al. appear nevertheless disappointing. The authors have indeed described a series of 30 patients treated with a nitrosourea-based chemotherapy for low-grade gliomas failing initial treatment with temozolomide. Response rate was 10 % (three minor responses achieved in nonenhancing tumors). Tolerance was considered as acceptable. Median PFS was 6.5 months. Median OS from start of salvage treatment was 23.4 months. Chromosomes 1p/19q codeletion was not predictive for objective response to salvage treatment but correlated with a better PFS ($p=0.02$). The conclusion of the authors was that salvage NU chemotherapy provides disappointing results in TMZ-pretreated DLGG. They recommend in priority conventional radiotherapy, especially in DLGG that display contrast enhancement at progression [96].

Platinum salts [97] or CPT11 [98] seems also to have a modest effect. Therefore, the development of new drugs is highly desirable.

Conclusions and New Horizons

A DLGG is a tumor that, in the absence of treatment, shows a continuous growth resulting, usually within a few years and through a *crescendo* evolution of tumor aggressivity, in life threatening. During the initial period, contrary to a classical belief, this tumor entity alters so much quality of life through, most often, the existence of cognitive disorders and *crescendo* socially debilitating epilepsy. The idea of an initial "simple" monitoring ("wait and see" attitude) should definitely be abandoned in favor of an active therapeutic strategy implying the preservation of the core neurological sensory-motor and visual functions, but also the cognition and language, and thus the quality of life. In recent years, we have very clearly seen an improvement of the surgical component of care. It has been shown that the extent of surgery had a major impact on the delay before anaplastic transformation

and survival itself. Under the development of functional surgery (cortical and subcortical stimulations, intraoperative awakening), percentage of patients undergoing a subtotal or total excision has increased considerably. At the same time, morbidity was significantly reduced with a mortality tending to zero. Meanwhile, indications of early radiotherapy were reduced because of inducing late treatment risks, mainly related to cognitive disorders. In this chapter, we saw that chemotherapy could also take place in the armamentarium. Currently, two strategies remain advisable: monochemotherapy by temozolomide or PCV-type association (vincristine + procarbazine + cecenu). The first (temozolomide) offers the advantage of a better tolerance to short and medium terms (hematologic, gonadal, and general parameters). Serious complications (myelodysplasia, leukemia) remain exceptional. The limit is clearly based on tumor regrowth in the months following discontinuation of therapy, inciting to discuss an extension of the treatment duration. The second (PCV) is somewhat more toxic when administered. Serious hematological complications also exist. Nevertheless, it provides tumor control over long periods up to several months or years after treatment discontinuation.

In all cases, we now know that chemotherapy (1) allows a volume decrease in the vast majority of cases and (2) improves the neurological symptoms, in particular epilepsy and cognition. We have also shown that this treatment could make "the bed of surgery" allowing the realization of subtotal or total excision, of which we know the impact on survival and quality of life. We are thus entitled to imagine, although we have not formally demonstrated it, that chemotherapy could improve directly or indirectly the survival of patients, while preserving or even improving the quality of life. Much work remains, however, to perform. Two major prescription frames seem to be well considered: (1) tumors (at diagnosis or progression) for which surgery could be discussed in case of a more or less volume reduction eventually associated with a redistribution of functional areas able to afford a subtotal or total resection and (2) tumors that will remain, with regard to their topography and or extension (gliomatosis-like, multifocality), forever inoperable. In both cases, there are, to date, many unanswered questions regarding (1) timing of the prescription (after demonstrating a lesional scalability with two successive MRI at 3 or 4 months of interval? later in the disease, after a period of "wait and see" with the risk of being confronted with a bulkier tumor having accumulated a greater number of genetic abnormalities potentially promoting a chemoresistance?), (2) decision-making criteria for prescription assistance (pathological, molecular, or radiological predictors of chemosensitivity or chemoresistance criteria? parameters to exclude patients who are at risk for serious side effects like myelodysplasia or induced leukemia?), (3) specific strategies (conventional temozolomide? intensified temozolomide? PCV? alternative treatment in case of progression after PCV and temozolomide for a tumor remaining a real WHO grade II glioma? If so which one?), and (4) duration (when to operate after a chemotherapy has been established? from the time when the tumor seems, based on probabilistic maps, able to benefit from subtotal resection? when the tumor stabilizes from a volumic point of view, which implies the necessity of close volumetric monitoring? in the event of a definitive inoperable tumor, how long will temozolomide be continued if the choice fells on this molecule? in case of well hematological and general tolerance, until when will the tumor is at least stabilized? for defined periods and if so, how long? after discontinuation, by repeating the treatment at a new documented growth, can we be certain of the chemosensitivity persistence?).

Furthermore, it is obviously important to continue basic research, both from neurological and oncological points of view. Neurologically, as well as for surgery, we must, again and again, better assess the impact of our treatment strategies, for each topography and each patient on cognitive or language impairments and on the capabilities of the brain to redistribute itself, with chemotherapy

alone or in combination with parallel strategies of speech therapy and cognitive rehabilitation. Oncologically, a better understanding of energy processes or cellular and molecular mechanisms will allow the development of specific therapeutic targets able to extend the rather limited armamentarium at our disposal. Despite the relatively low number of DLGGs, this adventure remains exciting by itself, but also in that it will be able to open up original concepts declinable for a number of other entities, starting with WHO grade III and IV gliomas.

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Radiation Therapy for the Treatment of Diffuse Low-Grade Gliomas

26

Stephanie E. Combs

Abstract

The radiotherapeutic treatment of low-grade gliomas (LGG) has been a matter of discussion for centuries. Several studies have focused on the adequate dose, timing of radiation, or on combination treatments. Target volume definition is essential for high-end radiation therapy and requires strong interaction with imaging disciplines. Follow-up should be regular, including clinical assessment, imaging, and neurocognitive assessment.

Keywords

Highly conformal radiotherapy • Proton radiotherapy • Neurocognitive functioning • Target volume definition

Over the years there has been much controversy about the optimal treatment of low-grade gliomas with radiation therapy. Focus has not just been set on target volume definition, treatment planning, and evolving techniques but also on finding the appropriate timepoint for treatment in multimodal treatment schedules. Additionally, dose was a point of discussion for this subgroup of gliomas, presenting with a biology somewhere between pilocytic astrocytomas, and high-grade gliomas, such as anaplastic gliomas or glioblastomas (GBM).

The questions of time and dose have been addressed in prospective randomized trials; however, still some issues remain unanswered. LGGs are commonly associated with an infiltrative growth pattern, however, in contrast to high-grade gliomas, with slowly progressing tumor extension; therefore, often, clinical signs and symptoms develop later during the course of the disease, and patients remain asymptomatic for longer time spans. Also in contrast to high-grade gliomas, in which survival especially for GBM patients remains to be around 15 months, survival in patients with LGG ranges between 6 and 12 years, with survival rates of 60–70 % after 5 years [1–5]. The most predominant age group diagnosed with LGG are patients in their third or fourth decade, and they are more widespread in children and young adults than in elderly patients; in pediatric oncology, they account for about 40 % of all intracranial tumors. Combining the excellent prognosis with young age, reasons for minimizing toxicity associated with different

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treatment approaches can be easily brought into discussion; treatment alternatives must not only focus on long-term tumor control but also on quality of life, preservation of neurocognitive function, as well as clinical neurological status. However, these are the most critical points for evaluation in prospective trials and remain a matter of discussion due to a lack of sound and safe data, even in the era of data from prospective clinical evaluations.

Timing of Radiation Therapy

In the mid-1980s, EORTC initiated to date one randomized trial trying to answer the question of the effectivity of radiation therapy for LGG. This question is of high importance especially in younger and asymptomatic patients, or in patients presenting with seizures only. Within this EORTC 22845 trial (known as the "nonbeliever trial"), patients with histologically proven LGG (including incompletely resected pilocytic astrocytoma, low-grade oligoastrocytoma, or low-grade oligodendroglioma diagnosed according to the valid WHO classification system at that time) were included into the trial. Further inclusion criteria were WHO performance status of 0–2, Karnofsky performance index (KPI) of ≥60, and no significant other diseases requiring treatment. Excluded were patients with completely resected pilocytic astrocytoma, brainstem glioma, optic nerve glioma, third ventricular glioma, and infratentorial gliomas. Of the 311 patients enrolled, 154 were treated with early radiotherapy, and 157 were followed according to a wait-and-see approach with regular assessments. Both groups were followed every 4 months for the first 2 years, thereafter yearly.

Radiation therapy was applied with a dose of 54 Gy in single fractions of 1.8 Gy daily. The target volume was defined as the visible lesion on preoperative CT imaging adding a margin of 2 cm, and a volume shrinkage to a margin of only 1 cm was irradiated after the dose of 45 Gy had been reached.

In early follow-up, as well as in long-term evaluation published by van den Bent in 2005, early radiation therapy significantly prolonged progression-free survival [6, 7]: In the wait-and-see group, median progression-free survival was 3.4 years, whereas 5.3 years in the early radiation group ($p < 0.0001$). However, overall survival was comparable in both groups, with 7.4 years in the radiation group and 7.2 years in the wait-and-see group ($p = 0.872$).

Conclusions of this study were that even if progression-free survival can be clearly extended, survival does not depend on early initiation of radiotherapeutic treatment; thus, especially for selected subgroups with better prognosis, clinical guidelines may include a wait-and-see strategy and defer radiation until tumor progression. However, there is major criticism concerning some aspects of the trial; no formal quality of life and neurocognitive assessment have been performed, and, also, no advanced imaging such as modern MRI techniques had been included into the trial. Of note, neuropathological diagnosis was changed after reference pathology during the course of the trial, and in 26 % of the patients of which tumor specimens were available for evaluation, a high-grade tumor was diagnosed. And lastly, extent of surgical resection was only estimated by the surgeon himself, not by early postoperative imaging.

For further evaluation, preservation of quality of life and time to neurological deterioration should be also endpoints within prospective trials. Preventing tumor progression for a longer time may provide patients with better quality of life, and tumor progression can lead to deterioration of neuropsychological functioning [8]; both arguments would underline early initiation of radiation treatment. This is stressed by the fact that early radiation therapy in the EORTC 28445 trial lead to better seizure control [7].

Taking together individual risk profiles known for patients with LGG [9], patients with large, progressive, symptomatic lesions which are unresectable or only incompletely resectable should be treated with immediate radiation therapy. Also, for patients older than 40 years, immediate (postoperative) radiation has been shown to contribute on an increase in outcome [10–12]. Histologic subtypes, such as

gemistocytic components, are also an argument for early treatment. Younger patients, patients with asymptomatic and/or smaller lesions, as well as operable tumors, may be followed with a wait-and-see strategy.

Dose for Radiation Therapy

For high-grade gliomas, a clear dose–response relationship for radiotherapy has been demonstrated [13]. In LGG, mixed data has been published; some groups have shown poorer survival in patients treated with radiation doses higher than 40–50 Gy [14, 15]; others have reported that higher doses of 50–53 Gy or more lead to an increase in survival [10].

To evaluate the optimal dose for the treatment of LGG, two prospective randomized trials have been conducted in the past.

As a pendant to the "nonbeliever trial," EORTC 28844, which is known as the "believer trial," randomized high-dose radiotherapy with 59.4 Gy in 33 fractions to 45 Gy in 25 fractions [16]. Inclusion criteria were the same as EORTC 22845 (see above). Treatment was delivered to the preoperative tumor extension with a margin of 2 cm up to 45 Gy and with a reduced margin for the boost dose up to 59.4 Gy. In this study 379 patients were randomized. The overall survival rate at 5 years was 58 % with low-dose radiotherapy and 59 % with high-dose radiotherapy ($p = 0.73$). Progression-free survival rates were 47 % with low-dose radiation and 50 % with high-dose radiation at 5 years ($p = 0.94$). In this study, the extent of tumor resection demonstrated the strongest impact on outcome.

The second trial initiated by the North Central Cancer Treatment Group (NCCTG) was later joined by the Eastern Cooperative Oncology Group (ECOG) and the Radiation Therapy Oncology Group (RTOG). Patients with LGG, histologically confirmed, including WHO grade 1 and 2 astrocytoma, oligoastrocytoma, and oligodendroglioma; pilocytic astrocytomas; and other LGG variants, were excluded, and reference pathology was obtained. In one treatment arm, radiation was applied up to 50.4 Gy in 28 fractions, in the other up to 64.8 Gy in 36 fractions, over 7 weeks. The target volume was defined as the preoperative visible lesion (in earlier patients on CT imaging, later on MR imaging), adding a margin of 2 cm; for the high-dose treatments, an additional boost of 14.4 Gy was delivered to a reduced margin of 1 cm. Follow-up covered 4-month visits for the first 2 years, 6-month visits for the following 3 years, and then yearly follow-up until year 15.

The study included 203 patients (101 treated with low-dose radiation and 102 with high-dose radiation). After a median follow-up time of 6.4 years, survival was nonsignificantly higher in patients treated with low-dose RT: 94 and 72 % after low-dose RT and 85 and 64 % after high-dose RT, at 2 and 5 years, respectively ($p = 0.48$). Likewise, time to progression was comparable on both groups. Incidence of high-grade radiation toxicity (grades 3–5) was double in the high-dose treatment arm (2.5 % vs. 5 %). Prognostic factors included younger age, oligodendroglial component in histology, smaller tumor size, gross total resection, and a higher pretreatment mini-mental status examination (MMSE).

Taken together, it can be concluded that lower doses of radiation therapy, between 45 and 54 Gy, in single fractions of 1.8 Gy are sufficient for long-term local control in patients with LGG, without high rates of treatment-related toxicity. Thus, these dose concepts reflect the current treatment standard for the treatment of LGG.

Radiation and Chemotherapy

For high-grade gliomas, chemotherapy is an established treatment alternative, and after the study of Stupp and colleagues, radiation in combination with chemotherapy has been established as the treatment standard [1, 2]. For LGG, the data still remain controversial, and several approaches have been studied, or are still under investigation. The rationale of combining both modalities is of course to achieve an increase in progression-free survival and overall survival, providing a more effective target potentially to higher-malignant areas within the tumor. Several

substances of different working mechanisms have been approached, as single-agent treatment or as combined regimens.

An early Southwest Oncology Group Study (SWOG) randomly assigned patients with LGG to postoperative radiation alone or in combination with lomustine (CCNU; [17, 18]). Inclusion criteria included subtotally resected or biopsied LGG including astrocytoma, oligoastrocytoma, and oligodendroglioma, aged 18 years or older. Radiation was applied up to 55 Gy to the tumor location, and CCNU was given at a dose of 100 mg/m^2 every 6 weeks. The median survival time was 4.45 years for radiation alone and 7.4 years for radiation plus CCNU; however, at 10 years, survival rates were 40 % for radiation alone and 20 % for the combined treatment ($p = 0.7$), thus not demonstrating a clear benefit of the combined treatment; moreover, the trial was terminated early due to poor accrual after 60 patients had been randomized. Supporters argue that continuing recruitment and waiting for long-term follow-up of the projected patients might have lead to a result favoring radiation and chemotherapy.

A similar result was published from the RTOG 98–02 trial. In that study, multiagent chemotherapy with PCV (procarbazine, CCNU, vincristine was evaluated), a concept which was shown to be beneficial by smaller studies [19, 20]. In that study, 251 patients with unfavorable characteristics such as age over 40 years with partial resection or biopsy only were randomized to radiation only up to a dose of 54 Gy in 30 fractions, or radiation and six adjuvant cycles of PCV. Progression-free survival was comparable in both arms, and overall survival in the radiation group was 86 and 70 % and in the combined group 87 and 61 % at 2 and 5 years, respectively [21]. In long term observation, overall survival was increased in the group receiving radiation and chemotherapy, but progression-free survival was unaltered [22].

Several smaller studies have shown efficacy of the alkylating substance temozolomide in patients with LGG [23–26]. Based on these data, within the EORTC framework, a randomized study comparing radiation with 50.4 Gy in single fractions of 1.8 Gy compared to temozolomide in a 21/28 regimen is being conducted; patient recruitment has been completed, and patients are now during continuous follow-up. The chemotherapy regimen is based on prior work focusing on the evaluation of the response rate and toxicity of a continued schedule of temozolomide chemotherapy administered before radiation therapy, which also evaluated correlation between outcome and 1p/19q deletions and MGMT promoter methylation status [27]. A total of 30 patients with a median age of 45 years were accrued. The overall response rate was 30 % (9 partial responses); 17 patients (56.7 %) had disease stabilization.

Currently, within the ECOG–RTOG framework, a study focusing on LGG patients with high-risk features that evaluates combination of radiotherapy with temozolomide is recruiting patients (RTOG Study 1072).

Target Volume Definition

Today, modern MR imaging should be considered the standard for target volume definition in patients with LGG. Optimal coverage of infiltration zones is an essential factor for the treatment goal in radiation treatment. Following the conventions of ICRU, gross tumor volumes (GTV), clinical target volumes (CTV), as well as planning target volumes (PTV) depending on the technique performed should be defined.

The GTV generally consists of the tumor visible in T2-weighed or FLAIR–MR imaging, including the resection cavity. If present, areas of contrast enhancement in T1-weighted MRI are to be included. The CTV includes the GTV with a margin of 1–2 cm accounting for microscopic spread of the tumor cells. In the literature, CTV-margin recommendations vary, depending on the institution's policy and the experience of the radiation oncologist. However, it has been shown that the 1–2 cm surrounding the GTV is at high risk for tumor cell invasion and should be included into the CTV. These margins should respect anatomical borders, that is, midline crossing is not necessary except in selected cases, for example, with clear invasion of the corpus callosum. Also, other anatomical borders should be respected, including bony structures or meninges. The PTV

depends on the technique performed and on institutional setup variabilities and ranges ideally somewhere between 3 mm and 1 cm. With modern technologies, especially in the era of image-guided radiotherapy (IGRT), PTV margins are continuously being reduced since setup errors can be corrected prior to each radiation fraction.

Radiation Therapy with Photons: 3D-Conformal Radiotherapy

Conformal 3D-photon radiotherapy is the treatment standard in patients with LGG. Based on CT and MR imaging, target volume definition is performed, and treatment planning aims at a highly conformal coverage of the treatment volume with the 90–95 % isodose encompassing the PTV. Target volumes and organs at risk are displayed using the beam's-eye view technique; multiple fields are commonly used: nonaxial coplanar and noncoplanar, depending on the size of the volume and the shape, as well as vicinity to sensitive normal tissue structures. Individual beam arrangements are based on the anatomy of the tumor, and beam shaping is performed with a multileaf collimator.

For repositioning, individual custom-made mask fixation systems are used, mostly made of aquaplast materials.

Radiation Therapy with Photons: Stereotactic Radiotherapy, Radiosurgery, and IMRT

Stereotactic high-precision treatments have been established within clinical routine based on the work of the Swedish neurosurgeon Lars Leksell. Coming from the Greek words stereo + taxis (putting in order in three dimensions) were location of the patient and the treatment target in the three-dimensional space was developed. Initially, this was performed for neurosurgical intervention, such as biopsies. To achieve a steep dose gradient, multiple beam portals are employed, either formed by a fixed collimator with defined diameters or with individually shaped treatment formed fields by a micro-multileaf collimator. Stereotactic treatments can be applied as radiosurgery (stereotactic radiosurgery, SRS), as was developed by Leksell using the Gamma Knife technique based on 201 ^{60}Co sources; radiosurgery can also be applied using a linear accelerator.

As a patient setup, either fixation in an invasive ring system or individually manufactured mask systems allowing for high repositioning accuracy can be used [28, 29]. A stereotactic ring is placed with a localizer over the patients' head attached to the base frame of the localization system. Ficual markers within the frame help define the x, y, and z coordinates in the defined space and appear on the obtained imaging, for example, CT for treatment planning. With this setup, each point in the patient can be assigned an x, y, and z coordinate.

Radiosurgery can be applied using three approaches: high-energy X-rays generated by a linear accelerator, the Gamma Knife or Cyberknife technique, or charged particles, such as protons.

