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Malignant glioma: Neuropathology and Neurobiology

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Maligne Gliome: Neuropathologie und Neurobiologie

Zusammenfassung. Maligne Gliome können in jedem Lebensalter auftreten. Am häufigsten jedoch bei Erwachsenen, die das 40. Lebensjahr bereits überschritten haben. Männer sind häufiger betroffen als Frauen.

Maligne Gliome beinhalten ein Spektrum von Tumoren mit verschiedenen Subtypen. Im Wesentlichen handelt es sich um Glioblastome, anaplastische Astrozytome / Oligoastrozytome / Oligodendrogliome, die gemeinsam durch ein diffus infiltrierendes, rasches Wachstum und durch eine fatale Prognose mit wenigen Monaten oder Jahren gekennzeichnet sind.

Invasion ist eine der Hauptursache für das geringe therapeutische Ansprechen, was auch eine komplette chirurgische Resektion unmöglich macht. Die Invasion durch Tumorzellen benötigt eine Interaktion mit extrazellulärer Matrix und benachbarten Zellen des normalen Gehirns. Vaskuläre Proliferationen und Gewebsnekrosen sind charakteristische Merkmale, insbesondere des Glioblastoms. Diese Veränderungen sind wahrscheinlich die Konsequenz rasch wachsender, schlecht oxygenierten, Tumorgewebes.

Häufige genetische Veränderungen wie P53, EGFFR und RB pathway scheinen auch pathogenetisch relevant. Bei Patienten mit Glioblastomen ist der Methylguanine-methyltransferase (MGMT) Promoter Methylierungs Status und bei Patienten mit anaplastischen Oligodendrogliomen der 1p19q Status relevant für das Ansprechen auf Chemotherapie.

Die Rolle der Neuropathologie und Neurobiologie in der Neuroonkologie besteht erstens in der klinisch relevanten Klassifizierung von Hirntumoren auf der Basis pathobiologischer Faktoren und zweitens in der Klärung der Ätiologie und Pathogenese von Hirntumoren und drittens das Übertragen von klinisch relevanten molekularen Parametern in die klinische Praxis.

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Schlüsselwörter: Maligne Gliome, Pathogenese, Histologie.

Summary. Malignant gliomas may manifest at any age including congenital and childhood cases. Peak incidence is, however, in adults older than 40 years. Males are more frequently affected than females. The sole unequivocal risk factor is therapeutic ionizing irradiation.

Malignant gliomas comprise a spectrum of different tumor subtypes. Within this spectrum, glioblastoma, anaplastic astrocytoma and anaplastic oligodendroglioma share as basic features preferential location in cerebral hemispheres, diffuse infiltration of brain tissue, fast tumor growth with fatal outcome within months or years.

Invasion is regarded as one of the main reasons for poor therapeutic success, because it makes complete surgical removal of gliomas impossible. Invasion of glioma cells requires interaction with the extracellular matrix and with surrounding cells of the healthy brain tissue. Vascular proliferates and tissue necrosis are characteristic features of malignant gliomas, in particular glioblastoma. These features are most likely the consequence of rapidly increasing tumor mass that is inadequately oxygenized by the preexisting vasculature.

In malignant glioma, distinct molecular pathways including the p53 pathway, the RB pathway and the EGFR pathway show frequent alterations that seem to be pathogenetically relevant. Methylguanine-methyltransferase (MGMT) promoter methylation status in glioblastoma and 1p19q deletion status in anaplastic oligodendroglioma are associated with response to chemotherapy.

The role of neuropathology and neurobiology in neurooncology is 1. to provide a clinically meaningful classification of brain tumors on basis of pathobiological factors, 2. to clarify etiology and pathogenesis of brain tumors as rational basis for development of new diagnostic tests and therapies, and 3. to translate testing for new clinically relevant molecular parameters into clinical application.

Key words: Malignant glioma, histology, pathogenesis.

Introduction

Classification of brain tumors is based on a histogenetic concept (1). According to this concept, malignant gliomas derive from astrocytic, olidogendroglial, or ependymal cells. Malignant gliomas comprise a spectrum of different tumor subtypes. Within this spectrum, glioblastoma, anaplastic astrocytoma, and anaplastic oligodendroglioma share as basic features preferential location in cerebral hemispheres, diffuse infiltration of brain tissue, fast tumor growth with fatal outcome within months or years.

