REVIEW

Glioblastoma, a Brief Review of History, Molecular Genetics, Animal Models and Novel Therapeutic Strategies

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Abstract Glioblastoma (GBM) is the most common and lethal primary brain tumor. Over the past few years tremendous genomic and proteomic characterization along with robust animal models of GBM have provided invaluable data that show that "GBM", although histologically indistinguishable from one another, are comprised of molecularly heterogenous diseases. In addition, robust pre-clinical models and a better understanding of the core pathways disrupted in GBM are providing a renewed optimism for novel strategies targeting these devastating tumors. Here, we summarize a brief history of the disease, our current molecular knowledge, lessons from animal models and emerging concepts of angiogenesis, invasion, and metabolism in GBM that may lend themselves to therapeutic targeting.

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Historical Overview of Gliomas

Gliomas are the most common form of primary brain tumors with glioblastoma (GBM) being the most malignant form of glioma (Kleihues and Cavanee 2000). Before the advent of molecular biology and large-scale genomic studies, which most recently have allowed for detailed sub-classification of primary brain tumors, research into primary brain tumors consisted of two main eras, the classical period (~1860–1920) and the "histological period" (~1920–1940) (Scherer 1940). Gliomas were originally known as "medullary sarcomas" by the English medical community and "fungus medullare" by the German medical community and this era of research in gliomas is largely known as the "macroscopic era" (Scherer 1940). GBM was first identified in 1863 by Dr. Rudolf Virchow as a tumor with glial cell origins using macroscopic and microscopic techniques which ultimately gave rise to the modern day classification of brain tumors (Globus and Strauss 1925; Virchow 1863). In addition to coining the term glioma, he ushered in the "classical period" of glioma research, which combined macroscopic observations with microscopic observations. In the 1920s, neurosurgeon, Dr. Walter Dandy took a radical step by removing the entire hemisphere of two comatose patients suffering from GBM. Despite this intervention, these patients ultimately succumbed to the disease, providing the first evidence of how truly invasive GBM is (Dandy 1928). GBM was originally

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known as spongioblastoma multiforme, but in 1926 neurosurgeon/neuropathologist Dr. Percival Bailey and neurosurgeon Dr. Harvey Cushing renamed the tumor as GBM (Bailey and Cushing 1926). Bailey and Cushing were instrumental in leading the "histological era" of glioma research. Bailey and Cushing noted that many patients after incomplete removal of a cerebral glioma survived longer than expected compared with others (Bailey and Cushing 1926). This fact combined with their observation that not all gliomas have similar microscopic features led them to make a thorough study of the structure and clinical history of over 400 verified gliomas. In their work they were able to classify over 13 glioma types and observed varying degrees of prognosis associated with different gliomas (Bailey and Cushing 1926). Bailey and Cushing's careful characterization laid a foundation on which the field of neurooncology and neurosurgery would grow. These

studies from past eras coupled with tremendous advances in clinical, pathological, molecular, and genetic research today reveal a detailed identity of GBM.

Epidemiological Features of GBM

The incidence of all of primary brain and central nervous system (CNS) tumors is estimated at 18.71 per 100,000 individuals per year (CBTRUS 2010). Primary brain tumors are one of the top ten causes of cancer related deaths accounting for $\sim 2.3\%$ of cancer-related deaths in Europe and North America (Canadian Cancer Society's Steering Committee 2010). The most common primary brain tumor is the glioma, accounting for 32 % of CNS tumors and 80 % of malignant CNS tumors (Fig. 1). Gliomas are classified according to their presumed cell of