Besides single-fraction radiosurgery, the radiobiological benefits of fractionation can be exploited while adhering to millimeter precision (fractionated stereotactic radiotherapy, FSRT); for fractionated treatments, commonly individual mask fixation systems are used [29–33]. Especially for larger lesions the risk–benefit ratio shifts towards FSRT, since the risk for treatment-related side effects increases with irradiated volume. For SRS, doses applied depend on the diameter and volume treated, keeping in mind the risk–benefit ratio. Within RTOG 90–05, doses of 24 Gy ≤21 mm diameter, 18 Gy for diameters between 21 and 30 mm, and 15 Gy for diameters larger than 30 mm are recommended; lesions exceeding 4 cm should preferentially be treated using fractionated techniques [34, 35]. Thus, the maximum tolerated dose (MTD) was set in 18 and 15 Gy for patients with lesions 21–30 mm and 15 Gy for lesions greater than 30 mm in diameter. These doses are a guideline; however, it should be kept in mind that all patients included into that study were pre-irradiated patients.

SRS plays only a minor role for the treatment of LGG except possible in recurring lesions with small areas of contrast enhancement (see

paragraph on re-irradiation). FSRT, however, has been widely implemented for the treatment of LGG. Several systems have been developed with an overall accuracy of 1–3 mm [36–38]. The main hypothesis is that the benefit of the steep dose gradient is sparing of normal tissue, and thus contributing to overall outcome especially with respect to quality of life and neuropsychological outcome. It could be shown that stereotactic radiotherapy can significantly reduce the dose to normal tissue [30, 39]; additionally, it could be demonstrated that the rates of marginal or outfield recurrences are not increased with these techniques, which is often the fear when high-precision techniques are implemented.

Intensity-modulated radiotherapy (IMRT) can offer a benefit especially in complex target volumes, often adjacent to organs at risk. In LGG, the benefit of IMRT is not overtly visible; however, for temporal lobe lesions reduction of dose to normal tissue might be better accomplished using an IMRT technique. However, it must be kept in mind that integral dose to the patient is likely to be increased, which might be of special clinical importance in pediatric patients and young adults. However, advanced IMRT techniques may help in concepts sparing eloquent regions, such as the hippocampal region, aiming at minimizing long-term toxicity [40, 41].

Particle Therapy

Particle therapy offers distinct physical characteristics potentially leading to a clinical improvement in patients with LGG. Within the entry channel of the particle beam, low doses of radiation are deposited into tissue, which is commonly normal tissue in this region. High-local-dose depositions can be directed precisely into the defined tumor volume, and these dose peaks are termed Bragg peak, after the British physicist William Henry Bragg. This peak is followed by a steep dose falloff, sparing all tissues behind the target volume from dose. These physical properties lead to a significant reduction of dose to normal tissue and overall to a reduction of integral dose the patient.

The biological effectiveness of a proton beam can be roughly compared to that of photons, with a relative biological effectiveness (RBE) considered to be about 1.1. Therefore, when available, proton beams might contribute, perhaps not to long-term tumor control, but to a reduction in long-term toxicity. Heavier-charged ions, such as carbon ions, are additionally associated with an increased RBE; for high-grade gliomas, clinical feasibility and effectivity has been shown and is currently under evaluation within a randomized clinical trial [42, 43]. However, due to the infiltrative nature of LGGs and the large percentage of normal brain tissue to be included into the target volume, heavier ions play a minor role for these patients, since treatment might possibly be associated with an increase in toxicity.

To date, no clinical data have been generated that demonstrate a clear clinical advantage compared to advanced photon techniques. Focus of evaluation may include endpoints such as reduction in normal tissue toxicity, including neurocognitive deficits.

It is known that specific areas within the human brain contribute to essential cognitive functions. For example, the hippocampal region has been shown to be responsible for the development of neurocognitive deficits [40, 41, 44, 45]. Therefore, sparing of such eloquent regions during treatment planning may contribute to clinical outcome. The benefit of particle beams is that commonly fewer treatment portals are necessary; thus, precise identification of areas of risk, such as the hippocampus, or areas where neuronal stem cells are localized, and avoidance of such structures can be performed more easily with particle radiation [45]. In Fig. 26.1 a typical treatment plan for a patients with LGG treated with protons applied with intensity-modulated raster-scanning is shown. Irrespective of dose to specific brain regions, it has been reported that radiation therapy in LGG can be associated with significant hormonal dysfunction, even if the pituitary region is not the region of primary interest during radiation treatment. This was shown predominantly in pediatric patients and young adults [46]. By reducing integral dose to the patient, these side

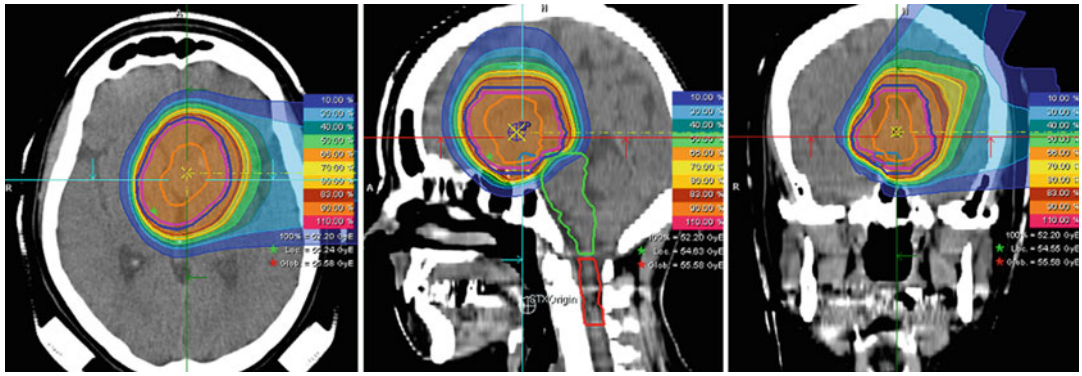

Fig. 26.1 Proton therapy applied with intensity-controlled rasterscanning at the Heidelberg Ion Therapy Center (HIT) for a young female patient with histologically confirmed LGG treated with a total dose of 54 Gy E in single dose of 1.8 Gy E

effects might also be reduced when treating LGG with particle therapy.

The real clinical benefit of proton radiotherapy in LGG has to be evaluated in a prospective clinical trial. For this purpose, the correct and relevant endpoints must be defined, and modern imaging for target and risk volume definition must be included. Quality of life, neuropsychological assessment, observation of hormonal aches, and clinical neurological status are an essential prerequisite, besides the "common endpoints," survival and quality of life.

Treatment of Recurrent Tumors

In the past, re-irradiation of gliomas had been considered to be possible in only selected clinical situations, and the treatment effect was only modest [47]. The evolvement of modern precision radiation techniques, such as stereotactic treatments, has enabled the radiation oncologist to perform second courses of radiotherapy, with effective dose levels and convincing clinical results. Individual treatment decisions, of course, have to be based on the tumor volume and overall performance status of the patient and on pretreatment variables.

In general progressive LGG often consists of large areas of T2-enhancing lesions, which progress only slowly over time. These areas including large safety margin have commonly been included into previous radiation series. However, during the course of the disease, patients often develop contrast-enhancing lesions, which represent more malignant areas of the tumor and are associated with a more rapid growth pattern. In selected cases, the T2-enhancing lesion can be targeted for re-irradiation. However, the concept of focusing on contrast-enhancing areas leads to more confined volumes safely amenable to second courses of radiotherapy.

The clinical effect of fractionated stereotactic radiotherapy (FSRT) for recurrent gliomas could be shown in a large group of 172 patients. The study included patients with recurrent or progressive low-grade gliomas, anaplastic gliomas, as well as glioblastomas. The target volume consisted of the contrast-enhancing lesion on T1-weighted MR imaging as the GTV, adding a safety margin of 1 cm for the CTV. A median total dose of 36 Gy was applied in 2 Gy single fractions. In patients with LGG, a median progression-free survival of 12 months was observed, with progression-free survival rates of 80 and 54 % at 6 and 12 months [31]. Median survival after re-irradiation was 22 months, with survival rates of 77 and 55 % at 1 and 2 years. Several other techniques and fractionation schemes are in clinical application for re-irradiation. Currently, the role of carbon ion radiotherapy is being evaluated in a randomized trial compared to FSRT with 36 Gy in 2 Gy fractions (CINDERELLA trial; [48]) at the Heidelberg University Radiation Oncology Department.

Special Subgroup: Brainstem Glioma

Brainstem gliomas are most predominant in the pediatric population, where they account for about 15 % of all pediatric brain tumors [49]. In adults, they are less common; however, when diagnosed, they represent a difficult-to-treat patient population.

Brainstem gliomas can be divided into subgroups, and besides histological classification, location and extension within the brainstem play a major role. Diffuse intrinsic gliomas are generally high-grade gliomas, either anaplastic astrocytomas or GBM. In pediatric patients, pontine diffuse gliomas are considered high grade even without histological confirmation and are therefore treated with radiation and chemotherapy [50, 51]. On the other hand, focal gliomas, often dorsally exophytic, are mostly low-grade lesions associated with a better prognosis [52–54]. Histological confirmation should be attempted in spite of the critical lesions, since PNETs or atypical teratoid–rhabdoid tumors (ATRT) might also be located in the same region, especially in pediatric patients [55, 56]. However, precise planning is necessary since neurosurgical intervention can be associated with substantial morbidity and mortality.

With brainstem LGG, patients may remain asymptomatic for longer time spans; however, due to the intricate anatomy, rapid progression of neurological signs and symptoms including cranial nerve deficits often affecting multiple nerves can be observed. Commonly, the VI and VII nerves are affected; however, all others can also be involved. Cranial nerve palsy is often associated with ataxia or hemiparesis.

Most brainstem gliomas are non-enhancing lesions, and in pontine gliomas commonly 50 % or more of the pons are involved (50 %). Enhancing lesions are typical for juvenile pilocytic astrocytomas.

Radiation therapy is performed as involved field radiation, treating the visible lesion plus a safety margin depending on histological subtype. The GTV is usually best defined on T2-weighted MRI or FLAIR–MRI, and a margin of 1–1.5 cm should be added for the CTV. For diffuse infiltrating lesions, the whole circumference of the brainstem should be treated.

Total doses up 54 Gy should be applied in single fractions of 1.8 or 2 Gy. To date, no advantage of higher doses even using hyperfractionated regimes has been clearly shown [51, 57, 58]. Overall survival ranges between 45 and 66 % at 2 and 5 years [59–63]. In a group of patients treated with FSRT supporting the rationale that surrounding normal tissue at the skull base, such as the cochlear region, can be spared of dose, local control of 70 % at 12 months and 63 % at 24 months and overall survival of 77 % at 12 months and 70 % at 24 months could be reported in 57 patients [64].

Commonly brainstem gliomas are less aggressive in adults; however, few data are available only focusing on the adult population; perhaps this is due to the more favorable histologies observed in adults [65]. A study performed by ANOCEF grouped 48 adult patients based on the radiological, clinical, and histological features: About 50 % presented with non-enhancing diffusely infiltrating tumors and demonstrated symptoms that were present for more than 3 months [63]. Of these 22 patients, 11 underwent biopsy, of which 9 were diagnosed with LGG. Median survival was 7.3 months, and most patients underwent radiation therapy. Patients presenting with rapid progression of symptoms associated with an enhancing lesion on MRI; of these, 14 underwent a biopsy intervention, and all of these were shown to have high-grade gliomas. In this group, median survival was 11.2 months, in spite of radiation therapy.

Special Subgroup: Gliomatosis Cerebri

Gliomatosis cerebri is formally defined by a glioma affecting three or more lobes of the brain. Taken this definition, the numbers of patients diagnosed with gliomatosis cerebri might be higher than usually thought.

Treatment indication for patients with gliomatosis cerebri is based on histological classification. When LGGs are diagnosed, a wait-and-see

strategy might be followed, indicating radiation therapy for tumor progression.

For radiation therapy, it is commonly recommended to perform whole-brain radiotherapy (WBRT), and doses range from 40 Gy in 2 fractions to 45 Gy in 1.8 Gy fractions. Depending on the growth pattern, an additional boost may be applied, for example, to contrast-enhancing lesions, up to 54–60 Gy. To spare normal brain and to avoid treatment-related toxicity, discussions have focused on treating involved areas only. Until now, no randomized studies exist comparing these two strategies. The rationale can be sparing regions of stem cells, for example, in the cerebellum or ventricular regions, or sparing areas responsible for neurocognitive functioning, such as the hippocampus, if not affected by the tumor.

In a group of 30 patients treated at M.D. Anderson in Houston, 87 % of patients treated with radiation therapy demonstrated disease stabilization, and 70 % clinical improvement [66]; histology significantly influenced survival.

Due to the large radiation volumes commonly recommended, some physicians are in favor of chemotherapeutic treatment in these patients. In a French trial patients with gliomatosis cerebri were treated with PCV or temozolomide; objective responses were seen in 33 % and radiological responses in 26 % [67]. No difference between both regimes was observed. In general patients with an oligodendroglial component in histology show a greater benefit from chemotherapy.

A retrospective analysis on almost 300 patients with gliomatosis cerebri published by ANOCEF reported a median survival of 14.5 months, with prognostic factors being younger age under 42 years, higher KPS, low-grade histology, or oligodendroglial component in neuropathological diagnosis. However, in spite of the large study, the real value of radiation therapy could not be defined.

Therefore, depending on histological subtype and the clinical course of the disease, treatment can include chemotherapy, or radiation therapy, while radiation still remains the treatment standard. Some controversy exists on the target volume; however, to date, no formal clinical studies evaluating this question have been performed.

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New Individualized Strategies in DLGG

27

Hugues Duffau and Luc Taillandier

Abstract

Although diffuse low-grade glioma (DLGG) is a chronic disease of the brain, the vast majority of studies investigated the role of only one specific treatment (e.g., impact of surgery, or impact of radiotherapy, or impact of chemotherapy) without a global view of the whole management. Here, our aim is to switch toward a more holistic concept, based on the anticipation of a personalized and long-term multistage therapeutic approach, with online adaptation of the strategy over years through the feedback provided by clinical, radiological, and pathologico-molecular monitoring at the individual scale. Indeed, we need to elaborate and then to adjust this tailored management during the follow-up, both on the basis of real-time oncological result (control of the glioma) as well as on the basis of functional outcome (preservation or even improvement of quality of life). Thus, we propose new individualized strategies dealing with the chronic interactions between the natural course of the DLGG, the reactional brain plasticity, and the onco-functional modulation elicited by serial treatments.

Keywords

Diffuse low-grade glioma • Surgery • Chemotherapy • Radiotherapy • Individualized management • Multistage therapeutic approach • Quality of life

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Introduction

In previous chapters, it was emphasized that diffuse low-grade glioma (DLGG) was not a tumor mass within the brain but a progressive, invasive, and chronic disease of the central nervous system. As a consequence, although the "wait and see" dogma should definitely be abandoned to evolve toward a preventive therapeutic attitude in order to delay malignant transformation, such a strategy should be adapted to the complex biological course of DLGG at the individual level. Indeed, in the traditional literature, the vast majority of studies investigated the role of only one specific treatment (e.g., impact of surgery, or impact of radiotherapy, or impact of chemotherapy) without a global view of the whole management. In addition, when different therapies have nonetheless been associated, a classical order (surgery followed by irradiation in cases of incomplete resection, followed by chemotherapy at recurrence) has rigidly been applied to the "group of DLGG patients" (as it was homogeneous), generally with no attempt to tailor the sequence of treatments to each patient.

Here, our aim is to switch toward a more holistic view, based on the anticipation of a personalized and long-term multistage therapeutic approach, with an online adaptation of the management over years through the feedback provided by clinical, radiological, and pathologico-molecular monitoring at the individual scale. This dynamic strategy challenges the traditional attitude regarding different issues: by proposing earlier therapy, by repeating treatments (e.g., 2–4 surgical resections spaced by several years or periods of 6–12 months of chemotherapy spaced by periods of single follow-up), and by reversing the "classical order" of therapies (e.g., neoadjuvant chemotherapy followed by surgery after tumor shrinkage, no early radiotherapy), with the ultimate goal not only to increase the overall survival but also to preserve the quality of life (QoL). To this end, beyond the benefit-to-risk ratio of each isolated treatment, the impact of the whole therapeutic strategy on the cumulative time with QoL versus time to malignant transformation (and not on the sole survival independently of the functional status of the patient) should be taken into account [1]. It means that we need to elaborate and then to adjust this tailored management during the follow-up, both on the basis of real-time oncological result (control of the glioma) as well as on the basis of functional outcome—QoL being preserved, thanks to (1) cerebral plastic mechanisms to compensate the growth/invasion of the tumor and to compensate the effect of the different therapies taken alone and together (2) the selection, with a similar efficacy, of the best tolerated treatment, at the right time, for the right patient [2]. In other words, we propose new personalized managements dealing with the chronic interactions between the natural course of the DLGG, the reactional brain remapping, and the onco-functional modulation elicited by serial treatments.

Proposal of Individualized Multistage Therapeutic Strategies in DLGG

The first step in the management of DLGG is to accurately investigate the tumor behavior and its possible consequences on brain functions (Fig. 27.1). Therefore, it is crucial (1) to calculate the volume of the tumor and its velocity diameter expansion (VDE, i.e., its growth rate on two consecutive MRIs at least 3 months apart—or eventually 1.5 month apart in cases of poor prognostic factors) [3], (2) to analyze the precise location of the glioma both at cortical and subcortical level, and (3) to perform extensive neuropsychological assessments (even in asymptomatic DLGG incidentally discovered) [4, 5]. All these issues have already been detailed in previous chapters and will be not discussed here. However, we would like to insist on the fact that such a baseline is essential to elaborate a personalized strategy, and on the fact that to start too quickly a treatment without this initial functional and dynamic radiological examination will result in a loss of precious information—preventing to anticipate a long-term optimal management at the individual scale. It is worth noting that this delay does not represent a risk in itself in this specific phenotype of DLGG, since they are in essence slow-growing tumors (conversely to glioblastoma).

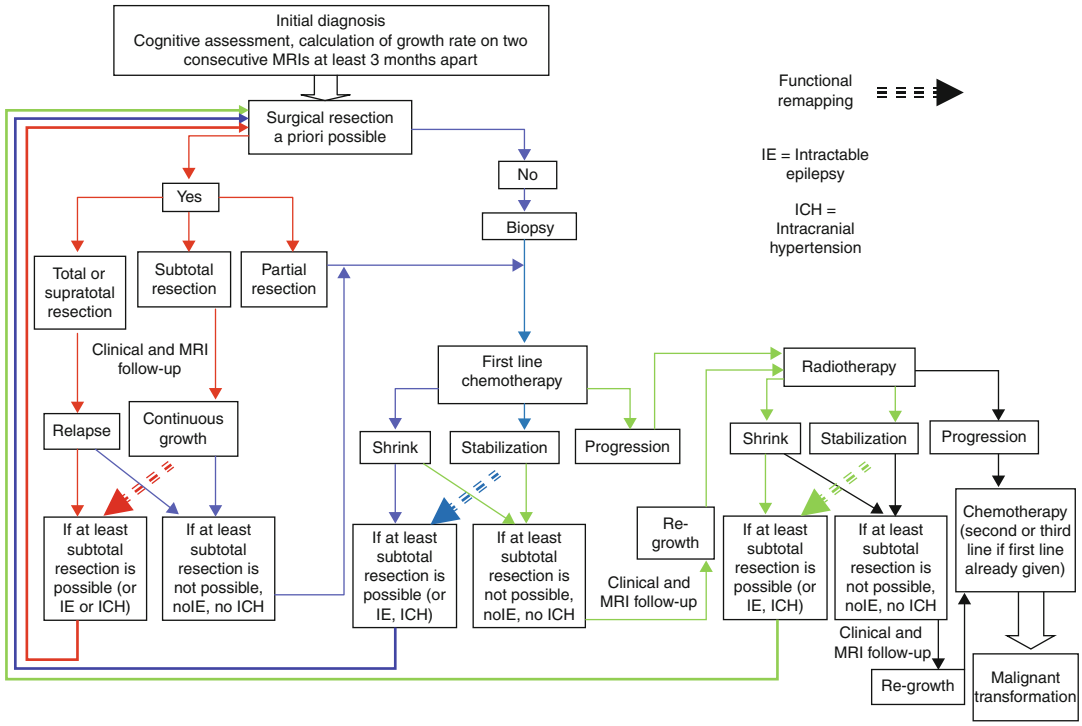

Fig. 27.1 Personalized dynamic multimodal therapeutic strategy in DLGG to prevent malignant transformation while preserving quality of life

The Multistage Surgical Approach

As extensively demonstrated in other chapters (see, for instance, the Chap. 23 by Duffau), surgical resection is the first option to envision in DLGG—as recommended by the European Guidelines [6]. Indeed, it was clearly demonstrated that radical surgery had a significant impact on overall survival by delaying malignant transformation [7, 8] while preserving or even improving the QoL, thanks to the use of awake mapping [9–11].