The role of neuropathology and neurobiology in neurooncology is 1. to provide a clinically meaningful classification of brain tumors on the basis of pathobiological factors, 2. to clarify etiology and pathogenesis of brain tumors as rational basis for development of new diagnostic tests and therapies, and 3. to translate testing for new clinically relevant molecular parameters into clinical application.

The major aspects on these issues in malignant gliomas are summarized in this paper.

Definitions of malignant glioma types

Glioblastoma is the most malignant astrocytic tumor composed of poorly differentiated neoplastic astrocytes. Histopathology shows brisk mitotic activity, microvascular proliferations, thrombosis and necrosis. Glioblastoma may develop from diffuse low grade or anaplastic astrocytomas (secondary glioblastoma), but more frequently, they manifest de novo, without a less malignant precursor lesion (primary glioblastoma) [1].

Anaplastic astrocytoma is characterized by focal or dispersed anaplasia and a marked proliferative potential. Anaplastic astrocytomas arise from low grade astrocytomas, but are also diagnosed at first biopsy without a less malignant precursor lesion. Anaplastic astrocytomas tend to progress to glioblastoma.

Anaplastic oligodendroglioma is a diffusely infiltrating tumor composed of oligodendroglia-like tumor cells, with focal or diffuse histological features of malignancy (Fig. 1).

Epidemiology and etiology

Malignant gliomas may manifest at any age including congenital and childhood cases. Peak incidence is, however, in adults older than 40 years. Males are more frequently affected than females [1].

Except for inherited tumor syndromes (approximately 10 % of brain tumors have a hereditary basis, 90 % are sporadic) the etiology of malignant glioma is still largely unknown [2, 3]. The sole unequivocal risk factor is therapeutic ionizing irradiation. E.g., children receiving prophylactic CNS irradiation for acute lymphatic leukemia (ALL) may develop malignant glioma. No association of exposure to electromagnetic fields and development of brain tumors has been established so far. Further, no definite association between viral infection or diet (e. g. intake of N-Nitroso compounds) and malignant glioma has been proven.

Although the etiology of brain tumors remains unclear, there is some evidence that enhanced generation of oxygen radicals by intrinsic and/or extrinsic factors may play a crucial role as initial tumorigenic event. The oxygen radicals may induce chemical modification of DNA bases, leading to spontaneous mutation of, e. g., tumor suppressor genes and/or oncogenes. Such initial events may induce further genetic events, ultimately leading to neoplasia of the brain.

Histogenesis

Traditionally, it was believed that the adult human brain does not contain precursor cells and it was assumed that brain tumors derive from mature parenchymal cells [4]. Recently, neural stem cells have been detected in the adult human brain which could also give rise to brain tumors [5, 6].

Indeed, a population of immature cells capable of self-renewal have been isolated from human brain tumours [7]. These cells have been termed cancer stem cells. Cancer stem cells are distinct from neural stem cells in that they are tumorigenic after implantation into mouse brains. It has been suggested that cancer stem cells are the main proliferating cell population in malignant gliomas responsible for tumour growth and progression.

Invasion

Glioma cells show a strong tendency to invade the brain tissue [8]. Invasion is regarded as one of the main reasons for poor therapeutic success, because it makes complete surgical removal of gliomas impossible. Migrating glioma cells are relatively resistant to cytotoxic therapy (chemotherapy, irradiation) because their turn-over is reduced as compared to non-migrating tumor cells ("go or grow"). Invasion of glioma cells requires interaction with the extracellular matrix (ECM) and with surrounding cells of the healthy brain tissue. The brain ECM is composed mainly of soft components such as glycosaminoglycans, proteoglycans, tenascin-C, and throm-

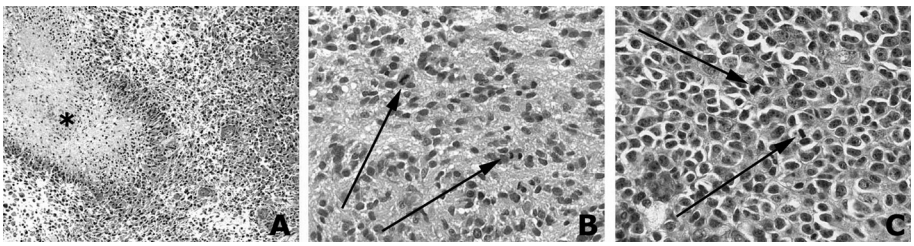

Fig. 1. A. Glioblastoma: histopathologic hallmarks are necrosis (asterisk) and microvascular proliferations. **B.** Anaplastic astrocytoma: characteristic features are anaplasia of astrocytic tumor cells and brisk mitotic activity (arrows). **C.** Anaplastic oligodendroglioma: tumor cells are oligodendroglia-like and show signs of anaplasia and brisk mitotic activity (arrows).

bospondin. Rigid components like collagen, fibronectin, or laminin are limited to perivascular and vascular areas in the brain. Migrating glioma cells may degrade soft components of ECM by proteases, namely the matrix metalloproteases, serine proteases, and cystein proteases [9].