Fig. 1 Primary brain tumor distributions. Percent distribution of all **a** primary brain and CNS tumors by histology, **b** primary brain gliomas in the United States from 2004 to 2006
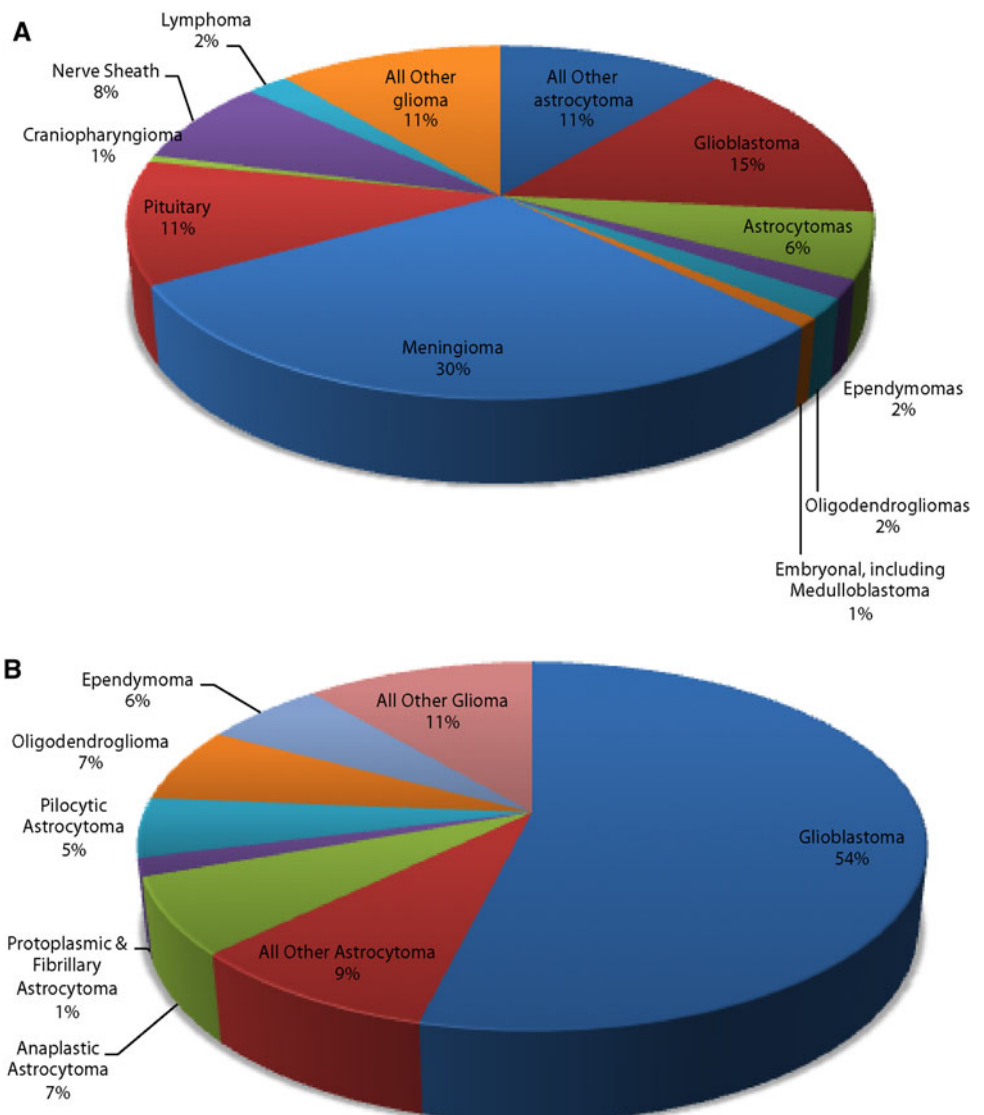
origin and extent of brain infiltration (circumscribed or diffusely infiltrating) and include astrocytomas, oligoden-drogliomas, and ependymomas. The most common type of glioma is astrocytoma, which account for about 76 % of gliomas (Fig. 1). GBMs are the most malignant glioma and common astrocytoma accounting for ∼54 % of all astro-cytic tumors (CBTRUS 2010). GBMs are more common among men than woman (male:female; ratio: 1.58:1) and twice as common in Caucasians versus African-Americans (CBTRUS 2010). The median age of patients at the time of diagnosis is approximately 64 years. No underlying cause has been identified for the majority of malignant gliomas. Although there has been some concern about an increased risk of gliomas in association with the use of cellular telephones, the largest studies have not demonstrated this assertion (Deltour et al. 2009; Rickman et al. 2001). Approximately 5 % of patients with malignant gliomas have a family history of gliomas. Some of these familial cases are associated with rare genetic syndromes, including neurofibromatosis types 1 and 2, and Li-Fraumeni syndrome (Farrell and Plotkin 2007).