Nonetheless, it is worth noting that DLGG cannot (yet) be cured by surgery, even in cases of "supratotal" resection. Yordanova et al. [12] reported that removal of a margin around the FLAIR-weighted signal abnormalities prevented malignant transformation (with no adjuvant therapy) in a subset of 15 patients with DLGG located outside eloquent brain areas, with a median follow-up of 35.7 months. However, 4 of 15 cases experienced a recurrence with a mean delay about 38 months, due to isolated tumoral cells still present far beyond the "glioma" visible on MRI [12]. Once again, it means that DLGG is a chronic brain disease and that this issue should be explained to the patient since the diagnosis in order (1) to inform him/her about the fact that additional treatment(s) will be regularly given over years and (2) to improve, in parallel, him/her compliance: indeed, this actual "honest information," very well accepted, permits the establishment of a trust that will last throughout the disease. Typically, following an initial "maximal" surgery, it is very likely that DLGG will recur after several years, even after supracomplete or complete resection—and a fortiori in all cases following subtotal resection (i.e., with a residue less than 15 ± 5 cc), because the growth rate of the residual glioma was demonstrated as being similar to its presurgical kinetics [13]. Therefore, thanks to the linear progression of the mean diameter of DLGG, it becomes possible to predict at the individual scale when the volume will reach 15 ± 5 cc, which represents a threshold with a higher risk of malignant transformation above

this limit. Consequently, it is also possible to propose a second "preventive" treatment just before to reach this threshold, but not in the preceding years, in order (1) to not prematurely use therapies which will be very useful in the future (2) to preserve the QoL of patient by limiting too much treatment(s) (3) while controlling the tumor by avoiding evolution toward a higher grade of malignancy. Interestingly, regarding the timing of second (or third) surgery, we reported a series of 19 DLGGs patients who underwent reoperations: 11 tumors had progressed to high-grade glioma in a median time between the two surgeries of 4.1 years. Therefore, because there was no permanent morbidity associated with reoperations, we suggested to "over-indicate" an early re-intervention than to perform a late surgery when the tumor has already transformed into a malignant glioma [14].

In this state of mind, again, reoperation is to consider as a priority, on the condition nonetheless that at least subtotal resection can be achieved. This can be possible, even in so-called eloquent areas, due to mechanisms of cerebral remapping induced by (1) the first surgery itself, (2) the post-surgical functional rehabilitation, (3) and the slow regrowth of the DLGG [15, 16]. As mentioned, regular neurocognitive assessments as well as serial functional neuroimaging can provide helpful data to predict the extent of a second (or even third or fourth) resection, in the mind of a "multistage surgical approach" [17]. The aim remains to reduce the glioma volume in order to prevent malignant transformation while preserving brain functions (or even improving them, e.g., by controlling seizures), thanks to repeated resections. Therefore, the onco-functional balance of surgery can be optimally found for each patient only if the strong relationships between the DLGG course and the brain adaptation are taken into account (see Chap. 22 by Duffau).

On the other hand, we have to insist again on the fact that a significant oncological benefit of surgery was actually demonstrated only when the resection was at least subtotal, that is, leaving a postoperative residual volume less than 15 ± 5 cc [8, 18]. Therefore, when the glioma is very diffuse, that is, with wide invasion of the "minimal common brain" (i.e., the cortico-subcortical structures which cannot be removed whatever the patient due to limitations of cerebral plastic potential, see [19]) and/or with bi-hemispheric infiltration, it is possible to predict using probabilistic atlas before any treatment whether the surgical removal will be only partial—thus with no or mild oncological impact [20]. As a consequence, in these specific cases, there is no indication to perform surgery first (or reoperation if a subtotal resection was already performed several years before, followed by tumor relapse with a very diffuse pattern)—except (1) in patients with intractable epilepsy, because even partial resection may allow a relief of seizures, especially when the insula and/or mesiotemporal structures are involved [21], and (2) in rare cases of intracranial hypertension. Therefore, in these invasive DLGG and at this moment, alternative treatment should be considered.

The Place of Chemotherapy in a Dynamic Multimodal Therapeutic Strategy

As previously described (see Chap. 25 by Taillandier), whatever the protocol used (PCV versus temozolomide), chemotherapy may diffuse in the entire brain, that is, even in eloquent areas, without inducing functional (neurological and cognitive) deficits [22]. In essence, it means that this therapy is perfectly adapted in cases of widely invasive DLGG, typically when (re-)operation is not possible. If one or multiple surgeries have already been performed, chemotherapy can be considered when the tumor regrew with a volume reaching 15 ± 5 cc (the same threshold as discussed regarding reoperation) and when it invaded critical structures which cannot be functionally compensated (such as the subcortical white matter connectivity and/or in cases of bilateral extension). The goal is at least to stabilize the DLGG, while preserving QoL, that is, to give chemotherapy before the occurrence of neurological symptoms. To this end, temozolomide is generally preferred, because of less adverse effects (see previous chapter by Taillandier). In

other words, the principle is to control the tumor volume, in order to delay malignant transformation, in patients who should continue to enjoy a normal life [1]. Interestingly, we have also shown that, in patients with intractable seizures, chemotherapy was able to improve QoL by controlling seizures, thus leading us to give earlier temozolomide in these specific cases [23].

In addition, chemotherapy may generate shrinkage of DLGG. In fact, tumor regression with negative VDE is very frequent under chemotherapy (see Chap. 17 by Mandonnet). In this setting, when the shrinkage is important, especially with regression of the tumor invasion within eloquent structures, chemotherapy may open the door to a subsequent surgery. This original concept of "neoadjuvant chemotherapy" in neuro-oncology may be considered following previous surgical resection(s) when the DLGG relapsed with a more invasive pattern or as the first therapeutic option at the time of diagnosis in very diffuse gliomas—sometimes mimicking a gliomatosis [22]. In these particular presentations, a surgical biopsy is recommended to benefit from neuropathological as well as molecular diagnosis.

In summary, this dynamic strategy shows that serial multidisciplinary discussions are crucial over the years for each patient, because a treatment which seemed impossible (e.g., surgical resection because of too invasive DLGG) a several months or years ago can become possible, thanks to a shrinkage elicited by temozolomide. In other words, it is not reasonable for a tumor board to give a "rigid and definitive" decision regarding the resectability of a DLGG, due to strong links between tumor behavior/brain plasticity/treatment: such equilibrium is dynamic and can be modified by administrating the right therapy at the right moment in a given patient, potentially leading to a subsequent surgery initially thought to be impossible. We have nonetheless to acknowledge that the question concerning the potential for chemotherapy to induce brain plasticity remains unanswered: therefore, it should be extensively investigated in the near future.

When chemotherapy allows (only) a stabilization of the tumor volume, without opening the window to a (re-)operation, the duration of temozolomide is still a matter of debate (indeed, PCV is stopped in all cases after a maximum of six cycles). Indeed, it is currently very difficult to predict the DLGG behavior after interruption of chemotherapy, since distinct patterns have been described: continuation of shrinkage [24], prolonged stabilization, or rapid regrowth [25]. Several criteria should be taken into account in order to try to solve this problem at the individual level. First, with the aim of preserving QoL, chemotherapy will be interrupted if it is (or if it becomes) poorly tolerated. Nevertheless, when the patient has no adverse effects, oncological considerations should be the major criterion. The tumor volume is again one of the most important markers. If it is more than 15 ± 5 cc, the tendency is to give temozolomide longer, because the risk of malignant transformation is directly related to the volume [8]—and thus the need to stabilize DLGG is crucial in comparison with a smaller glioma with a volume less than 15 ± 5 cc. Moreover, the VDE before administration of chemotherapy should be taken into account. Indeed, Pallud et al. demonstrated that VDE was a significant prognostic marker of overall survival [26]. Therefore, for DLGG with a higher growth rate (especially more than 8 mm/year), chemotherapy should be administrated earlier and longer. Neuropathological parameters are also interesting, in particular when micro-foci of malignancy have been detected within a DLGG (personal unpublished data), leading to interrupt temozolomide later—especially in bigger and rapid growing DLGG. It is worth noting that, as already mentioned in the Introduction of this book, the current WHO classification based on morphology is not sensitive enough to reflect the continuum between the so-called grade II/III gliomas. This is the reason why molecular marker might also been useful to monitor chemotherapy. Although we demonstrated that the decision to begin chemotherapy should not be based on molecular biology due to its poor predictive value of tumor response (personal unpublished data), significant correlations between 1p19q status and delay of relapse after interruption of temozolomide have been reported [25]. More recently,

Guillevin et al. [27] found that metabolic imaging based on proton magnetic resonance spectroscopy could be helpful to predict efficiency of chemotherapy on DLGG and to monitor patients under temozolomide. Indeed, the spectroscopy profile seems to change more widely and rapidly than tumor volume during the response and relapse phases and thus represents an early predictive factor of outcome over 14 months of follow-up [27]. This may provide a new parameter for making improved clinical decisions. In this setting, prospective studies are now mandatory to optimize the management of DLGG under chemotherapy, especially in order to evaluate the possible benefit-to-risk ratio of new protocols alternating periods of 6–12 months with temozolomide broken by periods of single clinical and radiological follow-up. It is likely that biomathematical modeling for each DLGG could bring precious additional information in the near future (see Chap. 28 by Mandonnet). In all cases, our decisions about chemotherapy are supported by an evaluation of the benefit-to-risk ratio of chemotherapy (e.g., short-term myelotoxicity and long-term myelodysplasia or leukemia) versus other treatment(s) as well as versus no treatment—all attitudes being weighted by the risks of tumor progression at short, medium, and long terms.

When to Irradiate DLGG?

A prospective randomized trial has strongly demonstrated that early radiotherapy has no impact on overall survival [28]. Although "progression free survival" was significantly increased, one should acknowledge that this issue has no any interest for the patient. Indeed, according to the onco-functional balance which must be optimized for each treatment in DLGG, it is worth noting that in early radiotherapy, not only the survival was not improved, but the QoL was worsened due to late cognitive decline induced by irradiation [29]. Finally, to date, conversely to surgery and chemotherapy, radiotherapy cannot be regularly repeated, in order to avoid neurotoxicity. On the basis of these objective data, one could be surprised to see that many DLGG patients have nonetheless continued to be irradiated on an early phase of the disease. Indeed, in the era of "evidence-based medicine," it is puzzling to note that on one hand, clinicians claim that they would like to benefit from more class I evidences but, on the other hand, that they do not apply the recommendations when such data have finally been obtained. However, it is now clear that early radiotherapy (before or after surgical resection) should not be considered in the new strategies which aim of optimizing the cumulative time with preserved QoL while preventing malignant transformation. As a consequence, irradiation should be kept in reserve for progressive DLGG which cannot be (re-)operated and which relapsed under chemotherapy (i.e., with positive VDE despite PCV and/or temozolomide).

On the other hand, again, because the goal is to avoid malignant transformation while preserving QoL as long as possible, it seems not reasonable in this "preventive" mind to wait for a too long time before irradiation in cases of growing DLGG not controlled by alternative therapy. To this end, the RANO criteria [30] are not adapted to DLGG, because they are not sensitive enough. First, it is crucial to measure 3D volume and not only two diameters (size) in this kind of invasive tumor (because it is well known that glioma can migrate in one specific direction along the white matter pathway) [31]. Secondly, VDE, which is calculated by comparing the evolution of median diameter (extracted from volume) over time, is more objective and sensitive at the individual level rather than to wait for the increase of 25 % of the size of the glioma (which is a subjective threshold with no value at this time in a given patient) on two consecutive scans at least 6 months apart before clinical decision. Of note, even in high-grade gliomas, it was demonstrated that, based on the high degree of intra-observer variability, tumor measurements producing an increase in bidimensional products of >25 % can routinely be obtained solely by chance [32].

In DLGG, it means that irradiation should not be proposed too late, especially not once the tumor has already evolved toward a higher grade of malignancy, but should be anticipated at this

stage of the disease (as surgery and chemotherapy have been anticipated in earlier stages)—even if radiotherapy was not given as a first treatment in this multistage strategy. In addition, besides its possible oncological impact, radiotherapy may also have potential benefit on intractable epilepsy [33].

Recently, Pallud et al. showed that after radiotherapy in a subset of 33 DLGG, all patients demonstrated a tumor volume decrease (negative VDE, mean −16.7 mm/year), with a significant longer overall survival for slow responders [34]. Such data lead to propose, in fast responders, an earlier postirradiation re-treatment. Although a (re-)operation could be considered following DLGG shrinkage, one must be aware about a higher rate of cognitive disorders (personal unpublished data). This could be due to a limitation of the brain plastic potential. Indeed, radiotherapy is known to increase apoptosis, to decrease cell proliferation, and to reduce stem/progenitor cell differentiation into neurons within the neurogenic regions (especially in the subventricular zone) [35, 36]. As a consequence, a new line of chemotherapy seems to represent the best option to consider in cases of DLGG relapse after radiotherapy. However, it is worth noting that, thanks to technical advances, re-irradiation might be possible in selected patients (see the Chap. 26 by Combs). In particular, the place of radiosurgery remains to be defined [37], for instance, by performing stereotactic radiotherapy on a very focal enhancement occurrence (personal unpublished data).

Of note, whatever the technical advances in radiotherapy, especially regarding its accuracy, one should keep in mind that, after maximal resection according to functional boundaries, in essence, adjuvant radiotherapy will imply the irradiation of the eloquent connectivity—thus with a higher risk of cognitive decline.

Conclusions

The current philosophy in DLGG patients is to anticipate (before neurological or even cognitive worsening) a personalized, multimodal, and long-term management from the diagnosis to the malignant stage of the disease, with online treatment adjustment over time on the basis of regular functional feedback and radiological monitoring. The ultimate aim is not (yet) to cure this tumor but nonetheless to be preventive by delaying malignant transformation as longer as possible while preserving an optimal QoL. To this end, a multidisciplinary discussion is crucial at every stage of the glioma, thanks to strong relationships between medical and surgical neuro-oncologists allowing open discussions and elaboration of new strategies. Such attitude has already enabled a significant improvement of both functional and oncological outcomes in the past decade. Our proposal is now to generalize this new state of mind against the dogmas, in order to continue to elaborate and validate original strategies through national and international networks, with the goal to give to DLGG patients a real life—including long-term project such as pregnancy.

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Part VII

Prospects

Biomathematical Modeling of DLGG Behavior

28

Emmanuel Mandonnet

Abstract

In this chapter, we review recent advances in biomathematical modeling of glioma growth, based on the proliferation-diffusion equation. We show how the computational simulations from this equation can be compared with real tumor evolution on MRI and how these simulations progressively integrate more realistic anatomical knowledge, improving the accuracy of the virtual tumor evolution. The Achilles' heel of this model comes from the lack of quantitative relation between cell density and abnormal signal on conventional MRI, although future methods could overcome this limitation by taking advantage of multimodal sequences. In its simplified version, the model offers a practical way to monitor tumor dynamics, by estimating the velocity of the tumor front. We also envision applications to the management of DLGG, regarding model-based personalization of treatment sequence and evaluation of treatment efficacy in clinical studies. Finally, we propose a three-pathway model of malignant progression. One of these pathways has been recently mathematically modeled by the proliferation-invasion-hypoxia-necrosis-angiogenesis (PIHNA) system of equations. We show how this model leads to the important concept of kinetic grade, which is complementary to the usual histological grade.

Keywords

Biomathematical modeling • Diffuse low-grade glioma • Computational models • Malignant progression

Introduction

Since the pioneering work of Murray and Alvord in the 1990s [2, 29, 31], there has been an increasing interest for biomathematical modeling of glioma growth. It is remarkable that the proliferation-diffusion model initially proposed by

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these authors still constitutes the core of elaborated approaches more recently developed. The specific case of low-grade glioma could be especially favorable for modeling purpose, as their biological behavior seems to be relatively constant during the "low-grade" phase. Moreover, since "watch and wait" policy has been until recently a standard recommendation in many centers, series of patients radiologically monitored over several years can be retrospectively collected, and serial MRI data sets before any treatment are available for comparison with model predictions.

In this chapter, we will give a brief overview about the proliferation-diffusion equation and explain the difficulties in personalizing the model based on serial MRI images. We will also detail current and future clinical applications, with special emphasis on the key approximations that should be improved in future works. Finally, we will also discuss why the complex problem of modeling the malignant transformation constitutes a real challenge.

Modeling the Low-Grade Period: The Challenge of an Image-Based Personalized Model

Modeling Proliferation and Migration of Glioma Cells: From a Mathematical Equation to Computational Simulations

On a biological point of view, the behavior of glioma cells is twofold: proliferation and migration. Mathematical models translate these two characteristics into an equation. The variable in the equation is a coarse-grained tumor cell density (c), which represents the average concentration of tumor cells in each cubic millimeter of the brain. The generic form of the equation, initially introduced in the 1990s [29, 31], is the following:

$$\frac{\partial c}{\partial t} = \rho c + \nabla \cdot (D \nabla c)$$

that is, evolution with time of tumor cell density (c) at each position in the brain = proliferation (ρc) + diffusion ($\nabla \cdot (D \nabla c)$).

The direct problem consists to compute this equation numerically, on a digital brain template, for given values of ρ and D. The results of the simulations give the evolution over time of maps of tumor cell density (see Fig. 28.1). The template is usually a generic atlas. Over the past decade, advances has been made in integrating a more precise anatomy in this atlas (see Fig. 28.1): whereas the very first templates were built from a 2D CT scan [29, 31], just outlining the brain surface and the ventricles, more recent works are based on 3D-MRI atlases (on which CSF, white, and grey matter segmentations are performed [25]), eventually including detailed white matter architecture via DTI sequences [3, 10]. In that case, the D in the equation should read as a tensor of cell diffusion, which can be built from the tensor of water diffusion, introducing a factor r of anisotropy increase between the two tensors. To our knowledge, as there is currently no DTI atlases, images from an individual healthy volunteer are used [3, 10], but future studies could incorporate own patient DTI MRI. Whatever the elected template, it is of utmost importance that an expert validates its anatomical accuracy. For example, as explained in [10], a wrong segmentation of the subarachnoidal spaces can create artificial bridges of grey matter, especially between the frontal and temporal operculum, leading to unrealistic growth patterns in the simulations.

The Visibility Threshold Hypothesis

The tumoral cell density, which is the variable in the proliferation-diffusion model, is not directly measured on MRI. One has to make the reasonable and simple assumption that the tumor is visible on FLAIR MRI at the condition that tumor cell density is above a given value (visibility threshold). Hence, the link between simulated and real tumors relies on the comparison between the thresholded isocontour on cell density maps and the effective contours of the tumor on MRI. Unfortunately, there are very few data in the literature about the value of this visibility threshold. Only one study correlating histological analysis with hypodensity on CT suggested a value of

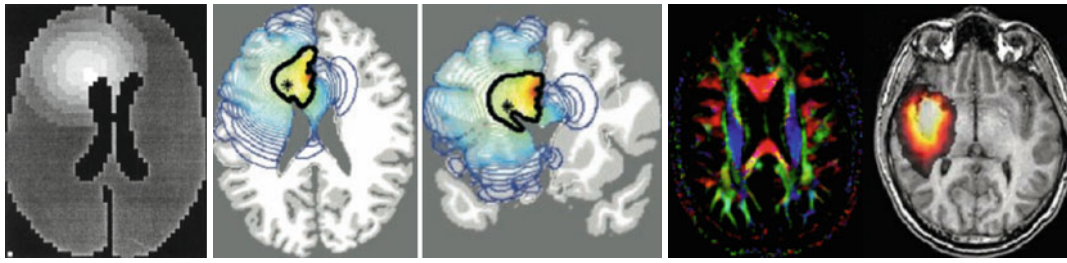

Fig. 28.1 Advances over a decade in the anatomical accuracy of the simulations. The very first templates in 1995 were built from a 2D CT scan [29, 31], just outlining the brain surface and the ventricles. More recent works in 2000 were based on 3D MRI atlases (on which CSF, white, and grey matter segmentations were performed [25]). Finally, in 2005, detailed white matter architecture via DTI sequences was eventually included [10]

8,000 cells/mm^3 [2]. Actually, current studies on this topic suggest that the MRI FLAIR hypersignal is not only dependant on the cell density but is also correlated to the intra- and extracellular water content (Badoual M, Personal communication, 2012). Nevertheless, even if it should be kept in mind that the visibility threshold hypothesis introduces a certain degree of uncertainty in the computational model, most of the following results will be based on this assumption.