Hypoxia and angiogenesis

Vascular proliferates and tissue necrosis are characteristic features of malignant gliomas, in particular glioblastoma [1]. These features are most likely the consequence of rapidly increasing tumor mass that is inadequately oxygenized by the preexisting vasculature. Tumor cells up-regulate hypoxia-associated factors that induce angiogenesis. In malignant gliomas newly formed vessels do not show normal phenotype but appear as bizarre vascular proliferates. It is assumed that these bizarre vascular proliferates are not suitable to sufficiently oxygenise the tumor tissue, resulting in a vicious circle of hypoxia and aberrant angiogenesis [10].

The most important hypoxia-associated factor is hypoxia inducible factor 1 (HIF-1), which is formed by association of HIF-1α and HIF-1β [11]. HIF-1α is stable under hypoxia and rapidly degrades under normoxic conditions. HIF-1β is constitutively expressed. HIF-1 is translocated to the nucleus and induces transcription of target genes involved in angiogenesis, migration, proliferation, and cell survival. Downstream effectors of HIF-1 include vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor (VEGFR). VEGF is a major angiogenic growth factor. It binds to and activates the tyrosine kinase receptors of the VEGFR family. Tyrosine kinase activity activates downstream pathways that induce proliferation and migration of vascular cells.

Pathogenetic pathways

Neoplastic disorders are in general genetic diseases [1]. The genetic alterations are associated with deranged regulation of cell proliferation, apoptosis (programmed cell death), senescence, migration, and cell-to-cell communication. Genetic alterations in malignant gliomas, as visualized by cytogenetics, are extremely complex and diverse. However, distinct molecular pathways show frequent alterations, which seem to be pathogenetically relevant [12].

a) p53 pathway ("sorting-out function")

p53 is considered as the "guardian of the genome". p53 is a transcription factor that protects the cell from malignant transformation by inducing cell cycle arrest, senescence, or apoptosis as a response to intrinsic or extrinsic stress, such as DNA damage, inflammation, hypoxia [13].

Disruptions of the p53 pathway increase genetic instability and may lead to malignant transformation. In gliomas, mutations of p53 are considered as an early lesion in the evolution of low-grade diffuse astrocytomas [1]. Over time, low grade astrocytomas progress to anaplastic astrocytoma and glioblastoma ("secondary" glioblastoma). Germline mutations of *TP53* are associated with familial tumor syndromes, in which also malignant gliomas arise, e. g. in the Li Fraumeni syndrome. Such associations further underline the relevance of p53 mutations in pathogenesis of malignant gliomas (Fig. 2).

b) RB pathway ("tumor suppressor function")

The RB pathway controls in the cell cycle transition from G1 to S phase. The RB pathway is a complex cascade involving many molecular factors [14]. The ultimate effector of this pathway are the transcription factors of the E2F family, which act as mitogens by targeting DNA replication proteins. RB complexes with E2Fs and inhibits their function. Phosphorylation of RB releases E2Fs.

Loss of RB leads to elevated levels of E2F. Likewise, abnormally enhanced phosphorylation of RB increases E2F levels. Increased E2F levels induce cell proliferation.

A considerable number of anaplastic astrocytomas and nearly all glioblastomas have alterations of the RB pathway [1] (Fig. 3).