Pathological Features of GBM

Astrocytomas are heterogeneous in nature and may show diffuse infiltration of adjacent and distant brain structures (Kleihues and Cavanee 2000). These neoplasms are histo-logically graded based on the criteria set by the World Health Organization into four prognostic grades (Fig. 2). Pilocytic astrocytomas, which are benign and relatively circumscribed, are classified as grade I tumors. Low-grade diffuse astrocytomas (LGA; grade II) are histologically characterized by moderate cellularity, mild nuclear atypia, and rare or absent mitotic figures and are moderately pro-liferative and invasive. Grade III astrocytomas, or anaplastic astrocytomas (AA), are characterized histologi-cally by increased cellularity, nuclear atypia, and mitotic activity. These tumors are more proliferative and infiltra-tive compared with grade II gliomas. GBMs, classified as grade IV gliomas, are histologically similar to AA but in addition are characterized by the presence of necrosis and glomeruloid microvascular proliferation. GBMs are sig-nificantly more proliferative, invasive, and angiogenic compared with grade II and III astrocytomas (Kleihues and Cavanee 2000). Malignant gliomas typically contain both neoplastic and stromal tissues, which contribute to their histologic heterogeneity and survival heterogeneity.

Molecular Genetics of Malignant Gliomas

Traditionally, GBMs have been classified into primary or secondary subtypes on the basis of clinical presentation and features although both subtypes are indistinguishable at a

morphological level. Approximately, 95 % of GBMs present as de novo primary tumors and typically occur in older patients (Ohgaki et al. 2004). Secondary GBMs arise and progress from lower grade astrocytomas, are quite rare, and tend to occur in patients below the age of 45. It is now known that both subtypes constitute distinct diseases with specific genetic differences documented between primary and secondary GBMs (Fig. 2). Primary GBMs are char-acterized by epidermal growth factor receptor (EGFR) gene amplification and mutation; loss of heterozygosity (LOH) of chromosome 10q containing phosphatase and tensin homolog (PTEN); overexpression of mouse double minute 2 (MDM2); and deletion of p16. The hallmarks of secondary GBMs include mutations of TP53 and retino-blastoma (RB); overexpression of platelet-derived growth factor A, and platelet-derived growth factor receptor alpha (PDGFA/PDGFRα); and LOH of 19q (Furnari et al. 2007; Wen and Kesari 2008). Recent studies from The Cancer Genome Atlas (TCGA) Research Network have reinforced our existing knowledge of the molecular genetics of GBMs and provided additional insight. TCGA is a multi-institute effort to accelerate understanding of the molecular basis of GBMs and other cancers through the use of genome analysis technologies. Ongoing gene copy number, gene sequencing, epigenetic methylation, gene expression pro-file, and micro RNA level characterization of a large cohort of GBM specimens have provided preliminary results that are reshaping our understanding of this disease.

Initial findings confirmed that three core pathways are deregulated in most, if not all GBMs: receptor tyrosine kinase signaling, and the TP53 and RB tumor suppressor pathways (Cancer Genome Atlas Research Network 2008). In fact, 74 % of GBMs analyzed harbored alterations in all three pathways. This study, along with other integrated genomic analysis studies, has characterized the importance of mutations in NF1, and ERBB2 (Cancer Genome Atlas Research Network 2008). Interestingly, mutations in iso-citrate dehydrogenase 1 (IDH1) were observed in about 70 % of grade II/III astrocytomas and secondary GBMs that developed from these lower-grade lesions suggesting a potentially more specific marker of secondary GBMs (Yan et al. 2009). The most frequent mutation of IDH1 is a point mutation of R132H, which is a gain of function mutation that allows production of 2 hydroxyglutarate and contrib-utes to a CpG island methylator phenotype (Dang et al. 2010; Noushmehr et al. 2010; Xu et al. 2011). Three dif-ferentially expressed IDH isoforms exist in humans, IDH1 being cytosolic, whereas IDH2 and IDH3 are mitochon-drial. In addition to IDH1 mutations, IDH2 mutations at R172 have also been identified in low-grade gliomas and GBM. Only 3–7 % of primary GBM have been reported to have the IDH1 mutation compared with 60–80 % in sec-ondary GBM.