Parameter Values for DLGG

For low-grade glioma, values for parameters were initially extrapolated from the values found for high-grade glioma and expected to be centered around 0.438/year for ρ and around 4.75 mm^2/year for D [2]. A range of values has been proposed by Harpold et al. [8], with ρ between 1 and 10/year and D between 10 and 100 mm^2/year (see Fig. 28.2). In more recent paper aiming to estimate the individual tumoral birth dates in a series of DLGG glioma, a range of values for ρ and D was found, which significantly differed from the one previously proposed [7]. The only paper attempting to estimate D and ρ by fitting a real patient evolution with simulated images used the values ρ=0.438/year and D=3.65 mm^2/year [10], which lies within the set of values found by Gerin et al.

For the anisotropic version of the equation, it has been found that the ratio r of anisotropy of the tensor cells has to be increased about tenfold compared to the anisotropy given by the tensor of water diffusion measured on DTI, reflecting the well-known propensity of glioma cells to migrate longitudinally rather than orthogonally to the axonal pathways [10]. This value was indeed needed to reproduce finely the shape of the tumor (which was known to be correlated with the shape of the white matter fasciculus [12]).

Virtual Imaging: Seeing Beyond the Visible

Interestingly, the ratio D/ρ controls the extent of non-visible part of the tumor (i.e., the number of cells located in areas with a cell density lower than the visibility threshold): the higher the ratio D/ρ, the greater the radiologically non-visible part of the tumor [14] (see Fig. 28.3). Although such virtual imaging could be potentially powerful, its practical interest is currently limited for two reasons: the lack of reliability of the visibility threshold hypothesis, as mentioned previously, and the challenging problem of determining the personalized values of ρ and D for each patient (see below, solving the inverse problem).

Model-Based Assessment of Tumor Dynamics

To go a step further toward clinical application, one needs to solve the inverse problem [1], that is, to identify the pair of parameters ρ and D specific to a given patient, resulting in the best fit

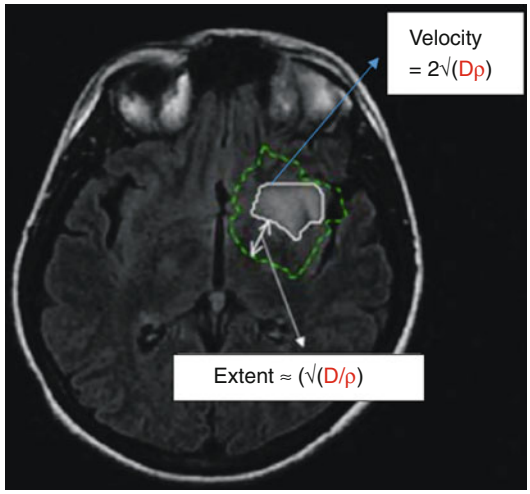

Fig. 28.2 The role of the product $D\rho$ and the ratio D/ρ. The two contours come from the simulation of a patient case. The *thick white* contour corresponds to the threshold of cell density visible on MRI. The velocity of this visible tumor front is given by the formula $2\sqrt{(D\rho)}$. The *dotted line* is the contour corresponding to a cell density five times smaller than the threshold. The extent of the non-visible tumor part is tuned by the ratio D/ρ

between simulations and a dataset of longitudinal MRIs of the patient. This field of research is also called model personalization. In a first approximate solution of this problem, it can be shown that the proliferation-diffusion equation states that the velocity of expansion of the visible front is a constant given by $2\sqrt{(\rho D)}$ (see Fig. 28.3). In other words, the slope of the linear evolution curve of tumor diameter is given by $4\sqrt{(\rho D)}$, where the diameter $d=(2\times V)^{1/3}$ is computed from the volume V. Note that V is estimated by full 3D segmentation of the hypersignal on FLAIR sequences. Thus, rather than expressing growth rates in terms of volumetric doubling times (which is the standard method for exponentially growing tumors), one should focus on the slope of diameter growth curves. Recent studies on low-grade glioma kinetics thus enabled to estimate the growth rate of tumor diameter (the so-called velocity of diametric expansion, VDE) for individual patients. The average VDE is about 4 mm/year, leading to a value of ρD close to

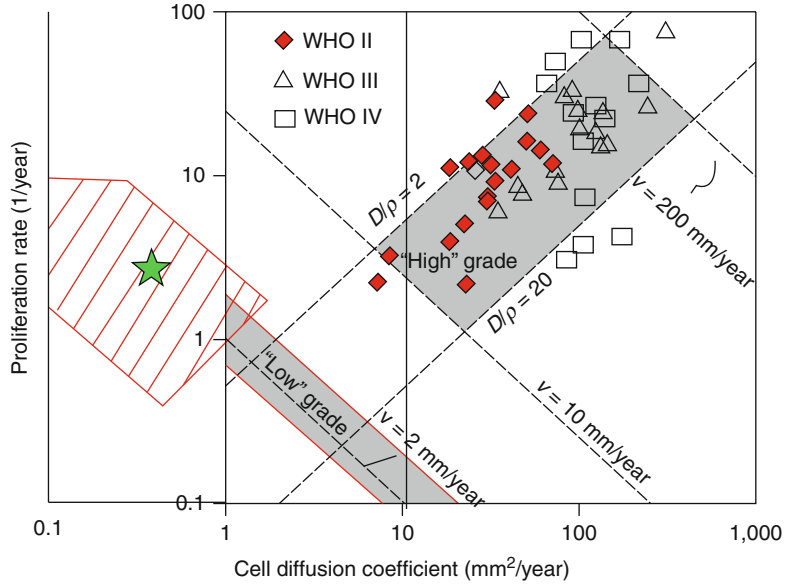

Fig. 28.3 Numerical values of D and ρ in a log-log plot. This diagram has been first discussed by Harpold et al. [8]. The diagonal $v=2$ mm/year corresponds to those glioma with a constant product $D\rho$, with a VDE of 4 mm/year, which is the average value for DLGG. *Red diamonds* correspond to the values found by Ellingson et al. [5] for WHO grade II glioma. Their values are probably irrelevant, as they fall within the expected range for high-grade glioma. The values found by Gerin et al. [7] in the *red dashed area* are correctly centered around the diagonal with a VDE equal to 4 mm/year (i.e., $v=2$ mm/year), but with values of the ratio D/ρ smaller than predicted by Harpold et al. Note that the *green star*, corresponding to the DLGG simulation performed by Jbabdi et al. [10], falls within the values found by Gerin et al.

9×10^{-6} mm^2·day^{-2}. Hence, this formula is a very simple and convenient way to estimate individually the product ρD from longitudinal MRIs. Finally, quantitative histological analysis could potentially allow to infer the ratio D/ρ: the steepness of the cell density decrease at the tumor margins can be linked to this ratio D/ρ [27]. Surprisingly, there are very few data in the literature on such quantitative histological measures.

More sophisticated tools are currently under development that will allow to estimate the optimized values of parameters, minimizing the difference between real and simulated time series of segmented contours. This inverse problem is numerically highly challenging and even not always solvable given the paucity of data (patients undergo usually two or at best three MRIs before treatment). At best, one can identify the two products ρD_w and ρD_g, D_w and D_g being the diffusion coefficients in white and grey matter, respectively [11]. Moreover, it implies the segmentations of the tumor on successive images, which is a time-consuming task. Hence, this method should be combined in the future with automated tools of segmentation.

Future Methods of Model Personalization

Apparent Diffusion Coefficient: The Missing Link Between MRI and Cell Density?

Ellingson et al. recently proposed an elegant and powerful method to estimate 3D individual maps of proliferation and diffusion parameters from at least three longitudinal diffusion-weighted sequences [5]. The key assumption is an inverse linear correlation between ADC and cell density ($ADC = \alpha c + \beta$, α being negative). These authors have indeed found a negative correlation between cell density and apparent diffusion coefficient, measured from diffusion-weighted sequences [4]. Assuming this relation, one can fully inverse the proliferation-diffusion equation, with $\rho(x)$ and $D(x)$ as the unknown variables ($\rho(x)$ and $D(x)$ are the proliferation and diffusion coefficient that can vary with position x). Three successive ADC maps are nevertheless required to estimate the time derivative terms in the equation. The results give nice color maps for proliferation and diffusion, showing spatial changes of these parameters. However, the link between cell density and ADC is not that clear, as ADC changes can be observed in relation to demyelination, edema, and disruption of normal brain architecture [18, 24]. This might explain why the values found by these authors for ρ and D in low-grade glioma are not consistent with the values estimated by the aforementioned approach based on longitudinal morphological follow-up (see Fig. 28.2).

Toward Integration of Longitudinal Multimodality Imaging in the Model

Spectroscopic magnetic resonance imaging also offers a means to estimate cell density and/or proliferation rate of a DLGG and to get a rough estimate of their spatial variations using multivoxel techniques [6, 16]. Similarly, indices derived from DTI sequences (p and q values, fiber density…) could also be linked to the cell density in the invasion part outside the FLAIR hypersignal. Hence, this information should also be used as inputs for the model personalization process. Promising methods, using a Bayesian framework, are under development to integrate these multimodality imaging and to manage the uncertainty inherent to these experimental data [17]. However, the key point in these methods still relies on the mathematical links between cell density and multimodal imaging parameters (ADC, Cho/NAA, CNI, p and q in DTI…), and more efforts should be devoted to their determination.

Future Applications of Personalized Models

Model-Guided Optimization of Treatment Sequence

Assuming that the inverse problem has been solved – that is, one is able to personalize the model based on (multimodal) MRI – treatment sequences can be simulated on the virtual tumor of

the patient, allowing to select an optimized scheme for each patient. This would be especially helpful for recurrent DLGG after initial surgery, as there is no standardized strategy in this setting. For example, it has been suggested that the benefit of gross total resection for tumors with high values of D/ρ is limited, since a lot of isolated tumor cells would be left even after a radiologically complete resection [26]. On the contrary, the model would predict that a supra-radical resection of these tumors would dramatically change the delay of recurrence, hence the prognosis [26]. Thus, the combined use of tools to predict individually the likely extent of resection [9, 13] and patient-specific simulations could assist the decision-making process of a second surgery versus another oncological treatment (chemotherapy, radiation therapy). To this end, the effect of chemotherapy and radiation therapy should also be included in the model. Some attempts have already been done for high-grade glioma, but their validity is not well established. Moreover, the prolonged effect of chemotherapy and radiation therapy in DLGG [20, 21, 23] warrants to develop specific models of DLGG response to these treatments [22].

Model-Based Evaluation of Treatment Efficacy

As explained in the next chapter, the evaluation of treatment efficacy in DLGG is in itself a real challenge. The usual methodology of evidenced-based medicine that prevails in other fields of oncology, that is, randomized studies comparing two treatment arms, is inadequate for DLGG (in their true low-grade period), given the very long survivals of these patients [15]. Moreover, most patients will ultimately also receive the treatment of the other arm, thus precluding to analyze separately the effect of each treatment. Personalized models can play an important role to quantify individual treatment response: for each patient, simulations can act as its own virtual control. Hence, response can be defined at any time as the difference between real (measured) tumor diameter in the patient under study and simulated (predicted) tumor diameter in its untreated virtual clone. In its simplified version, this method consists in comparing the slopes of tumor diameter growth curve before and during treatment. This idea will be further developed in the next chapter.

The Backward Extrapolation

Simulations can also be used to estimate the real biological birth date of a DLGG, which is anterior to the radiological birth date estimated by a simple backward linear extrapolation (see Chap. 17). It can be shown that, within some approximations of the proliferation-diffusion model, a corrective term of $20/v$ has to be added to the radiological birth date [7], v being the velocity of diametric expansion (VDE). Applying these principles to a series of 144 patients, it has been found that patients could be classified roughly in two groups: a group of patients with low velocities (v between 1 and 4 mm/year) and a group with high velocities (v between 4 and 8 mm/year). For the low-velocity group, patients are about 15 years of age at estimated biological onset, whereas for the high-velocity group, patients ages are centered around 25 years of age [7]. Even if these results should be considered very cautiously given the strong underlying hypothesis of the model, they could help to identify different molecular signature of these two groups of tumors and to target age groups for a screening policy.

Modeling the Transition Toward Higher Grade

The transition toward a glioma of higher grade is a somehow unforeseeable event, albeit unavoidable, in the natural history of a LGG. It has been well proven that the greater the initial tumor volume (or its residue after surgery), the higher the risk of imminent anaplastic transformation. Whereas the reference definition of anaplastic transformation is based on the histological criteria of a grade III or IV glioma, it is now widely admitted that it can be also diagnosed by *the appearance in the longitudinal follow-up* of a new contrast-enhanced nodule on T1-gado MRI.

Considering that there is no neoangiogenesis in grade II glioma, we conclude that innate vascularization of the brain parenchyma (probably combined with an optimized metabolic scheme) is able to fulfill the energetic needs of a tumor growing up to 4 mm/year.

On a fundamental point of view, three distinct pathways can lead to the onset of neoangiogenesis (which is the major criteria of malignant transformation):

1. A genetic mutation (or the cumulative effect of several mutations or any changes at molecular scale) can directly drive the building of neovessels, irrespective of the hypoxic state of the cells (as it can be observed in the model of tumorigenesis of hemangioblastoma in Von Hippel-Lindau patients).
2. Without any additional molecular changes, due to the progressive growth of the tumor, cells can enter a hypoxic environment (decrease of available energetics/oxygen resources per cell), triggering the neoangiogenic cascade.
3. A genetic mutation (or the cumulative effect of several mutations or any changes at molecular scale) can induce the appearance of a more aggressive cellular behavior (regarding proliferation rate and/or migration ability), which in turn will lead to a hypoxic focus within the tumor (increase of energetics/oxygen needs per cell).

This three-pathway model could explain why longitudinal imaging can fail to anticipate malignant progression. For example, in pathways 1 and 2, the VDE of the tumor measured on the FLAIR images should not increase before the onset of contrast-enhanced nodule. An increase of the VDE should precede the appearance of the contrast-enhanced focus only in pathway 3. Similarly, spectroscopic imaging, which is based on surrogate marker of cell density and/or proliferation (i.e., choline increase, NAA decrease, or Cho/NAA ratio increase), might not be able to predict anaplastic transformation in pathways 1 and 2. In pathway 1, one would indeed not expect an increase of such markers, as the cellular density and the proliferation rate remain stable despite onset of contrast enhancement, and in pathway 2, the increase in cellular density would be not significant enough to be detected by choline and NAA changes. Only in pathway 3 would changes in these compounds be an early marker of anaplastic shift.

Moreover, this classification could also be of importance regarding treatment selection. It would be expected that surgery is of crucial importance to stop pathway 1 and to a lesser extent to refrain the progression in pathway 2. Pathway 3 would rather require chemotherapy or radiation therapy, as it is likely that the change in cellular behavior has also spread to the cells in the radiologically non-visible part of the tumor.

Interestingly, microenvironment-driven progression has been modeled within the framework of the proliferation-invasion-hypoxia-necrosis -angiogenesis (PIHNA) model [28]. This model builds upon the proliferation-diffusion model, adding two other populations of cells: hypoxic and necrotic cells, as well as concentration of angiogenic factors and neovessels. Normoxic cells evolve toward hypoxic cells at a rate proportional to the concentration of cells and to the proliferation ρ. Hypoxic cells generate angiogenic factors, which in turn lead to an increase of vessel density. The advantage of this model is that it allows quantitative comparison with some histological immunomarkers, like the density of HIF1-α-positive cells or the density of VEGF-positive cells. The drawback of this approach is that several new parameters are introduced, the values of which are poorly known. As an interesting result, it is shown that a glioma with a product $D\rho$ in the typical range of a glioblastoma (corresponding VDE around 40 mm/year) can exhibit, at initial diagnosis, histological features of a grade II glioma at the condition its ratio D/ρ is very high. Of course, within the next 3 months, histological characteristics of a glioblastoma arise in the simulated tumor. The authors interpret this simulated tumor as a "secondary glioblastoma." We do not share this opinion, as the initial values of D and ρ were typical of a glioblastoma from the beginning. In other words, the tumor is a de novo glioblastoma, but due to the high ratio of D/ρ, the cell density was not high enough to generate hypoxic focus triggering the neoangiogenesis cascade

Fig. 28.4 Kinetic classification. The range for grade II is based on the results from Pallud et al. [19]. The range for grade IV comes from the work of Wang et al. [30]. Note that a grade IV kinetics might present at diagnosis with histological characteristics of a histological grade II (at the condition the ratio D/ρ is high, hence the cell density is quite low)

during the first months of growth. In our view, these tumors correspond to "false" grade II (because of high VDE) despite true histological characteristics of grade II: they belong to the 10–15 % of histological grade II glioma with an initial VDE higher than 8 mm/year as described in a series of 143 patients [19]. This underlines the importance of a kinetic grading based on VDE, independently of the histological grading (see Fig. 28.4).

Conclusion

Biomathematical modeling applied to glioma is still in its infancy. But the joined advances of computational modeling and multimodal MRI should offer in the near future powerful tools enabling to build realistic patient-specific virtual tumors. This would open new avenues to develop model-based virtual imaging and to progress toward the individual optimization of treatment planning.

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Toward the Definition of New Endpoints

29

Emmanuel Mandonnet, Luc Bauchet, Luc Taillandier, and Hugues Duffau

Abstract

Despite considerable advances in the different treatment modalities, there is no evidence-based consensus in the therapeutic management of diffuse low-grade glioma (DLGG). The typically long overall survival of patients with DLGG makes the usual methodology in oncology of randomized trials inadequate. Moreover, any evaluation of treatment efficacy on overall survival has to be associated with an analysis of functional status in these young patients, who are often not that much affected in the initial phase of their disease. In a first step, we propose a new method to compare the efficacy of the different strategies (advocated in different centers): plotting the time to malignant progression versus the time with quality of life. In a second step, new criteria of response to a single treatment are defined on morphological MRI. Contrarily to previous work, our approach takes into account not only the tumor size evolution but also the dynamic changes induced by the therapy.

Keywords

Endpoint determination • Functional status • Low grade glioma • Malignant progression • Neuro-oncology • Neurosurgery • Quality of life

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Introduction

Endpoints in oncology are of utmost important as they can serve three distinct but complementary and interdependent goals:

- Helping treatment monitoring (hence decision making) in daily clinical practice
- Defining criteria (on radiological, clinical, cognitive, and quality of life points of view) to quantify the effect of a single treatment in retrospective and prospective studies
- Comparing the overall clinical benefit experienced by the patients, for different treatments sequences, in prospective series or clinical trials

In this chapter, we will first propose new endpoints to address the last point. For the first two points, we will also introduce a new methodology, offering an alternative to the McDonald's criteria that were introduced to this intent in 1990 in the context of high-grade glioma [1] and recently adapted by the RANO group to the specific case of diffuse low-grade glioma [2].

Endpoints in Long-Term Evaluation: Time with Quality of Life Versus Time to Malignant Progression

Overall survival (OS) is the gold standard endpoint when elaborating clinical trials in oncology. However, diffuse low-grade glioma (DLGG) patients are typically long survivors. This means that clinical trials using OS as endpoint will span over at least 10 years, rendering their practical organization highly difficult. Moreover, at the moment the final studies are published, their results are disputable given the advances in diagnostic and therapeutic techniques made in the meantime, and their conclusions are finally not always considered as evidence-based in clinical practice. The study EORTC 22845 on early versus late radiotherapy clearly illustrates this issue: launched in 1986, with inclusions up to 1997, the long-term results were published in 2005 [3]. Over this two-decade period, MRI completely changed the way of imaging a DLGG (whereas the study design was based on CT scan follow-up), radiation techniques greatly improved (e.g., IMRT), molecular biology defined new subgroups (1p-19q codeletion versus p53 mutations), and new drugs became available (temozolomide). Hence, even if randomized studies using OS as endpoint are theoretically the only way to make evidence-based medicine, there is a need to define endpoints over a shorter time interval.

To this end, image-based progression-free survival is commonly used. The criteria of progression have recently been revised for the specific case of DLGG [2]. Unfortunately, the definitions introduced in this paper are very confusing. First, malignant (onset of a new area of contrast enhancement) and nonmalignant (growth on FLAIR or T2 images) progressions are both included in the same common definition of progression. Second, as it will be explained below, there is a great intra- and inter-expert variability in size estimation based on the product of two greatest perpendicular diameters. Moreover, there is a paradox in qualifying a tumor with up to 25 % increase of its area as being stable. As detailed in the chapter on DLGG dynamics, these tumors are continuously growing when not treated, without any period of stability. Hence, after subtotal or partial resection, the residual tumor is still continuously growing (if no adjuvant therapy is administered) at the same rate as before the surgery [6]. In other words, there is, in our dynamic view of DLGG, no progression-free period after non-complete resection (which represents about 70 % of patients). Thus, the term PFS should be used only after complete resection, as progression can then be unambiguously defined as the time when T2 or FLAIR abnormality reappears on MRI. Last but not least, PFS is of limited value, given that it is not systematically correlated with OS [3].