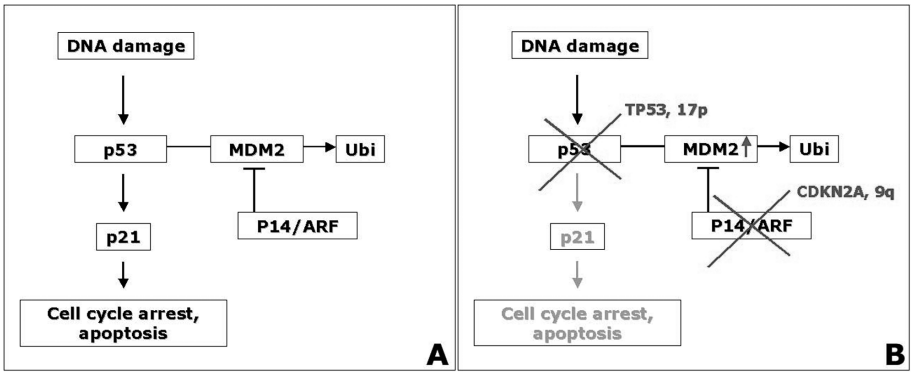

Fig. 2. A. p53 pathway under physiological conditions. p53 and its downstream effector p21 lead to cell cycle arrest or apoptosis after DNA damage. MDM2 regulates p53 activity by facilitating the ubiquitin-mediated degradation of p53. **B.** Pathological alterations of p53 pathway in glioma. Loss of functional p53 by *TP53* mutation or increased degradation of p53 due to increased MDM2 activity interrupt the cytoprotective p53 pathway.

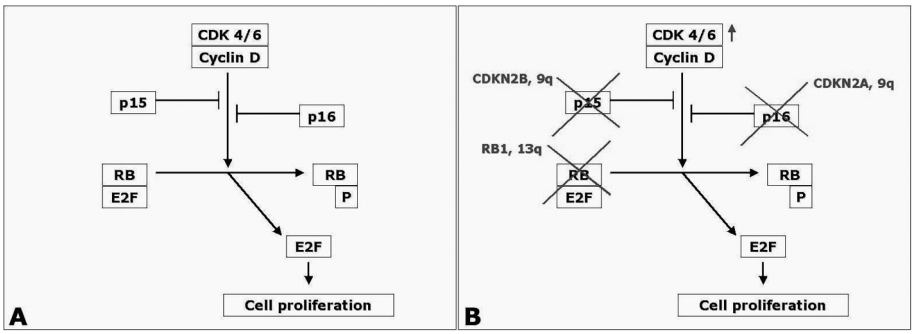

Fig. 3. A. RB pathway under physiological conditions. CDK4 and CDK6 form complexes with members of the cyclin D family and phosphorylate retinoblastoma protein (RB). Phosphorylation of RB releases transcription factor E2F, which facilitates cell proliferation by inducing transcriptions of genes that promote DNA synthesis. p16 and p15 inhibit CDK 4 and CDK6. **B.** RB pathway under pathological conditions. CDK4 or CDK6 gene amplification increase RB phosphorylation releasing E2F, which induces cell proliferation. Deletion or inactivation of CDKN2A or CDKN2B genes reduces inhibitors p16- or p15. Reduced p16- or p15 lead to increased RB phosphorylation, release of E2F, and induction of cell proliferation. Inactivation of the RB1 gene reduces RB protein, which releases E2F with the consequence of increased cell proliferation.

c) EGFR pathway ("proliferative signaling")

Epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor [15]. It belongs to the ErbB family of tyrosine kinase receptors, which includes ErB1 (EGFR or HER1), ErbB2 (Neu or HER2), ErbB3 (HER3), and ErbB4 (HER4). The most important endogenous ligands are EGFR and transforming growth factor alpha (TGFa). Activated EGFR propagates proliferative signaling via two major pathways, the Ras pathway and the PI3K pathway.

EGFR may be overactive due to gene amplification, over-expression, activating mutations, or activation of autocrine growth factor/receptor loops. The most common somatic mutation of EGFR lacks parts of the extracellular domain (residues 6–273) due to lack of exons 2–7 and is termed EGFRvIII. EGFRvIII is constitutively activated without ligand binding.

Several new therapeutic strategies aim at blocking EGFR signaling [16]. These strategies include cell surface blockade by monoclonal antibodies (e. g. Trastuzumab; Genentech), or intracellular blocking of the signaling cascade by small-molecule drugs such as ZD-1939 (Iressa; AstraZeneca) or OS-774 (Tarceva, Genentech) (Fig. 4).

Prognostic and predictive biomarkers in malignant gliomas

a) O^6-methylguanine-methyltransferase (MGMT) in glioblastoma

In recent studies it was shown that the addition of the alkylating agent temozolomide to radiotherapy for newly diagnosed glioblastoma resulted in a clinically meaningful and statistically significant survival benefit with minimal additional toxicity [17]. Molecular analysis showed that the glioblastomas containing a methylated MGMT promoter benefited from temozolomide, whereas those who did not have a methylated MGMT promoter did not have such a benefit [18].