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Fig. 2 Genetic and molecular pathogenesis of GBM. Genetic and chromosomal alterations involved in primary and secondary GBMs are shown. **a** Primary GBMs present de novo, whereas secondary GBMs arise and progress from a lower grade astrocytoma. Both primary and secondary GBMs contain similar aberrations, although certain aberrations are more prevalent in one subtype over the other (*bold*). **b** Recent integrated genomic analysis has defined primary and secondary GBMs into a further four distinct subtypes characterized by specific molecular alterations. *OLIG2* oligodendrocyte transcription factor 2, *PTEN* phosphatase and tensin homolog, *LOH* loss of heterozygosity, *Amp* amplification, *INK4A* inhibitor kinase 4A, *ARF* alternate reading frame, *MDM2* mouse double minute 2, *RB* retinoblastoma, *PDGFR* platelet derived growth factor receptor, *PI3K* phosphatidylinositol 3-kinase, *VEGF* vascular endothelial growth factor, *NES* nestin, *NF1* neurofibromin 1, *MET* met proto-oncogene (hepatocyte growth factor receptor), *CHI3L1* chitinase 3-like 1, *CD44* cd44 antigen, *MERTK* c-mer proto-oncogene tyrosine kinase, *TNF* tumor necrosis factor, *NFKB* nuclear factor kappa-B, *IDH1* isocitrate dehydrogenase 1, *PIK3R1* phosphatidylinositol 3-kinase, regulatory subunit 1
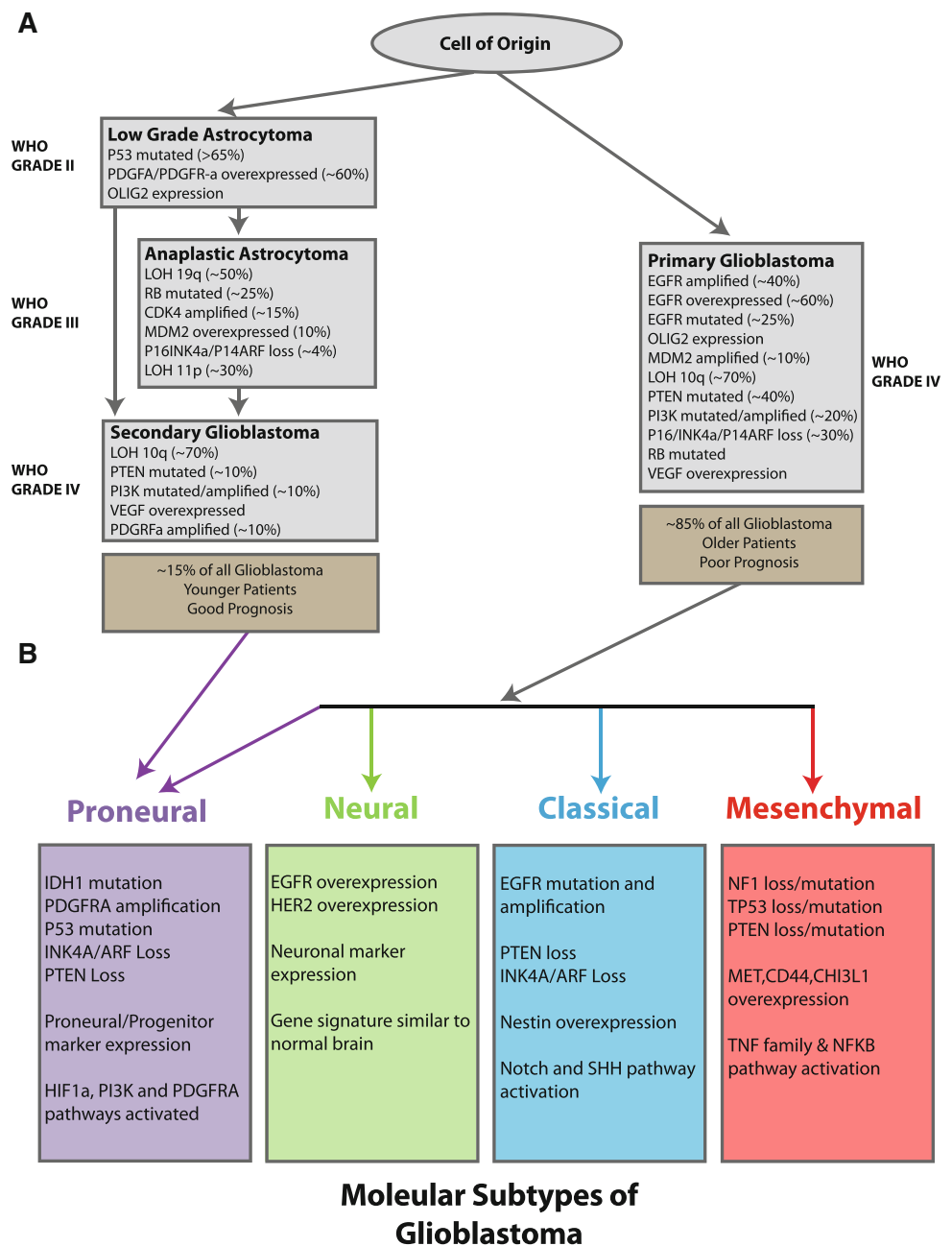
Molecular Subtypes of GBM