On the contrary, several studies have evidenced that overall and malignant progression-free survival curves are parallel [4]. In other words, any treatment delaying malignant transformation is expected to improve OS. Stricto sensu, malignant transformation is defined by a grade III or IV glioma on histopathological analysis. However, it is now widely admitted [4–6] that malignant transformation can be detected on

MRI changes: appearance of a new contrast-enhanced nodule on longitudinal follow-up.

Thus, time to malignant transformation (detected either histologically or radiologically) appears to be an objective and meaningful endpoint, enabling to overcome the logistics issues raised by the long OS of DLGG patients.

DLGG affects young adults that are socially and professionally fully functional, living a "normal life", and any therapy generating a dramatic change in quality of life (QoL) would be highly questionable in these patients. Still, as a result of disease evolution or adverse effects of treatments, patients may go through a period of time during which their QoL is hampered, up to the point that they cannot anymore enjoy a "normal life" (e.g., 3 months with uncontrolled epilepsy, 1 month of hemiparesis or 3 months of aphasia after surgery, 1 month of aplasia after chemotherapy). This functional deterioration cannot be regarded as an endpoint, as it is often transitory rather than definitive. It is thus necessary to add to the survival analysis a parameter monitoring continuously the functional status, in order to assess whether a gain in MPFS or OS is indeed associated with a better life. To this intent, we recently suggested to use a time with (good) quality of life (TQL) [7]. It corresponds to the cumulative periods of time during which the patient scores above a certain threshold, on a scale evaluating either cognitive functions, symptoms burden, seizure activity, or quality of life. By plotting TQL versus MPFS for each patient, one can measure how much treatments enable to delay malignant transformation while preserving patient's functions. This method does not come without any practical issues. First, there is no consensus on the ideal score evaluating whether a patient is "functioning" normally or not. Second, as there is a need of repeated evaluations over time, it is important that the testing can be done by any trained clinic personnel, in a reasonable amount of time. As we suggested, the simplest implementation would be to use the Karnofsky score, with a threshold at 80, despite the well-known fact that this score poorly reflects quality of life. More elaborated assessments have been proposed [8, 9], and thresholds could be defined for computation of several TQL, evaluating the time with high cognitive level (using, e.g., the Hopkins Verbal Learning Test, the Controlled Oral Word Association, and Trail Making Test Part A and B – see [8]) or with good quality of life (using, e.g., the Functional Assessment of Cancer Therapy [10]). Many other scores could also be used, including but not restricted to *EQ-5D*, *SF-36*, *and EORTC QLQ-BN20*/*C30* (see Chap. 14). A simple TQL can also be defined as the time without any neurological deficit and/or without any seizures. We emphasize that an optimal treatment would increase both the MFPS and the TQL, i.e., one would move forward on the diagonal of Fig. 29.1. A patient with a permanent postoperative hemiparesis after an extensive resection would have an oncological benefit but no functional benefit (point 3 on the figure). Conversely, a patient undergoing an initial wait and watch attitude would move forward on the diagonal, but the MPFS is shorter, meaning a low oncological benefit (point 1 on the figure). Point 2, corresponding to the patient treated on the Fig. 29.2, is close to the ideal scheme, with an optimum between oncological and functional efficacy.

All in all, if applied in different centers with distinct management of grade II gliomas (e.g., "watch and wait" policy versus upfront surgery), this tool would allow, by performing meta-analysis, to measure how much treatments enable to delay malignant transformation while preserving QoL.

Revising McDonald's Criteria for Short- and Midterm Evaluation of a Single Treatment Effect

Global endpoints such as MPFS or OS are reached after a sequence of multiple treatments (surgery, repeated surgery, first- and second-line chemotherapy, radiation therapy…). These endpoints can be used to compare strategies with different modalities or different orders in the treatment sequence. However, they cannot point out the contribution of each single treatment modality to overall efficacy. To this intent, McDonald's criteria have been introduced in 1990, with the aim to define objective criteria of response to a single

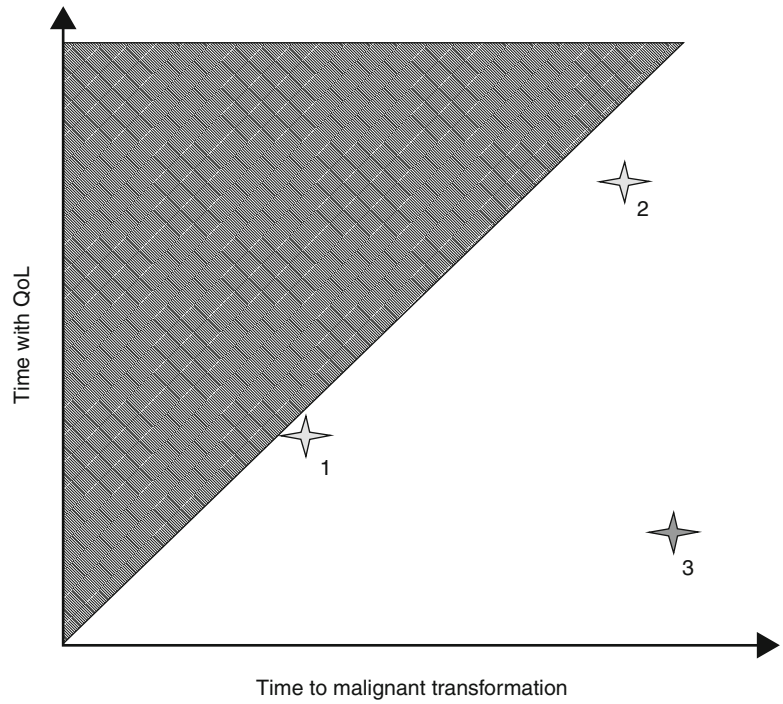

Fig. 29.1 Time with QoL versus malignant progression-free survival. Point *2* corresponds to the patient treated by the sequence of Fig. 29.2. The MPFS is 52 months, while the TQL is 49 months (due to 3 months with transient aphasia after initial surgery), close to the *ideal diagonal*. Point *1* and *3* are imaginary: they represent where would have been represented the same patient on the diagram, if wait and watch policy had been advocated (point 1, good functional score, but poor oncological result) or if extensive surgery without identifying the functional limits had been performed (point 3, good oncological result but poor functional score)

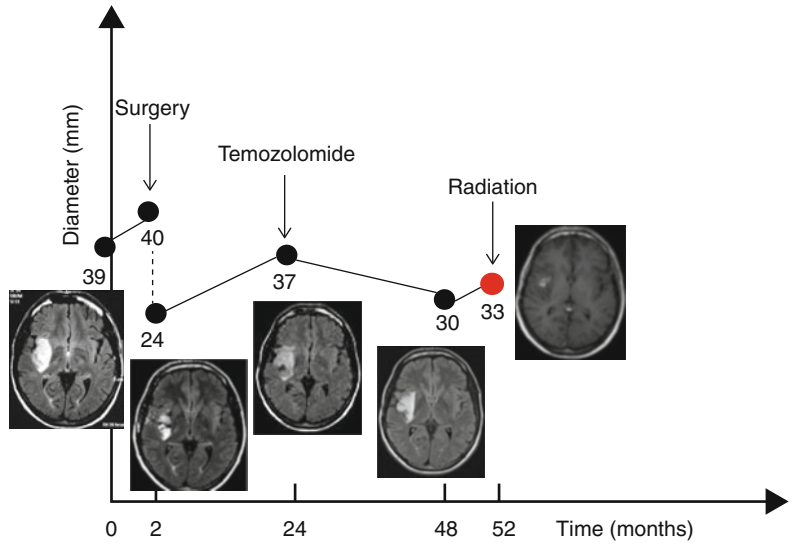

Fig. 29.2 Illustration of new endpoints and follow-up of the equivalent diameter. This 35-year-old patient was diagnosed with a grade II glioma of 39 mm (i.e., a volume of 29 cm^3). Patient was operated on 2 months later, at a size of 40 mm (i.e., a volume of 31.5 cm^3). The preoperative growth rate of 6.6 mm/year was indicative of a kinetics faster than the average (4 mm/year). After surgery, the continuous regrowth over the next 2 years, at a velocity of 7 mm/year, shows that the notion of *progression-free survival* is meaningless in this context. Because of this radiological growth rate and despite the patient was fully stable clinically, temozolomide treatment was initiated, for the next 2 years. Under temozolomide, patient belongs to the *reversed* group (7 mm/year before, −3.4 mm/year during). The intensity of the response is a decrease of 7 mm. An increase greater than 2 mm defines the treatment *relapse* after temozolomide treatment ended. This relapse was accompanied in this case by a malignant transformation – characterized by the development of a new contrast-enhanced focus on MRI – defining a *malignant progression-free survival* of 52 months. For this patient, for the whole sequence of treatment (surgery followed by chemotherapy), the *time with QoL* is 49 months (3 months of aphasia after surgery with KPS <80)

treatment modality for phase II study of high-grade glioma [1]. The novelty was to "stress imaging and steroids requirements and deemphasize, but not to ignore, clinical considerations." Those criteria have been recently modified for the specific case of DLGG [2]. However, this revision does not seem to be well designed. The criteria are mainly based on the evolution of tumor size on FLAIR (or T2) MRI. Unfortunately, these authors propose to estimate tumor size by the product of the two largest perpendicular diameters on axial slices. However, it has been clearly evidenced that estimating tumor size by segmenting (manually or semiautomatically) each axial slice on a computer reduces greatly the intra- and inter-reader variability [11]. Indeed, as stated by van den Bent et al. [2], "DLGG are often irregular in shape and grow anisotropically, resulting in poor reproducibility of area or volume estimation based on linear measurements." This is especially of importance in the postoperative setting, considering the complex tumor shapes, due to surgical cavity. The increasing availability of softwares allowing to perform segmentations on DICOM images (be they on dedicated stations in neuroradiology and radiotherapy department or even on PC – e.g., OsiriX, ImageJ) renders any other technique based on one-, two-, or three-diameter measurements old-fashioned.

Furthermore, in their scheme, response to treatment is categorized irrespective of the pretreatment dynamics of the tumor. But does it make sense to classify a tumor growing at 2 mm/year under treatment in the same response group of stability whether its pretreatment kinetics was 2 mm/year or 10 mm/year? Obviously, treatment does not change the tumor evolution in the former case, whereas it is quite efficient in the latter situation. Hence, treatment should not only be evaluated by size parameters but also by dynamic parameters. Whereas the gold standard to estimate tumor size has been established (i.e., full 3D volumetry), there is no consensus regarding the best parameter to assess tumor dynamics. There are good reasons to believe that volumetric growth rate is not the optimal parameter to this intent, because the mathematical law of volume evolution is far from being linear. That is the reason why we advocate the use of an equivalent diameter, computed from the volume, thanks to the formula (easily implemented on any pocket calculator, Excel, Google…): $d=(2\times V)^{1/3}$. By repeating MRI at regular intervals of 3–6 months, one can plot the evolution curve of this equivalent diameter. This curve of equivalent diameter evolution is a fundamental tool (see Fig. 29.2), both for daily practice and clinical research, as the different parts of this curve (before, during, and after any treatment) can be fitted by a linear law. The slope of each line segment corresponds indeed to the velocity of diameter evolution (VDE, positive for increasing lesions and negative for decreasing lesions). Of course, several time points spanning over a long period of time make the linear regression more reliable. Hence, ideally, *the pretreatment and undertreatment VDE should be estimated from at least three time points 6 months apart*.

Based on VDE changes, four groups of *treatment effects on dynamics* can be defined, as the tumor growth can be uncontrolled, slowed down, stabilized, or reversed by the therapy:

- *Uncontrolled*: unchanged or increased VDE compared to pretreatment VDE
- *Slowed down*: decreased VDE compared to pretreatment VDE, but still positive
- *Stabilized*: VDE is close to 0 mm/year
- *Reversed*: negative VDE

For the *slowed down* group, the undertreatment VDE can be reduced in various proportion compared to the pretreatment VDE. This allows a quantitative analysis of the slowdown.

For the *stabilized* and *reversed* classes, *treatment escape and relapse* need to be further defined: it corresponds to *a regrowth of the tumor greater than 2 mm*, during the course of treatment and after the end of treatment, respectively. Based on our own experience and the reported inter-rater variability in the literature, the absolute error in the "volumetric" measure of the equivalent diameter is indeed about 1 mm. Consequently, an increase greater than 2 mm between two images cannot be attributed to an error of measure. For the *reversed* class, the intensity of the response is then evaluated at the antepenultimate examination, i.e., at the nadir of the curve of diameter evolution, and is given by how much the

diameter has been reduced by the therapy. The duration of the response is given by the time period between treatment onset and treatment escape or relapse (see Fig. 29.2). Of course, the duration of response with QoL should be determined, as for the TQL.

This classification is also adequate to guide the clinician in his attempt to continuously adapt treatment over time. For a patient with *uncontrolled* growth, shifting for alternative treatment should not be deferred. For a patient with a *slowed down* response, depending on the exact proportion of slowdown, one could choose to go on, eventually by combining the current treatment with another one. For the *stabilized* and *reversed* classes, it seems reasonable to keep the treatment ongoing until escape. However, as adverse effects can be observed after long period of administration (e.g., theoretical risk of hemopathy with temozolomide), a washout is often proposed. Treatment can then be reintroduced at relapse.

Conclusion

Future multicenter prospective studies should implement systematically two endpoints, assessing, respectively, the oncological and functional efficacy of a therapeutic strategy: the time to malignant progression and the time with QoL.

The monitoring of a single treatment should be based on the longitudinal follow-up of equivalent diameter curve, with estimation of pre-, under-, and posttreatment VDE. Several types of response on the dynamics have been defined: *uncontrolled*, *slowed down*, *stabilized*, *and reversed*. This classification, based on objective quantification, can thus be used in retrospective studies and future clinical trials, as well as for guiding decision making in daily clinical practice. The main obstacles to a wider application of this method are the lack of studies establishing the exact intra- and inter-rater variability in VDE estimations, as well as the penibility of the time-consuming task that consists in contouring tumor at each time point. We envision that in the future, (semi-)automated algorithms of imaging analysis will solve these issues, by providing to the clinician an easy to use tool to compute the VDE.

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Functional Rehabilitation in Patients with Diffuse Low-Grade Glioma (DLGG)

30

Guillaume Herbet and Sylvie Moritz-Gasser

Abstract

A relevant and ethical management of DLGG patients can't refrain from taking into account cognitive disorders and proposing, if need be, a specific program of cognitive rehabilitation, to allow patients recovering -or maintaining- the best level of quality of life as possible. The slow-growing and infiltrating character of DLGG makes their associated cognitive disorders particularly amenable to rehabilitation, by potentiating or even constraining the mechanisms of functional brain reorganization within complex large-scale neural networks.

Keywords

DLGG • Cognitive disorders • Cognitive rehabilitation • Distributed interconnected networks • Functional brain reorganization • Neural plasticity • Quality of life

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Introduction

Patients with DLGG may present with functional impairments in various degrees, according to lesion location and size, treatments, and disease course. The term "functional" encompasses everything related with human functioning. Here we will focus on nonpharmacological rehabilitation of cognitive functioning, its efficacy, and its consequences on the level of quality of life (QoL) – indeed, sensory-motor rehabilitation is managed either by physiotherapists, occupational therapists, or even by orthoptists (e.g., in case of hemianopia). As mentioned in previous chapter, cognitive functioning encompasses language, attention, memory, and executive functions to which we may add social cognition.

Recent advances in therapeutic strategies allow increasing significantly the duration of survival in patients with brain tumor. Nevertheless, during these disease-free periods, most of the patients experience cognitive disorders, which may negatively influence the QoL. Moreover, given that DLGG occurs mainly in young adults, with busy socio-professional activities, a relevant and ethical management of DLGG cannot refrain from taking into account cognitive disorders, whatever their importance and their origin. Indeed, cognitive disorders, which may go from slight ones to broad impairments in different cognitive functions, might be caused by the tumor itself but also by related epilepsy and treatments [1]. Disorders may be related to the location of the tumor but also to disconnection mechanisms between functional networks induced by probable disturbances in functional connectivity due to the tumor [2]. Thus, disorders are often diffuse and not necessarily as they would be predicted by tumor location. Moreover, these disorders are different, for a given location, from those secondary to strokes [3]. Therefore, in the context of patient care as well as in the context of longitudinal follow-up, we absolutely have to assess periodically the cognitive functioning of DLGG patients (see Chap.19) and to propose, if need be, a specific program of cognitive rehabilitation, in order to prevent or treat cognitive disorders. It is worth noting that we may propose this program not only in patients with cognitive disorders highlighted by cognitive assessments but also in those who have subjective complaints concerning cognitive functioning, even if not objectivable by neuropsychological evaluations.

Although studies on cognitive rehabilitation have already a long history in neuropsychology, from the early twentieth century in the aftermath of World War I [4], its efficiency is currently a wide matter of debate because, despite early and sometimes intensive therapies, cognitive or neurologic disorders may persist chronically [5]. Several lines of explanation can be advanced to account for this lack of positive outcomes, from methodological, institutional to more neurophysiological considerations. With regard to the latter, it was long believed that the poor functional recovery could be explained by a limited potential of the brain to compensate from lesions [6]. However, observations from DLGG patients show in an exemplary manner that this statement may not be true. Indeed, it is now well acknowledged that cognitive disturbances are limited in patients harboring a slow-growing tumor, despite sometimes extensive lesions and resections [6]. Among the most striking clinical observations, it has been, for example, demonstrated that extensive frontal lobectomies did not induce any cognitive or behavioral dysexecutive syndrome [7] or that the surgical excision of Broca's area, a brain region thought yet as crucial for language processing, did not induce permanently a productive aphasia [8]. These provocative findings have led to revise the conception according to which the potential of brain plasticity is relative in the case of brain injury as not allowing a complete and efficient recovery. In the same way, they have challenged the conventional conceptions of neuropsychology which are not able to explain these important functional reorganization phenomena. For this reason, alternative view of anatomo-functional organization, according to which brain function is the result of functional orchestration and integration of large-scale and distributed networks, has emerged. The heuristic value of this framework is much higher to account for functional plasticity than the functional specialization framework.

If studies focusing on functional rehabilitation in patients with brain tumor are scarce, especially concerning cognitive rehabilitation (and even more concerning language rehabilitation), the few we found underline that rehabilitation interventions are associated with significant improvements in functional status (for a review, see [9–16]. These improvements in functional outcomes induced by rehabilitation justify, following some authors, the *delivery of rehabilitation services to brain tumor patients* [17, 18].

Theoretical Approaches and Mechanisms of Recovery

Cognitive rehabilitation encompasses all the modalities of nonpharmacological interventions to treat or prevent cognitive disorders. These interventions are administered to the patient by a speech therapist and/or a neuropsychologist. Two kinds of mechanisms underlying the recovery of cognitive functioning are described in the literature: compensation and restoration [19–21]. Their effectiveness has been addressed in several studies, concerning different brain injuries (traumatic, strokes, and more scarcely tumors) [22, 23]. Compensatory and restorative processes participate both in functional brain reorganization and may be induced by different strategies of cognitive rehabilitation. These different settings may be divided in two groups.

In the setting of compensation strategies, patients are taught to make use of external and internal strategies in order to bypass their cognitive disorders. Thus, they learn to achieve a given cognitive task in a different way as before by reorganizing functional networks in intact brain areas, close or distant to the lesion [24].

In the setting of restoration strategies, patients are taught to retrain specific cognitive skills, thanks to repetitive stimulation, in order to restitute at least partially the prior cognitive functioning. Thus, they learn to achieve the same behavior in a similar way as before, by enhancing residual functional capacities [25].

In any case, these strategies are not mutually exclusive, and actually, the mechanisms of recovering induced by the use of these different strategies remain unclear, certainly because no program of cognitive rehabilitation is based exclusively in one or the other strategy.