For understanding this therapeutic effect, the in-vivo biochemical effects of alkylating drugs on tumor cell DNA and the biological function of MGMT in tumor cells need to be considered:

MGMT protects against cell death induced by alkylating drugs. The cytotoxic effect of alkylating agents is

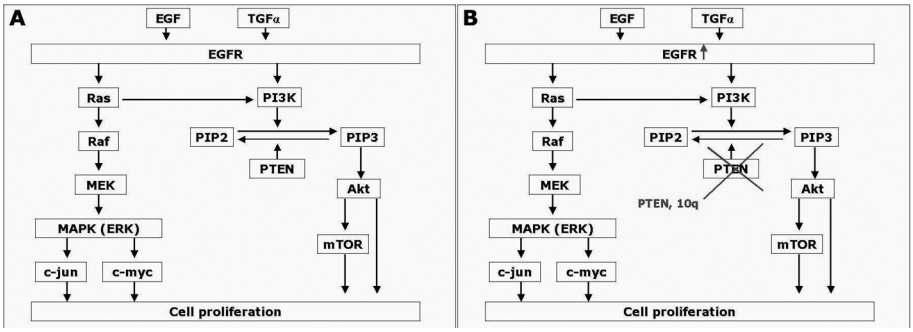

Fig. 4. A. EGFR pathway under physiological conditions. EGFR propagates proliferative signaling via two major pathways, the Ras pathway and the PI3K pathway. **B.** Tumorigenic alterations of the EGFR pathway. EGFR activation and PTEN inactivation lead to increased availability of PI3K. PI3K activates Akt, which leads to increased proliferative activity.
The EGFR pathway seems to play a crucial role in pathogenesis of "primary" glioblastomas (de novo glioblastomas without a less malignant precursor lesion). Germline mutations of PTEN are found in familial tumour syndromes, e. g. Cowden disease (associated with dysplastic gangliocytoma of the cerebellum Lhermitte-Duclos).

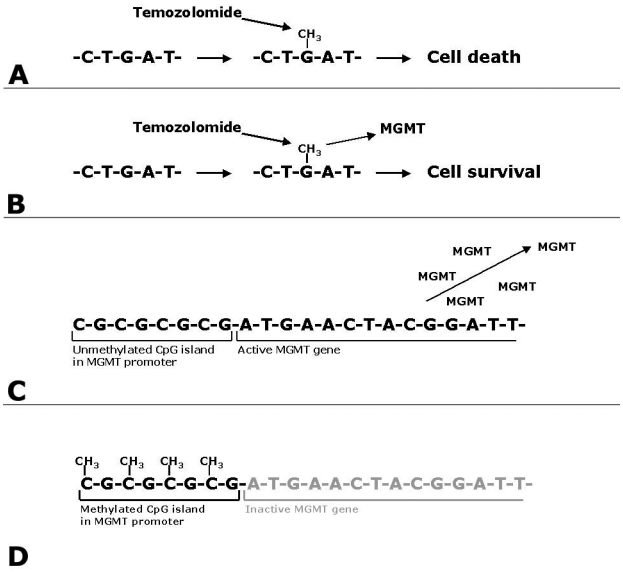

Fig. 5. A. Temozolomide adds a methyl group (CH_3) to the O^6-position of guanine in the DNA. Such DNA lesioning induces apoptotic cell death. **B.** MGMT protects from temozolomide-induced cytotoxicity by removing methyl groups (CH_3) from the O^6-position of guanine. **C.** Unmethylated CpG clusters ("CpG island") in the MGMT promoter are associated with active transcription of the MGMT gene and MGMT expression. **D.** Methylated CpG islands in the MGMT promoter are associated with transcriptional inactivation of the MGMT gene. MGMT is not expressed and its cytoprotective effect is lacking rendering tumor cells sensitive to alkylating agents.

mainly due to alkylation/methylation of the O^6-position of guanine in tumor DNA. Such DNA lesions induce apoptotic cell death. MGMT specifically removes alkyl/methyl groups from the O^6-position of guanine and thus protects against cell death induced by alkylating drugs [19]. It has been shown that MGMT expressing tumour cells are 4- to 10- fold more resistant to alkylating agents [20, 21].

MGMT promoter methylation suppresses MGMT expression. Change of genetic information in tumor cells is usually due to alteration of the sequence of DNA bases (deletion, amplification, base exchange due to point mutation). However, genetic information may be modified without changing the base sequence [22]. One mechanism of such "epigenetic" DNA modification is methylation of cytosine, especially if it is part of a cytosine-guanine dinucleotide (CpG; p denotes the phosphodiester bond joining the two bases) [23]. CpGs are unevenly distributed throughout the human genome. DNA stretches devoid of CpGs are interspersed by CpG clusters ("CpG islands"). Methylation of CpG islands in gene promoters is associated with transcriptional silencing of the affiliated gene [24]. Methylation of CpG islands in the MGMT promoter on chromosome 10q26 is associated with loss of MGMT expression. Loss of MGMT expression is associated with diminished DNA-repair activity. Tumor cells lacking MGMT are prone to cell death induced by alkylating substances.

Testing for MGMT promoter methylation in malignant gliomas in the clinical setting. The most widely used technique for analysis of methylation of CpG islands is methylation-specific polymerase chain reaction (MSP) [25]. It has been successfully applied both in native and formalin-fixed and paraffin-embedded tumour tissue. For establishing MSP as diagnostic test, systematic validation and definition of laboratory guidelines by task forces representing diagnostic neuropathology and also clinical neurooncology, e. g. European Association of Neuropathological Societies (EURO-CNS) and Brain Tumor Group of the European Organization for Research and Treatment of Cancer (BTG-EORTC) would be mandatory. Such consensus guidelines and validation of MSP are currently lacking (Fig. 5).

b) DNA deletions on chromosomes 1p and 19q in anaplastic oligodendroglioma

In contrast to other glioma types, anaplastic [26, 27] but also low grade [28] oligodendrogliomas are often chemosensitive. However, around 30 % of oligodendrogliomas are resistant to chemotherapy [29]. Molecular investigations showed that loss of DNA material on chromosome 1p is a frequent alteration in oligodendroglial neoplasms [30, 31]. Clinicopathologic analysis disclosed that combined allelic loss of DNA on chromosomes 1p and 19q is associated with chemosensitivity, longer recurrence-free survival after chemotherapy, and longer overall survival. A high frequency of MGMT promoter methylation and low or absent expression of MGMT has been described in oligodendroglial tumours, suggesting that these findings may contribute to the chemosensitivity of oligodendroglial neoplasms [32].

Clinical and histological features do not indicate which oligodendrogliomas are chemosensitive and which are not [29]. Therefore, it was suggested that molecular genetic testing may aid therapeutic decisions, e. g. selection of patients for chemotherapy and/or radiotherapy [29]. However, prospective trials are needed before such conclusions may be drawn [33]. In such trials, validated

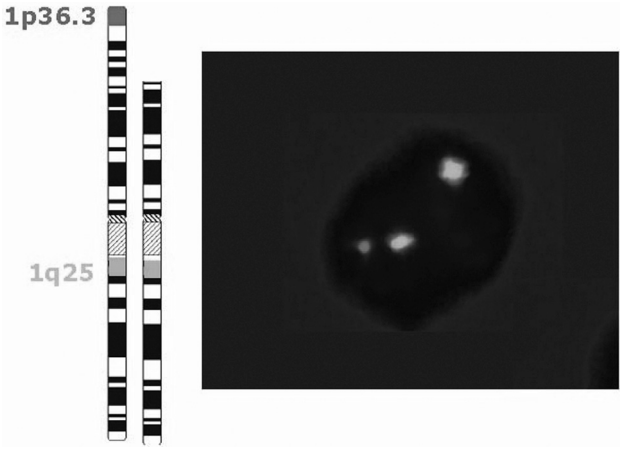

Fig. 6. DNA deletion on chromosome arm 1p in oligodendroglioma. A 2:1 signal ratio in double color FISH analysis indicates deletion status. (Dark signal: subtelomeric probe targeting chromosomal region 1p36.3, light signal: paracentromeric probe targeting chromosomal region 1q25).

methods for 1p/19q analysis should be used. Currently, a number of techniques are applied for 1p/19q testing, including loss of heterozygosity (LOH) analysis, fluorescent in situ-hybridization (FISH), comparative genomic hybridization (CGH), quantitative microsatellite analysis, multiplex ligation-dependent probe amplification (MLPA) [34]. It remains to be clarified which method is most suitable for 1p/19q diagnostic testing (Fig. 6).

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