What one has to keep in mind when managing a patient with cognitive disorders is that, on the one hand, cognitive functions interact with each other and that, on the other hand, a given cognitive deficit may be induced by a disturbance of different functional overlapping brain systems [26]. Then we assert, borrowing from Luria's thought [27], that a relevant and appropriate program of cognitive rehabilitation may be outlined only with a specific and accurate cognitive assessment. Indeed, we have to be able to highlight intact kinds and levels of cognitive functioning as well as damaged ones, in order to plan a program of cognitive rehabilitation. Moreover, this clinical highlighting must be confronted with theoretical models of cognitive functioning, to understand at what level the disturbance is located.

The brain is by nature highly plastic. In humans, development, rapid leaning, or quasi-spontaneous flexibility toward the environment are perhaps the most striking and visible evidence of this high potential in normal circumstances. In neurophysiological terms and at the macroscopic level, this means that the neural networks sustaining brain functions, although their general skeletons are probably already formed in childhood [28], are constantly modified and reshaped as one goes along the experience [29, 30]. This continuous process allows us to maintain and even improve the quality and the efficacy of our interactions with the environment. The brain is a dynamic evolving entity.

In the case of brain injury, trying to take full advantage of natural brain plasticity is the principle on which cognitive rehabilitation is based. In this context, the notion of plasticity slightly differs since it refers to the capacity to the brain to compensate for lesions. But, in many ways, plasticity induced by the lesion looks like natural plasticity [31]. In this sense, intensive cognitive or behavioral training is thought, at least to some extent, to constrain what the brain does naturally. Findings from natural plasticity studies in animals and humans have demonstrated that the

acquisition of a new skill or the development of a cognitive expertise induced morphologic changes in the brain, sometimes very rapidly, minute-scaled [32, 33]. Furthermore, neuroanatomical reorganizations (i.e., rewiring) have been identified after brain injury in humans [34], facilitating probably the functional recovery [35]. This means that the brain is not hardwired but can be, in some extent, "rewired" [36]. How to help the brain to change or even to create new neural representations to support brain functions following damage is a key issue for cognitive rehabilitation.

The Particular Case of DLGG Patients

Slow-Growing Tumor as a Paradigmatic Model to Study Functional Plasticity

Concerning the particular case of cognitive rehabilitation in DLGG patients, we have to keep in mind the slow-growing and infiltrating character of DLGG, which make their associated cognitive disorders particularly amenable to rehabilitation. Indeed, on the one hand, by infiltrating cortical and subcortical structures, the tumor may destroy some of them but also only displace others, and then residual function may be maintained [3, 37]. On the other hand, by growing slowly, the tumor induces a reactive reshaping and reorganization of functional networks [38]. It is precisely this key feature that may explain why cognitive or neurologic disorders can be more easily compensated as one goes along the disease compared to acute events like stroke [6]. In this respect, neurophysiological studies are really informative. Studies using functional magnetic resonance imaging (fMRI) paradigms have demonstrated different patterns of functional reorganization at the cortical level, showing that the brain recruits alternative areas not previously implied for the expression of cognitive function. Among these functional strategies, ipsilateral, perilesional as well as homonymous contralateral recruitments have been described (for a review, see [6]. In this context, DLGG offers a unique and exciting opportunity to better understand both the dynamics underlying functional plasticity and the neural implementation of cognitive processes, valuable data for cognitive rehabilitation.

Thus, cognitive rehabilitation might on the one hand enhance residual functional capacities and, on the other hand, potentiate the spontaneous functional brain reorganization.

Linking Cognition to Functional and Anatomical Connectivity

The dynamic and holistic organization that assumes functional plasticity finds its corollary in the studies of cerebral connectivity in normal brains. For over than 10 years, new techniques of data analyses more and more sophisticated, from functional and morphologic imaging, have emerged. In this setting, the idea according to which the brain is composed of complex large-scale neural networks became dominant. However, this view was already present in the middle of last century with Daniel Hebb [39] who suggested that high-level human functions are determined by the activity of complex neural networks composed of local and distant areas across the whole brain.

Data from spatial reconstruction of anatomical connectivity by the means of diffusion tensor imaging, a technique that measures the diffusion of water molecules though cerebral tissues, is perhaps the best illustration of this complexity. The visualization of connections between distant brain areas via the multiple white matter bundles (projection or association fascicles, U-shaped fibers) is really demonstrative about this organization [40]. Although these data are by nature anatomic and give no direct functional information, studies using direct electrical stimulations during awake neurosurgery [38], which induce transient disconnection syndrome, have proved their essential role for the complete and normal expression of the functions. In neuropsychology of strokes, injury of these subcortical fascicles can provoke severe cognitive disturbance, hardly compensable [41, 42]. In this context, it has been proposed that these subcortical structures are crucial for functional plasticity [43]. If so, we should find structural changes of these white matter fascicles in reaction to tumors and neurosurgery and

correlate their markers (i.e., fractional anisotropy and mean diffusivity) to cognitive functions. However, to the best of your knowledge, there is for the moment no study with DLGG patients in which structural integrity and changes have been assessed in a systematic manner by the means of a longitudinal design (pre-/postsurgical). As a consequence, this type of structural plasticity remains to be demonstrated in the framework of this brain pathology. Yet, recently, works in the field of the surgery of refractory temporal epilepsy are very interesting in this respect. For example, the team of Duncan [44] has found, using a pre-/postsurgical design, DTI, and language assessment, a correlation between the score obtained in verbal fluency after the surgery and an increase of fractional anisotropy in several regions including subcortical structures such as corona radiata. In other words, these results show that the degree of language recovery is related to structural changes implying some white matter pathways. These provocative data, by demonstrating for the first time that we can call functional subcortical plasticity, open exciting perspectives in the field of brain tumors.

In addition to the fact that DTI can be combined with functional data like cognitive scores, other methods are particularly promising, especially those implying functional connectivity computing, to track the phenomena of plasticity induced by the tumor and its resection. Functional connectivity (FC) is defined as "the correlation between spatially remote neurophysiological events" [45]. This means that temporal statistical interdependencies can be found between several cortical areas composing the neural networks sustaining cognitive functions.

In several studies, abnormality of FC has been correlated with cognitive disorders, demonstrating that temporal desynchronization (hypo- or hyper-synchronization) between distant brain areas is particularly deleterious for functions. It is, for example, the case in neurodegenerative diseases where several and distinct patterns of functional alteration within the different networks can be found and linked to neuropsychological phenotypes (for a review, see [46]. For example, memory loss has been related to FC decrease in Alzheimer's disease [47]. In the field of brain tumor and traumatic brain injury, several works have studied the impact of brain damage on FC (see the Chap. 17). Bartolomei and colleagues [2] have shown using resting MEG (magnetoencephalography) paradigm that synchronization was altered in a population of patients harboring a brain tumor. In a subsequent study, using the same experimental design, cognitive disturbances were shown to be correlated to abnormality of FC [48]. Very recently, FC-based resting MEG at the level of the tumor was evaluated before brain surgery. It was found that decrease resting-state FC was highly predictive to the lack of functionality of this region as evaluated by the means of direct electrical stimulation during awake surgery, suggesting that FC is a good measure of the integrity of brain functions [49].

In the same vein, patients with TBI showed altered FC [50]. Nakamura and colleagues [51] demonstrated using a resting-state fMRI that just after the injury rsFC was disturbed and that, during recovery, this disturbance tended to normalize. A more recent study showed that cognitive complaints were predictive of altered FC in the default mode network in semi-acute TBI patients [52]. Furthermore, Castellanos and his team [53] have evaluated FC-based rsMEG in a population of TBI patients. Data were recorded immediately following the traumatic event and after a specific cognitive rehabilitation program. The authors found that neuropsychological performances significantly improved after treatment. Interestingly, this cognitive recovery was correlated with the reorganization of neural networks as indexed by the comparison between the pre- and posttreatment. These results suggest that functional recovery is related to the reorganization/reconfiguration of neural networks.

Aims of a Cognitive Rehabilitation

Clinical Aims

Of course, the main goal we aim to reach when proposing a cognitive rehabilitation is to bring the patient to recover a satisfactory level of cognitive functioning. The question is as follows: What is a

satisfactory level of cognitive functioning? We think that there is no unique answer to this question and that it depends on the patient, his personality, and his expectations. Thus, the program of cognitive rehabilitation has to be established taking into account not only the objective assessments of cognitive functioning but also the subjective complaints and the expectations of the patient. In this state of mind, we approve and recommend applying the following proposal, borrowed from Kurt Goldstein's works [54–56], a precursor of great influence in cognitive rehabilitation:

- Recognition of the individuality of patients
- Need for standardized assessments and recognition of their limitations
- Importance of working with the problem of fatigue
- Careful observation of patients' response to the program
- Importance of periodical reevaluations and long-term follow-up
- Need to connect cognitive rehabilitation to personal and socio-professional activities

The relevance of taking into account the patient in his wholeness, and not only in his cognitive functioning, has been confirmed in a more recent study [57].

Moreover, the patient has to be informed about our objectives and how we project to reach them, in order to establish a real therapeutic alliance.

Understanding Functional Network Reshaping to Constrain Brain Plasticity: A New Door to Cognitive Rehabilitation

Taken together, the observations mentioned above suggest that brain injuries impact the functional coupling and integration between distant brain areas and that this alteration can be related to cognitive disorders. However, some results in studies with TBI patients show also that the spontaneous reorganization of neural networks is correlated in some extent with cognitive improvement. More interesting, cognitive rehabilitation with significant functional outcomes helps the brain to reorganize its functional networks [53]. Although these seminal results remain to replicate, they are particularly promising for cognitive rehabilitation. Understanding the dynamic of neural network reorganization and the optimal functional reconfigurations may have several important implications for the elaboration of cognitive rehabilitation strategies (e.g., which networks should be targeted).

If the first studies concern mainly patients with TBI, the field of slow-growing tumors may provide crucial data on how the brain can maintain a functional homeostasis, despite extensible lesions. As mentioned above, cognitive disorders in DLGG patients are classically limited before the surgery, what is particularly challenging for the brain. Through a specific cognitive management, these same patients recover most of the time after the surgery on the condition that functional structures are preserved, especially some elements of subcortical connectivity [58] essential in maintaining functional communication between distant brain areas. Consequently, how the brain changes the typology of its neural networks to continue sustaining brain functions despite extensive lesions and resections is an important step in cognitive neurosciences, both at the fundamental and clinical level.

In this setting, coupling brain functional and anatomical connectivity analyses with neuropsychological assessments in a pre-/postsurgical experimental design is essential to shed light on the neurophysiological underpinnings of what may be the optimal functional reorganizations. VBM-based and DTI-based analyses, if correlated with cognitive profiles, can provide valuable information on cortical and subcortical structural plasticity and how this latter is related to a good recovery at the behavioral level. Functional connectivity analyses at rest or during behavior tasks are essential to grasp the functional interactions between brain areas within neural networks and their efficiency for cognitive functions. These systematic analyses can allow, after a time, to find neurophysiological markers of optimum recovery.

It could help, at the clinical level, to anticipate, for example, the risks of cognitive decline after the surgery and to plan a priori hyper-individualized cognitive rehabilitation programs adapted to individual functional architectures

identified previously as at risk. It could otherwise incite clinician neuropsychologists/speech therapist to implement, in some patients, preoperative cognitive training to constrain functional networks to reshape in a better way and minimize cognitive disturbances after the surgery. More indirectly, this knowledge could participate to the fine-grained selection of cognitive tasks for cognitive programs, in the extent that they will be known to stimulate the desired functional networks.

Functional Rehabilitation in the Context of DLGG Patient Care: Perioperative Rehabilitation

Postoperative Rehabilitation

In the context of perioperative DLGG patient care, a postoperative cognitive rehabilitation has to be systematically administered to the patient. Indeed, as detailed in our previous Chap. 19 most of the time, the immediate postoperative cognitive assessment (between 3 and 5 days after the surgery) highlights disorders related to the brain area operated on [38] but also related to disconnection mechanisms between functional networks [2]. Immediate postoperative clinical presentations are various and may go from slight disorders to broad impairments in cognitive functioning.

Even if these disorders are mainly transient, due to the postoperative edema and probably to the functional reorganization in progress induced by the surgery itself, which may spoil transiently the functioning of a given functional network, all patients should benefit from a specific and intensive program of cognitive rehabilitation – performed right from their return at home by a speech therapist specialized in this management in order to potentiate spontaneous functional reorganization and thus to recover the best level of cognitive functioning as possible in a short delay.

Apart from "site-specific" disorders (e.g., articulatory or initiation disorders after a resection close to motor planning areas), we may observe different kinds of language and other cognitive disorders. Concerning language, the immediate postoperative assessment may highlight typical clinical presentations of various types of fluent and nonfluent aphasia. In any case, patients present always with slowness in information processing (i.e., increase of reaction time), associated with attention, memory, and executive functioning disorders.

Thus, in contact with the professional who managed the inpatient and thus administered cognitive evaluations (see our previous chapter), the specialized speech therapist establishes an individualized program of rehabilitation, depending on cognitive disorders highlighted by the immediate postoperative evaluation.

In all cases, the program of cognitive rehabilitation will be *intensive* and *specific*, at least during the three months following surgery. As much as possible, one should plan five sessions weekly, of which duration will depend on patient fatigue.

This program will first focus on the most prominent disorders observed. For instance, in the case of an akinetic mutism due to resection of the supplementary motor area, we will retrain intensively motor initiation of speech. But simultaneously, we will propose to the patient compensation training and retraining of attention, memory, and executive functions skills. Indeed, clinical studies [13] showed that improving the functioning of other cognitive functions may enhance the recovery of a given damaged function. This assertion is easily explainable by the fact that all cognitive functions interact with each other and that an efficient cognitive processing is possible, thanks to the involvement not of isolated brain areas but of parallel and distributed cortico-subcortical networks.

Alternatively, it is conceivable that, for some patients, additional therapies may be added. There is a growing literature on the use of transcranial magnetic stimulation (TMS) or transcranial direct current stimulation (TdCS) to improve functional outcomes, notably in patients with focal lesions like strokes. Some beneficial effects have significantly been obtained for motor, language, and visuospatial functions (for a review, see [59, 60] even if further controlled studies are needed to assess the actual effects of these techniques. The basic principle is always the same: inhibiting some cerebral areas to help the brain to reach a better

recovery strategy. For example, it has been demonstrated in patients with nonfluent aphasia that the recruitment of right hemisphere, especially at the level of inferior frontal gyrus (iFG), was a poor predictor of efficient language recovery. This "overactivation" has been analyzed not as a mechanism of functional plasticity but as a secondary effect of transcallosal disinhibition from the left damaged hemisphere. Therefore, in these patients, TMS have been applied over the right inferior frontal gyrus with some degree of success on language processes, leading to a best recovery [61].

From this perspective, given that tumors impact on functional connectivity and integration in patients, especially in those presenting with cognitive disorders, it could be interesting to cumulate TMS sessions and cognitive stimulations to constrain the brain to reshape neural networks that have been identified previously as dysfunctional. TMS may have a relative long-term effect on the functionality of a given brain region. In this state of mind, it is reasonable to think that the transitory inhibition of dysfunctional networks during cognitive stimulations might help the brain to recruit alternative networks, to remap its connections in an alternative manner, and thus to accomplish the cognitive processes required to success the tasks during cognitive training. This may be also a good strategy for some patients before surgery.

Ultimately, it is acknowledged that some types of pharmacotherapy may have a positive effect on cognitive functioning by modifying functional connectivity previously demonstrated as abnormal in patients with neuropsychiatric disorders [62, 63], stroke [64, 65], and dementia [66]. Even if we think that cognitive programs must be the first therapeutic option, these findings have to be taken into account.

As mentioned above, the aim of the program of cognitive rehabilitation will be the best recovery as possible of cognitive functioning, following patient expectation. Thus, periodical (twice a year) reevaluations of cognitive functioning and subjective complaints will be administered to the patient.

The end of cognitive rehabilitation will be decided, in common, with the therapist and the patient, when both of them will consider that the best level of QoL is reached, thanks to cognition recovery and/or, eventually, to the use of compensatory strategies (e.g., use of external aids, mnemonics, diaries, computer, prevention of distractions, adaptation of the environment).

It is worth noting that the therapist has to listen the complaints of the patient. Indeed, even if cognitive evaluation highlights normative scores, he has to consider the level of satisfaction of the patient regarding his QoL, including social and familial relations, return to work, and personal activities.

Toward a Preoperative Rehabilitation?

In some cases, depending on the results of the preoperative cognitive evaluation and on the location and the volume of the tumor, it may be useful to propose a program of cognitive training to DLGG patients before surgery, in order to constrain functional networks to reshape in the best way and to prevent cognitive disturbances that may occur after surgery – in such a way that postoperative recovery might be the more complete and rapid as possible, by preparing the functional networks that will be the most suited to sustain cognitive functioning.

Functional Rehabilitation in the Context of Longitudinal Follow-Up

Of course, cognitive rehabilitation is not only intended for patients who undergo a surgical management. It has to be considered also in the setting of longitudinal follow-up, depending on the results of periodic cognitive assessments and complaints inventory, with the same aims as those mentioned above, that is, to allow patients maintaining the best level of QoL as possible.

Conclusion: Toward a Hyper-Individualized Approach of Cognitive Rehabilitation

Cognitive rehabilitation is an essential step in the clinical care of patients with brain injury. In the case of DLGG surgery, it helps the patients to

reach its presurgical status and, even sometimes, to improve it and to resume most quickly as possible a normal socio-professional life. To be more efficient as possible, functional rehabilitation in DLGG patients has to be led keeping in mind both the interconnectivity of cognitive functions as well as the brain functional organization in parallel and distributed interconnected networks. The slow-growing and infiltrating character of DLGG makes their associated cognitive disorders particularly amenable to rehabilitation. Its main goal is to enhance residual functional capacities, to potentiate spontaneous functional brain reorganization, and even to constrain some patterns of neural functional reshaping by proposing specific programs of rehabilitation to each patient – depending on the results of cognitive assessments and complaint inventories, in order to allow them recovering the best level of QoL. Neuropsychological profiles have to be precisely delineated before and after the surgery in a systematic manner. By this method, we will be able in the future to better understand and anticipate at the individual level the risks of cognitive surgery aftereffects. These predictive data will help us to put forward hyper-individualized strategies of cognitive rehabilitation and also to build more adapted tests for awake surgery, especially for high-order cognitive functions.

Advances in cognitive neuroscience will be probably crucial in this clinical endeavor. Brain plasticity sciences have extensively learned from clinic of DLGG. By inducing impressive functional reshaping, DLGG can be seen as an exemplary paradigm to study and understand the principles that govern both functional plasticity and anatomo-functional organization of neural networks underlying cognitive functions. In a modern view, higher-order cognitive functions are thought as the result of sophisticated interactions between complex long-range and distributed cortico-subcortical neural systems. It is precisely this holistic organization that might explain the important functional reorganization phenomena in the case of slow-growing lesions such as DLGG. In this setting, the major development of data analyses from functional and morphological imaging, by extracting more and more precisely the temporal and spatial characteristics of such neural networks, will open new doors to cognitive rehabilitation. Indeed, coupling multimodal imaging with fine-grained cognitive evaluations in a pre-/postsurgical design will allow determining the efficiency of functional reconfigurations, so that we can target more specifically the networks to be stimulated by intensive cognitive therapies. In this state of mind, seminal works on functional connectivity in the field of brain pathology, particularly with patients with strokes, traumatic brain injuries, or brain tumors, have offered interesting results. It is well known that brain injuries alter functional links between distant and local brain areas and that this alteration can be correlated to cognitive disorders. We also know that the efficiency of cognitive rehabilitation programs may induce a reorganization of functional connectivity. Given that DLGG patients show little or sometimes no cognitive disorders, this patient population appears to be of major interest to reveal the neurophysiological underpinnings of efficient brain plasticity mechanisms across the whole brain.

In a translational context, other approaches seem to be interesting to improve cognitive functioning. Some studies have, for example, shown with some degree of success that the use of transcranial magnetic stimulation or transcranial direct current stimulation, two noninvasive techniques, was promising for helping to recover from cognitive disorders. In the context of neurosurgery, while cognitive presurgical status is doubtless a marker of postsurgical recovery, this approach could be particularly interesting for some patients who present with a cognitive decline and lack of plasticity before surgery. In this sense, coupling this therapy with intensive cognitive training could allow to take full advantage of neuroplasticity by constraining functional networks to reshape and minimize the risk of postoperative disorders. However, further controlled studies are needed to assess precisely the effects of these techniques.

Functional rehabilitation will probably take advantage of the rapid advances in cognitive neurosciences which will help to a better care of the patients, notably in the DLGG population.

The treatment of cognitive disorders is a specific and accurate exercise that requires an individualized approach that is time-consuming. Its efficacy is guaranteed by expert clinicians with large knowledge in clinical neurosciences. In this state of mind, it requires an institutional structuration to allow a maximal efficacy. It means that medical institutions should develop specific departments dedicated to cognitive rehabilitation.

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31 Early Detection and Management of Incidental Diffuse Low-Grade Gliomas (IDLGG): Toward a "Prophylactic Oncological Neurosurgery"

Hugues Duffau

Abstract

Maximal resection is the first treatment to propose in DLGG, since allowing a delay of malignant transformation with a significant increase of overall survival. The use of intraoperative mapping enabled a minimization of the postsurgical morbidity while increasing the extent of resection. On the basis of these new concepts of "functional neurooncological surgery," the future step will be to evolve toward a "preventive neurosurgery" in incidentally discovered DLGG. Indeed, because of the development of noninvasive MRI, one can observe an increased number of incidental DLGG (IDLGG) for unrelated complaints (e.g., headaches, traumatic brain injury) or research studies. In the current literature, the incidence of IDLGG ranges from 0.025 to 0.3 %, and its prevalence is evaluated about 0.05–0.2 % in the healthy population.

The natural history of IDLGG was recently analyzed, showing that it was not a benign tumor, but an entity that will grow in all cases (with a growth rate very close to that of symptomatic DLGG), and with a risk of malignant transformation ultimately leading to the death. Thus, because IDLGG likely represents an earlier step in the natural course of a glioma than the symptomatic DLGG, their behavior supports the idea that they should be managed as symptomatic DLGG. In this state of mind, several authors proposed to achieve surgical removal of DLGG diagnosed in asymptomatic patients. The rate of total and supratotal resections was improved in comparison with surgical series of symptomatic DLGG, while

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the rate of permanent deficit was very low, even in critical areas. Interestingly, microfoci with endothelial proliferation were identified in the middle of the tumor in around a quarter of the cases, demonstrating that beginning of malignant transformation may occur before any symptoms. Therefore, these data plead in favor of an early surgical treatment in asymptomatic patients, to maximize the extent of resection before glioma growth and migration, even in eloquent regions. In summary, the similarity of the natural course of DLGG during the silent and symptomatic periods supports "prophylactic" surgical resection of IDLGG.

Keywords

Incidental DLGG • Early detection • Surgery • Supratotal resection • Overall survival • Malignant transformation • Extent of resection • Functional mapping

Introduction

In previous paragraphs, it was extensively demonstrated that maximal resection was currently the first therapeutic option to consider in DLGG, as recommended by the European Guidelines [1], because allowing a significant delay of malignant transformation with a significant increase of the overall survival [2–4]. In addition, the use of intraoperative mapping enabled a minimization of the postsurgical morbidity, even in eloquent areas, while increasing the extent of resection [5, 6]. Therefore, the resection should be functional mapping guided, with no margin around the crucial structures, i.e., with a tumor removal pursued until functional areas have been encountered *and not before* [7, 8] – even for DLGG located within non-eloquent areas, in order to perform a supratotal resection [9].

On the basis of these new concepts of "functional neurooncological surgery," the future step will be to move toward a "preventive neurosurgery" in incidentally discovered DLGG. Indeed, due to the development of noninvasive MRI, an increased number of IDLGG is discovered for unrelated complaints (e.g., headaches, traumatic brain injury) or research studies [10–13]. In the current literature, IDLGG incidence ranges from 0.025 to 0.3 % [10–12, 14, 15]. Its prevalence is evaluated about 0.05–0.2 % in the healthy population, e.g., 1 case in a population of 2,000 healthy volunteers [16] or 2 cases in a population of 1,000 healthy volunteers in whom MRI was performed for investigational protocols [14]. In a series of 4,309 gliomas from the French Brain Tumor Databank, Bauchet et al. [17] reported a 3.0 % rate (130 cases) of incidentally diagnosed gliomas. This rate is in agreement with Olson et al. [18] who observed 5 cases of IDLGG from 105 grade II oligodendrogliomas and mixed gliomas (4.7 %), with Kamiguchi et al. [19] who reported 9 cases from 185 gliomas (4.9 %) as well as with Pallud et al. [20] who described 47 asymptomatic DLGG from 1,296 WHO grade II gliomas (3.8 %).

In this new setting, which is based on an increased frequency of MRI, the probability for a neurosurgeon to encounter IDLGG will likely continue to increase in the near future. As a consequence, it is time to raise the question of management of IDLGG.

Characteristics and Natural History of IDLGG

In recent studies specifically dedicated to IDLGG, it was shown that this entity represented a distinct population from its symptomatic counterparts, in that there was a female predominance, with younger patients; a significant lower volume of the tumor; a lower rate of contrast-enhancing

lesions; and a lower frequency of gliomas involving eloquent brain areas [20, 21].

The natural course of DLGG incidentally discovered on imaging was recently analyzed for the first time in the literature [20]. This series showed that incidental DLGG was a progressive tumor in all cases (median velocity of diametric expansion around 3.5 mm/year, i.e., very close to the growth rate of symptomatic DLGG), leading to clinical transformation toward symptomatic DLGG at a median interval of 48 months after radiological discovery. Moreover, almost one-third of patients (14 among 47) presented malignant transformation at a median interval of 5.7 years after radiological discovery, and 4 patients died at a median interval of 8.9 years after radiological discovery. Thus, these data demonstrated that even small IDLGG is not a benign tumor, but an entity that will grow in all cases, with a risk of malignant transformation ultimately leading to the death of the patients. In other words, because IDLGG likely represent an earlier step in the natural history of a glioma than the symptomatic DLGG, their behavior supports the idea that they should be managed as symptomatic DLGG [20].

Surgical Management of IDLGG

Oncological Results

Based on the better understanding of natural course of IDLGG, several authors have recently proposed to achieve surgical removal of DLGG diagnosed in asymptomatic patients. In the series by Pallud et al., resection was performed in 57 % of patients, with a higher rate of gross total resection in comparison with a control group of symptomatic DLGG as well as a better OS [20]. In the same state of mind, the UCSF group demonstrated that patients operated on for incidental DLGG were more likely to undergo gross total resection (60 % vs. 31.5 %, $p=0.001$) and had improved OS on Kaplan-Meier analysis ($p=0.039$, Mantel-Cox test) with a mean follow-up of 5.1 years [21]. In a third experience by Duffau [22], beyond the fact there was no partial resection in the subgroup of IDLGG (no residual volume more than 10 mL), it was possible to perform not only a total resection in 36 % of cases but also a supratotal resection in 27 % of cases, whereas tumors were located in eloquent areas (Fig. 31.1). This issue is crucial because a supratotal removal (i.e., the resection of a margin around the tumor visible on FLAIR-weighted MRI) may prevent malignant transformation in LGG [9]. Such results are much better as compared with a series of 115 symptomatic patients who underwent surgery by the same author for a DLGG involving language areas, since only 32 % of resections were complete or supratotal (with 17 % of partial resections) [23]. In summary, in all surgical series of IDLGG, a greater rate of gross total resection was achieved in IDLGG in comparison with a control group of symptomatic LGG.

It is likely that such high rate of maximal resection in IDLGG was made possible, even in critical areas (see below), because the tumor volume was smaller. In a previous personal surgical series of symptomatic LGG [23], whatever the location of the tumor, the preoperative volume was 55 mL, whereas the mean volume was 32.6 mL in the recent experience with asymptomatic patients [22]. Such a lower volume of IDLGG in comparison with symptomatic DLGG is in agreement with the two other series on IDLGG [20, 21]. This argument is an additional one supporting that IDLGG likely represent an earlier step in the natural history of symptomatic LGG. It is also worth noting that, in the experience by Duffau [22], one microfocus with endothelial proliferation was identified in the middle of the tumor in 27 % of cases, demonstrating that beginning of malignant transformation may occur before any symptoms. Last but not the least, Pallud et al. [20] as well Potts et al. [21] demonstrated that patients operated on for IDLGG had improved overall survival in comparison with a control group of symptomatic DLGG ($p=0.039$, Mantel-Cox test, with a mean follow-up of 5.1 years in the University of California, San Francisco, experience) [21]. Therefore, taken together, these data plead in favor of an early surgical treatment in asymptomatic patients, to maximize the extent of resection before glioma growth and migration.

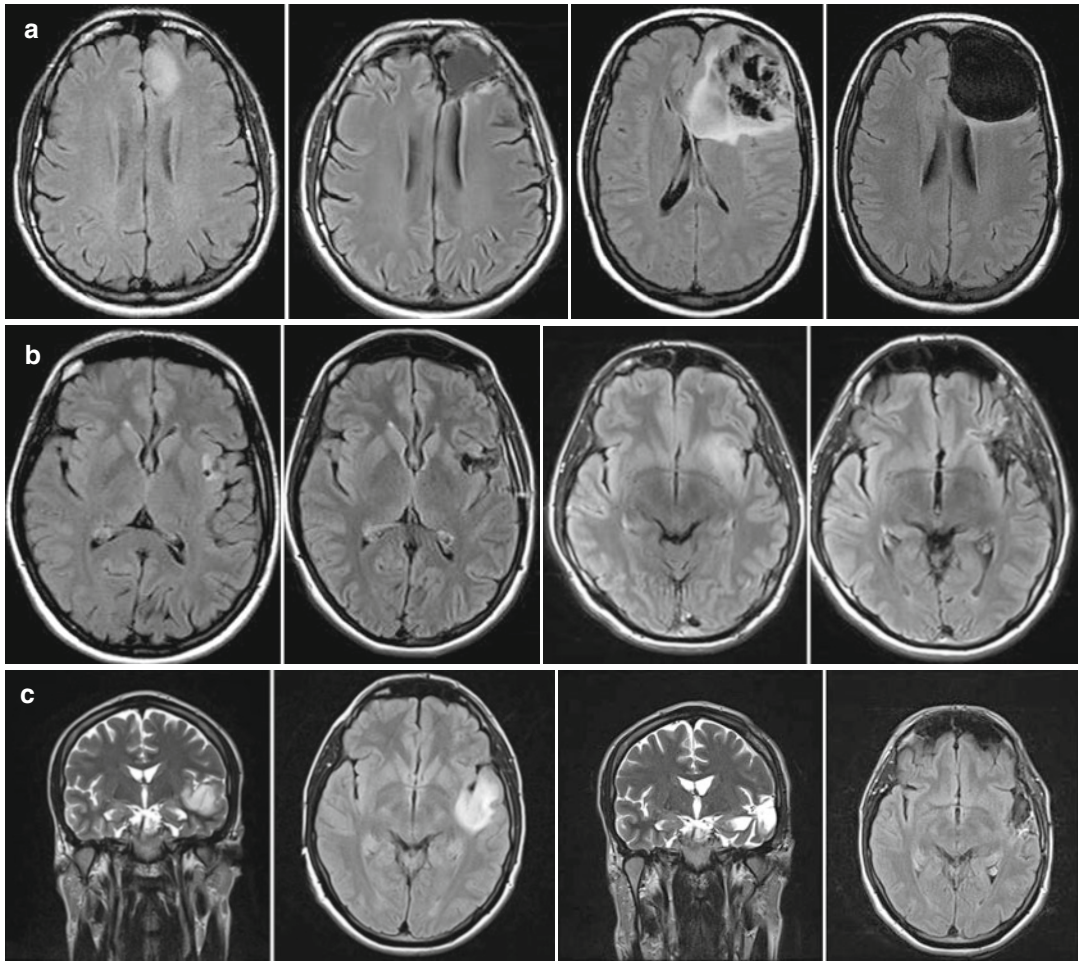

Fig. 31.1 Illustrative cases of total or supratotal resections for IDLGG involving the left dominant hemisphere: (**a**) Two examples of left frontal IDLGG. (**b**) Two examples of left insular IDLGG. (**c**) One example of left temporo-insular IDLGG

Functional Issues

Even if there is a lower frequency of IDLGG within functional structures compared with symptomatic DLGG (thus making surgery easier) [20, 21], it was nonetheless demonstrated that early and maximal resection was also possible for IDLGG involving eloquent areas, especially in the left dominant hemisphere (Fig. 31.1) [22]. This was made possible, thanks to the use of intraoperative mapping in awake patients, since direct stimulation enables the achievement of an optimal resection according to functional boundaries and not according to "oncologic" limits [5, 6, 23]. A transient neurological worsening occurred in 63 % of cases after resection, especially with respect to language function. Such results are not surprising due to the absence of margin between functional cortico-subcortical structures and the edge of the surgical cavity [24]. However, all patients recovered their preoperative neurological status within a few weeks after surgery thanks to a specific functional rehabilitation, and they returned to a normal social and professional life (Karnofsky Performance Status score of 100 in all cases, no seizures, no antiepileptic drugs following surgery). Thus, these results demonstrate that surgical resection of incidental DLGG can be performed with a high rate of preservation of quality of life in asymptomatic patients, despite the involvement of critical

areas such as the left insula or the left frontal lobe (including the inferior frontal gyrus, i.e., the so-called Broca's area).

Conclusions and Perspectives

These original findings support what was recently suggested by Kelly [25], i.e., that it could be important to find (incidental) DLGG when they are small, before they turn malignant, and when they may still be "curable" by some minimally invasive surgical method – because it is much easier and safer to operate on a small lesion. Indeed, the three surgical series on IDLGG in the current literature have shown that (1) surgery was possible with a low rate of morbidity, even in critical areas, thanks to the use of intraoperative mapping; (2) the extent of resection was significantly greater in IDLGG in comparison with symptomatic DLGG; (3) with a significantly longer overall survival in IDLGG in comparison with symptomatic DLGG.

Of course, it seems reasonable to give the patient time to think after the incidental diagnosis and before surgery. During this period, it is recommended to perform (1) an extensive neuropsychologic assessment, as already advised in symptomatic DLGG [26] (several DLGG patients may have cognitive disorders that usually are not detected during a standard neurologic examination; see Chap. 19 by Moritz-Gasser and Herbet); (2) a preoperative functional as well as metabolic neuroimaging (see Chap. 20 by Bizzi and Chap. 15 by Guillevin); (3) and finally a second MRI approximately 3 months after the first one – which allows the calculation of the velocity diameter expansion, i.e., a very reliable prognostic marker in DLGG [20, 27].

Finally, because the growth rate is constant during the initial presymptomatic period, it is possible to extrapolate backward in time, leading to approximate the glioma date of birth in early adulthood, as recently demonstrated [28, 29]. This method might enable to better understand the origin of DLGG and thus to screen the best population for an early MRI detection, for instance by focusing on young people between 15-year-old (early adulthood) and 40-year-old (because more than one-half of symptomatic patients are less than 40-year-old at time of diagnosis) [2, 23]. Indeed, as mentioned, if the prevalence of incidental DLGG is currently estimated around 0.05–0.2 % in the healthy population [14, 16], their early detection should be increased as access to MRI broadens worldwide and since surgical resection could be rapidly proposed following the radiological diagnosis. Such an early detection could also allow a better knowledge of the origin of glioma, especially regarding the relationships between initial tumor location (brain areas involved, contact with the subventricular zone and/or cortex) and molecular biology [30–32] (see next Chap. 32 by Gozé et al.).

In summary, the similarity of the natural course of DLGG during the silent and symptomatic periods and the favorable oncological and functional surgical outcomes should lead neurosurgeons to evolve toward a personalized "prophylactic" resection of IDLGG, with the aim of preventing histological upgrading while offering to the patient a normal and prolonged quality of life [33].

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32 The Origins of Diffuse Low-Grade Gliomas (DLGGs): "Functional Theory" Versus "Molecular Theory"

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Abstract

The improved understanding of the natural course of DLGGs has allowed a paradigmatic shift in their management from a "wait-and-see" attitude to an early, individualized, and dynamic therapeutic strategy. However, optimization of this management implies to better understand the origin of DLGG. First, because its growth rate is constant during the initial presymptomatic period, it was possible to extrapolate backward in time, leading to approximate the glioma date of birth in early adulthood. Another way to improve our knowledge about the mechanisms of DLGG genesis is

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to study their spatial distribution. Indeed, this tumor has preferential cerebral locations, especially in the supplementary motor area or the insular lobe – while occipital DLGG is very rare. On the basis of strong relationships between DLGG development and the eloquence of brain regions frequently invaded by this tumor, we propose a "functional theory" to explain the origin of DLGG. In addition, anatomo-molecular studies showed significant correlations between the DLGG locations and tumor genetics, especially with a lower rate of 1p19q codeletion in the insula. Here, the ultrastructural mechanisms of such "molecular theory" based on a cortical origin of DLGG will be reviewed. Finally, future directions such as potential interactions between environment–brain function–tumoral genes, the study of genetic susceptibility variants in DLGGs and the possible role of hormones in their etiology will be discussed. These crucial issues illustrate very well the close relationships between the pathophysiology of gliomagenesis, the anatomo-functional organization of the brain, and the personalized management in DLGG patients.

Keywords
DLGG • Anatomo-molecular correlations • Eloquent areas • Brain–tumor interactions • Ultrastructural mechanisms • Extracellular matrix • Oligodendroglial progenitor cells • Subventricular zone

Introduction

In the previous chapters, it was extensively demonstrated that the improved understanding of the natural course of DLGGs has allowed a paradigmatic shift in their management from a "wait-and-see" attitude to an early, personalized, and dynamic therapeutic strategy. However, optimization of this management implies to better understand the origin of DLGG. As already mentioned, because its growth rate is constant during the premalignant symptomatic period [1] as well as during the initial presymptomatic period [2], it was possible to extrapolate backward in time, leading to approximate the glioma date of birth in early adulthood (around 20 years of age) [3]. In other words, it seems that DLGG arises more likely "ex nihilo" rather than from a preexisting congenital lesion. Interestingly, recent refinement of the biomathematical model, based on a differential equation describing the diffusion–proliferation process, has enabled the identification of two types of DLGG: the first corresponds to very slowly growing tumors that appear during adolescence, and the second type corresponds to slowly tumor that appears later, during the young adult period [4]. This means that the genesis of DLGG is complex, with likely different subgroups, which can explain the heterogeneity of this entity and the need to tailor the sequences of treatment to each patient. To this end, the aim of this chapter is to review the possible mechanisms underlying tumorigenesis.

DLGGs Have Preferential Brain Locations

One way to improve our knowledge about the mechanisms of genesis of DLGG is to study their spatial distribution. Indeed, it seems that this tumor has preferential cerebral locations. A first report showed a frequent involvement of the supplementary motor area (SMA) (27.3 %) and insular lobe by DLGG (25 %), with a significant difference when compared with de novo glioblastomas, suggesting a possible different origin between these two kinds of gliomas [5]. This preliminary

observation was confirmed by a study demonstrating a higher rate of DLGG in anterior regions of the brain [6]. On the other hand, it is puzzling to note that this kind of tumor scarcely involves the occipital lobe. In a large consecutive series of DLGG recently reported by the UCSF team, on 281 patients, only two had an occipital tumor involving visual regions (i.e., 0.71 %) [7]. The results are almost similar in our consecutive experience with about 400 DLGGs, since only six patients had an occipital glioma (i.e., 2 %) [8] – in agreement with our preliminary data showing 0.75 % of occipital DLGGs in the previous series of 132 GIIG reported in 2004 [5].

However, in these reports, the spatial classification was based on cerebral lobe or gyri. Therefore, this kind of classification lacks accuracy. More recently, we proposed a novel statistical approach to infer position of DLGG using a graphical model and to build a graph based spatial position mapping [9, 10]. Interestingly, the previous findings have been confirmed, with very few tumors located in the occipital and prefrontal lobes (7 %), while we found (symmetrically) the higher amount of DLGG around the insula/paralimbic system (27 % in the right hemisphere and 33 % in the left hemisphere) from 95 patients.

The "Functional Theory"

Several hypotheses may be considered to interpret the reasons for such "preferential" brain locations of DLGG, such as the paralimbic system – in comparison with their "non-preferential" brain locations, such as the occipital lobe.

First, the cytoarchitectony of the visual cortex is not the same, since the insula is constituted by a mesocortex, making a link between the allocortex and the neocortex [11]. Furthermore, regarding chemocytoarchitectony, dedicated studies demonstrated a lightly stained region within the insular lobe, with an intermediate profile between that of a primary area and a high-order association area [12].

In the same vein, the insula, as well as the SMA, represents a functional interface between the limbic system (mesiotemporal structure and cingulum) and higher-order supra-modal areas (such as the temporal pole or the prefrontal regions). On the contrary, the occipital lobe is not a "transitional" area, and it does not make a link between the limbic system and the neocortex. Moreover, from a functional point of view, both insula and SMA play a role in planning of movements and language [11, 13], while the occipital lobe is not a region involved in planning. As a consequence, it could be hypothesized that interactions between neurons and glia are different in the SMA and insula in comparison with the occipital lobe. Indeed, glial cells are known to play a role (1) in neuronal migration, which may explain the existence of migration disorders in some cortical epilepsy, including the extratemporal epilepsy that often originates from the SMA and insula – but rarely from the occipital region; (2) in the regulation of synaptic transmission; (3) in the control of synapse numbers; and (4) in the energy metabolism of the neuron, explaining the neurovascular and metabolic decoupling in gliomas.

Therefore, if we consider that the insula/SMA and occipital lobe have different structural and functional profiles, some repercussions concerning the biology of the local glia cells are likely. For example, recent neuroimaging studies using voxel-based morphometry in healthy volunteers showed that learning could generate a significant increase of gray matter volume in areas specifically involved in tasks extensively repeated [14]. It was suggested that proliferation of glial cells might be a mechanism underlying such structural changes in the eloquent regions recruited by the task [15]. Thus, we might suggest that such modifications in the local glial properties may favor (or may prevent) the development of DLGG in some specific brain locations.

More recently, it was also demonstrated that NogoA, which regulates axonal fiber growth and regeneration, is mainly expressed by oligodendrocytes but also by subpopulations of neurons, in particular in plastic regions such as the hippocampus [16]. As a consequence, it seems that this regulator of functional and structural plasticity in mature neuronal networks is heterogeneously secreted throughout the brain.

We call this hypothesis, based on the eloquence of cerebral regions involved by DLGG, the "functional theory."

The "Molecular Theory"

Beyond this "functional theory" based on the fields of cytochemoarchitectonics, developmental and structural–functional relationships, as well as neuron–glia communication, a "molecular theory" can also be hypothesized. Indeed, anatomo-molecular studies showed significant correlations between the location of DLGG and the tumor genetics. For example, DLGG with chromosome 1p-19q loss and IDH1/2 mutation were found more significantly in the anterior part of the brain, especially in the frontal lobe, as compared with non-frontal gliomas such as occipital DLGG [6, 17]. In the same state of mind, we have recently demonstrated the lower rate of DLGG with 1p19q codeletion in tumor involving the insula [18]. This could account for a different genetic behavior in insular DLGGs, with possible clinical implications (see below).

In addition, regarding the gliomagenesis, it was recently suggested that oligodendrocyte progenitor cells could represent the origin of DLGG [19], at the cortical level – and not stem cells from the subventricular zone (SVZ). This is in line with our recent data, showing that all DLGGs had a constant relationship with the cortex, but not with the ventricle [20]. Because DLGGs that came in the contact with the SVZ had a significant larger volume, we hypothesized a centrifugal tumor growth, with a cortical origin of DLGG and a subsequent migration to the ventricle. On the contrary, several studies have suggested a relationship between the human stem cell regions, with particular emphasis on the SVZ, and the development of high-grade gliomas [21, 22] (see below). Our data could therefore suggest a different cellular origin of DLGGs compared with de novo high-grade gliomas, in accordance with preliminary biological results obtained in animal models [23]. These findings may explain the radically different natural history of low-grade versus high-grade glial tumors as well as for the differences retrieved in molecular patterns of de novo GBMs and secondary GBMs. Indeed, it is worth noting that we also observed a distinct genetic pattern in non-insular DLGG coming into the contact of the SVZ in comparison with non-insular DLGG not in contact of the SVZ, with a significant lower rate of 1p19q deletion in the latter [20].

In this hypothesis of a cortical origin of DLGG, the microstructural organization and eloquence of each cortical area might play an important role, making the link with the "functional theory" and providing possible explanatory mechanisms enabling to better understand why some brain regions such as the insula/SMA are "preferential" locations of DLGG.

The Cellular and Ultrastructural Mechanisms

The context influences deeply the way of considering the relationship between functional and molecular theory. In the healthy brain, these interactions could be thought as different scales of integration (global level or individual level) of pieces devoted to cooperate and to create something benefic for brain functioning. Individual cells, with their own molecular characteristics related to the lineage they belong to, group them into functional networks in order to execute a particular function depending on the cerebral location. The function will condition the interactions between the different kinds of cells and will organize their spatial relative distribution. The system is able to adapt to changes required by the function implementation needs.

When tumor arises, the equilibrium is disrupted. Genetic changes occurring in a cell transform it in the cell of origin of cancer. The transformed cells are devoted to multiply and to spread. Tumor cells do not develop in isolation but coevolve with stromal cells and tumor-associated immune cells in a tumor microenvironment mediated by an array of soluble factors, forming a complex intercellular signaling network. The tumor diverts the environment out of its functional mission in order to fulfill its growth

need. A perverted cooperation becomes established between tumor cells and the surrounding ones. On the confrontation of the elements involved in this interplay, that is, molecular characteristics of the origin cancer cell (molecular theory) and microenvironmental features shaped by function dedicated to the involved brain area (functional theory), depends the successful development of DLGG resulting in function destruction and tumor expansion.

Functional and Molecular Theories at the Cellular and Molecular Levels

The functional theory relates to the influence of microenvironment or stroma on the tumor development, whereas the molecular theory refers to tumor molecular patterns determined by the identity of cell of origin of cancer and the genetic changes these cells underwent.

Similar to tumors in other organs, brain tumors are composed of an evolving ecological community composed of nonneoplastic and preneoplastic/neoplastic cells. The environment of the brain is highly specialized and consists of blood vessels, extracellular matrix (ECM), microglia, progenitor cells, mature oligodendrocytes, astrocytes, and neurons. The neurofibromatosis type 1 (NF1) provides a convincing example of necessary interplay between stroma and cells harboring cancer-initiating genetic events. This frequent inherited genetic disease of the nervous system results in the development of benign neurofibromas that can progress, in 15–20 % of cases, to brain tumors mostly prevailing in the optic pathway. Zhu et al. [24] recapitulated NF1-associated brain tumors in *NF1* genetically engineered mice. They demonstrated that both events were required to obtain brain tumors: nullizygous ($NF1^{-/-}$, homozygous *NF1* gene loss) Schwann cells evolving in a haploinsufficient state ($NF1^{+/-}$ with heterozygosity for *NF1* gene) of the surrounding tissue (mast cells). Their study identifies a non-cell autonomous role for the development of tumors in NF1. The preferential spatial pattern of NF1-associated gliomagenesis (optic chiasm) raises the possibility that tumor birth is defined by the presence of cell types and signals unique to a specific region of the brain during a given time of development, that is, a regional and developmental permissive context in which bi-allelic NF1 inactivation will lead to glioma formation [25].

It is now admitted that surrounding stroma plays an active role in tumor progression. The complex interactions between tumor and some of the composing elements of its microenvironment have been described fast exclusively for high-grade diffuse glioma. We have very little knowledge of what happens specifically in DLGG, but low-grade as well as high-grade gliomas develop in a comparable brain parenchyma. Thus, it can be hypothesized that characteristics of the main components of tumor microenvironment involved in tumor–stroma interplay remain the same in both cases, at least in the beginning. These steady features of surrounding stroma allow considering that accumulated knowledge for high-grade gliomas in this field could be extrapolated to low-grade gliomas.

One of the major players involved in the stroma supporting role in tumor spread is microglia. Microglial cells are an abundant portion of the healthy central nervous system cell population. Gliomas have been shown to accumulate many microglial cells along with a small population of lymphocytes. This accumulation is thought to be due to the local production of chemo-attractants and growth factors by glioma cells. Microglial cells are in turn a cellular source of matrix metalloproteases (MMPs), ECM-degrading enzymes. Even if the legitimate function of these enzymes is to allow microglial cells to infiltrate tumor in order to play their immune gatekeeper role, the MMP release into the tumor environment may similarly help the glioma cells to easily invade the surrounding parenchyma and thus assist its growth. Furthermore, microglial cells secrete tumor proliferation promoting factors including epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF). These findings suggest that microglia have an interactive role in the tumor progression by supporting at once migration (MMP), angiogenesis (VEGF), and proliferation of glioma cells. Thus, microglia

are intended to fight against glioma but its function seems to be overcome [26, 27].

A first evidence of distinct microglia phenotypes in various regions of healthy adult brain has been demonstrated in mice. Microglia isolated from the striatum, hippocampus, spinal cord, cerebellum, and cerebral cortex of healthy young adult mice were analyzed for regional diversity in a panel of markers expressed in control or inflammatory conditions. Qualitatively, they were not different but expression levels were markedly distinct on microglia derived from different brain regions [28]. These data indicate that the phenotype of microglia might be shaped by region-specific microenvironmental signals. If such findings can be extrapolated to human brain, this regional diversity might be of relevance in the microglia–tumor interaction issue.

Other cell–cell interactions have been described between glioblastoma cells and stromal cells, but these cues remain poorly understood in DLGG. Most glioblastomas secrete chemokines which frequently results in the recruitment of inflammatory cells within tumor. In the same way, glioma-secreted motility factors are responsible, in part, for the phenomenon of neuronal progenitors homing to tumor. The last ones release PDGF which has a potent oncogenic effect on glioma cells. Tumor cells as well as oligodendrocytes product autotaxin (ATX), a motogenic protein. This protein reduces oligodendrocyte adhesion during the initial stages of myelinization. Glioma-derived ATX may provoke the de-adhesion of oligodendrocytes all along the path of invading tumor cells [29]. Oligodendrocyte-rich white matter tracts are one of the preferred conduits of invasion by glioblastoma but also by DLGG.

Phenotypic cell heterogeneity observed in high-grade glioma, in advanced stages, can be related to the presence of numerous different cell types infiltrated in the growing tumor mass, where they have been attracted by chemotactic cues: endothelial cells, pericytes, fibroblasts, inflammatory cells, microglia, and neuronal precursors. But another source of tumor cell heterogeneity could be due to glioma cell ability to fuse with no transformed cells of the surrounding stroma. Mercapide et al. reported an in vitro homotypic fusion of cells from U87-MG glioblastoma cell line with cocultured fibroblasts. The cells formed by fibroblast/glioma cell recombination were polyploid, exhibited long-term viability, and demonstrated phenotypic characteristics distinct from the parental tumor cells [30]. These in vitro results indicate that phenotypic modifications might be intrinsically bound to the biology of glioma cells on the basis of their fusogenic propensity. In vivo results reported by Clavreul et al. might be also related to the ability of glioma cells to promote emergence of specific cells around the tumor [31]. These authors recently reported isolation from patients' brain of a new stromal cell population surrounding glioblastoma. These cells are diploid and exhibit phenotypic and functional properties in common with the cancer-associated fibroblasts described in the stroma of carcinoma, despite brain parenchyma contains a very low proportion of fibroblasts. The authors named these cells GB-associated stromal cells. Their origin remains elusive at now. These cells have tumor-promoting effects [31]. But it is completely unknown if such mechanisms exist also in DLGG.

Glioma cells are highly invading cells. Their invading power is conditioned by multiple interactions with ECM. The ECM in normal brain is composed principally of hyaluronan, proteoglycans, tenascin-C, and thrombospondin. Because of the paucity of fibrillary collagen, fibronectin, and laminin, ECM in the brain has a softer consistency compared with other systemic organs. In primary brain tumors, hyaluronan and other ECM components are upregulated within the tumor stroma. Tenascin-C is increased at the same time as tumor grade is increasing; this ECM component is predominately localized to blood vessels walls [32]. The ECM qualitative composition is then remodeled during tumor spread.

Interactions between ECM and tumor cells are molecular (cytoskeleton–integrin–ECM two-way communication and release of a spectra of varied matrix-degrading enzymes) but also mechanical. Biophysical inputs such as the density, rigidity, and geometry of the ECM have powerful influence on cell fate, migration, and morphogenesis [33].

As demonstrated by intraoperative ultrasound, tumors and their surrounding stroma are stiffer than normal brain parenchyma. Normal and tumor brain tissue display contrasting mechanical microenvironments. Assays performed on glioblastoma cell lines demonstrated that increasing ECM rigidity can induce phenotypic changes in human glioma cells with enhanced cell spreading, faster motility, and increased proliferation [34]. Bulk brain tissue has an elastic compliance almost the lower of the matrices tested in the study. These authors envision that glioblastoma cells stiffen their surroundings as they invade. Important anatomic variations of stiffness within the rat brain have been reported [35]. The infiltrative path of GBM cells tends to favor interfaces between mechanically distinct structures such as the basement membrane of blood vessels and white matter tracts. Such invasive features are shared with DLGG.

Watkins and colleagues examined on glioblastoma cell lines whether glioma cells modulated their cell volume during invasion through extracellular spaces in the living brain. After stereotaxical injection of D546MG and U251-MG glioma cells in frontal brain of *scid* mice, they imaged cell invasion [36]. Tumor cells decreased their volume by 30–35 %. They performed assays in three other models, but magnitude of the volume changes did not vary and was independent of the cell size and the barrier size encountered. Thus, it could be hypothesized that this value (30–35 %) represents the lowest readily achievable volume of the cell. This decrease requires cells to release essentially all unbound cytoplasmic water and involves the coordinated efflux of Cl^- and K^+ ions along with obliged cytoplasmic water. The authors provide the first evidence that invading glioma cells modulate their volume to adapt to the spatial constraints that migrating cells encounter in the brain. The magnitude of the cell volume loss being constant, spatial constraints imposed by regional anatomic features depending on and varying with brain locations could impact on invasion ability of tumor cells in the different brain regions.

When comparing molecular patterns of glial tumors, regional differences have been described for a long time between supratentorial and infratentorial gliomas using DNA arrays providing complex profiles: distinct gene expression profiles among pilocytic astrocytomas [37] and whole-genome profiling of pediatric diffuse intrinsic pontine gliomas [38]. In adult supratentorial DLGGs, anatomo-molecular studies established also significant correlations between location and basic molecular patterns. As already mentioned, oligodendrogliomas with chromosome 1p loss were found more significantly in the anterior part of the brain, especially in the frontal lobe [6, 39]. Similarly we have demonstrated the lower rate of DLGG with 1p19q codeletion in tumors involving the insula [18]. Complex genetic patterns remain to be determined in DLGG by using microarrays analyses. The molecular differences yet observed in DLGG have already consequences on outcome of these tumors because prognosis value has been assigned to some of them. In any case, molecular regional differences in DLGG should be linked to the cell they are derived from. Nevertheless, cell of origin and cancer stem-cell concepts are distinct: it is noteworthy that the cell of origin, the normal cell that acquires the first cancer promoting mutation, is not necessarily related to the cancer stem cell, the cellular subset within the tumor that uniquely sustains malignant growth [40].

For diffuse low-grade oligodendrogliomas, several complementary studies performed in transgenic mice marked out oligodendroglial progenitor cell (OPC) as cell of origin of DLGG. At first Persson et al. showed that low-grade oligodendrogliomas in transgenic mice arose from NG2-expressing OPC which also has tumor-initiating potential when injected orthotopically into adult mice: these mice developed tumors within 2–3 months [19]. These results have been further confirmed and elucidated. Normally NG2+ OPC exhibits asymmetric divisions accompanied by an asymmetric EGFR distribution leading to two distinct progenies: NG2+ EGFR + OPC with self-renewing and proliferating properties and NG2- EGFR-OPC with differentiating capabilities moving on to expression of Olig4. Gliomagenesis might be related to a defect in asymmetric cell division of OPC, since asymmetry regulators

proteins are misexpressed in DLGG. Some of the deregulated genes map to chromosome 1p or to chromosome 19q which are very often codeleted in oligodendrogliomas [41].

OPC are widely dispersed in the SVZ and represent a resident population throughout the gray and white matter [19, 42]. White matter tracts in mice are enriched for OPC. In the same way, NG2+ Olig2+cells like OPC are the major cycle-related population of the adult normal brain [43]. OPC, located in white matter tracts, as possible precursors of DLGG is a finding in good accordance with clinical observations reported by Vergani et al.: a systematic cortical involvement in a series of 43 DLGGs suggested a centripetal tumor growth [20]. All these observations reinforce the thinking that DLGG do not arise from neural stem cells from the SVZ [20].

In glioblastomas, stem cells with self-renewing properties have been isolated from tumors. They exhibit many shared features with neural stem cells originating from the SVZ. The SVZ maintains the ability to generate neurons and glia throughout adulthood. In animal models, high-grade gliomas can be generated from this cell population [44]. It has been reported more aggressive patterns of glioblastomas recurrence associated with tumors that contact the SVZ [22]. It was suggested that cells responsible for gliomagenesis might be produced in the SVZ but migrate toward the cortex before tumorigenesis [45]. These data suggest that low-grade and high-grade diffuse gliomas could have distinct cellular origin. Genetic differences in primary de novo glioblastomas and secondary glioblastomas developing from lower-grade gliomas, in spite of similar histologic diagnosis, give great credibility to this concept. The discovery of the mutation in isocitrate dehydrogenase 1 in codon 132 (R^{132}) has shed a new light on distinction between these two kinds of glioblastomas. Indeed, *IDH1*^{R132MUT} and *IDH1*^{R132WT} GBMs differ in their demographic, anatomic, phenotypic, epigenetic, and genomic presentation and follow a different clinical course, supporting a model of two disease entities arising as a result of vulnerability of different stem-cell and progenitor populations to particular oncogenic alterations [46].

The Clinical Implications

Such spatiotemporal, functional, and molecular considerations may have important implications with regard to the therapeutic strategy.

First, the dynamic interactions between DLGG and the brain may also vary depending on the eloquence of the areas involved by the tumor. Indeed, it was shown that slow-growing DLGG might induce cerebral plasticity, explaining why most of patients had no neurological deficit despite voluminous glioma, even in the so-called critical regions [47, 48]. Nonetheless, a recent atlas of resectability of DLGG demonstrated that some cerebral areas had low compensatory capabilities [49], constituting a "minimal common brain" among patients [50]. As a consequence, the extent of surgical resection (and thus the median survival) is correlated with the glioma location (with regard to the cortex as well as the white matter pathways, therefore the distance with the SVZ), that is, with a better tumor removal in "non-eloquent" than in "eloquent" areas and in "compensable" rather "non compensable" structures [7, 51–54]. Indeed, larger tumors in contact with the SVZ appeared to be more difficult to treat surgically, with a higher percentage of partial resections because of invasion of functional subcortical pathways such as the superior longitudinal fasciculus, the arcuate fasciculus, and the inferior fronto-occipital fasciculus in the dominant hemisphere as well as the corticospinal tract, the thalamocortical tract, and the optic radiations in both hemispheres [20].

In addition, the presence of a higher proportion of 1p19q codeletion for DLGG not into the contact of the SVZ, thus with a higher rate of radical resection [20], might also represent a more favorable spontaneous prognostic factor [55, 56]. It could be therefore suggested that in patients with SVZ tumors that are less amenable to complete resection, which is currently the first treatment in DLGG [57], neoadjuvant chemotherapy might be discussed first [58, 59]. In the same vein, different genetic pattern in insular DLGG, namely, with a significant lower rate of 1p19q codeletion, could account for a more aggressive behavior. Indeed, in a recent series on 1097 DLGGs, tumor location was an independent

prognostic factor, with a significant worse prognosis for insular gliomas in comparison with frontal gliomas [60]. As a consequence, because the better understanding of the functional anatomy of the insular lobe as well as the use of intraoperative functional mapping allowed a dramatic decrease of posturgical morbidity [11, 61], surgery should become a relevant early treatment in insular DLGG.

Future directions will explore the possible involvement of external factors, with potential interactions between environment–brain function–tumoral genes, which could explain the heterogeneity in the geographic distribution of DLGGs in France [62]. Furthermore, studies of genetic susceptibility variants in diffuse (low-grade) gliomas should be more widely developed to better understand the origin of this entity [63–66]. Finally, whether hormones may play a role in the etiology of DLGGs should also be elucidated [67], taking into account the modification of their behavior during pregnancy [68, 69].

These crucial issues illustrate very well the close relationships between the pathophysiology of gliomagenesis, the anatomo-functional organization of the brain, and the personalized management in DLGG patients [70]. Moreover, they will allow a better screening of the population in order to perform early detection of DLGG – and thus to propose a more precocious and more efficient treatment [71, 72].

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