Our latest insight into the molecular pathogenesis of GBMs comes from very recent TCGA data. Integrated genomic analysis described a gene expression-based molecular classification of GBMs into classical, mesenchymal, pro-neural, and neural subtypes characterized by aberrations and gene expression of *EGFR, NF1, PDGFRα*, and *ERBB2*, respectively. The researchers of this study also found that response to aggressive chemotherapy and radiation differed by subtype (Verhaak et al. 2010). Using unsupervised hierarchical clustering, Verhaak et al. (2010), identified four molecular subtypes of GBM using an 840 gene

signature which was validated in separate data sets. The four subtypes identified are proneural, neural, mesenchymal, and classical. Each subtype was enriched for different mutation, genomic, and transcript alterations. The proneural subgroup was enriched for mutations in IDH1/2, mutation in TP53, and amplifications of PDGFRA, CDK6, CDK4, and MET. Additionally this group contained the highest percentage of young patients, likely due to the enrichment of IDH1 mutations which is associated with younger age. The classical sub-type is characterized by EGFR amplification and loss of PTEN. The classical sub-type also harbors the mutant EGFR variant III or EGFRvIII mutation, which is a constitutively active and has in frame

deletion of exons 2–7. The messenchymal subclass is associated with poor overall survival and contains NF1 mutations and loss of TP53 and CDKN2A. Last, the neural subtype has elevated levels of neural markers such as NEFL but has no unique distinguishing alterations from other classes, although elevated rates of ERBB2 mutation were observed. Figure 2 summarizes the four subclasses of GBM.

The Cell of Origin in GBM and the Cancer Stem Cell Hypothesis

Here, we define the cell of origin as the original cell or group of cells that acquired neoplastic lesions to induce transformation or cancer. The putative cell of origin of GBM is weighted around three possible theories: first, mature glia will dedifferentiate to acquire unregulated "stem-cell" like properties through acquisition of mutations or epigenetic lesions. Second, restricted neural progenitors, which have limited self-renewal potential need to acquire mutations which lead to the gain of unregulated "stem-cell" like properties, and third, adult neural stem cells (NSCs) which normally have tight regulation over their proliferative and differentiation potential acquire

mutations which render them tumourigenic (Dirks 2001, 2008; Stiles and Rowitch 2008) (Fig. 3). This concept in GBM where a subset of cells within the tumor that has stem-cell like properties, limitless expansion potential and can drive tumor formation, has been designated the cancer stem cell (CSC) hypothesis. The stem cell compartment of tumors has been termed CSCs, tumor initiating cell (TIC), or tumor-propagating cell (Hadjipanayis and Van Meir 2009). The definition of brain TICs (BTICs) does not encompass the cell of origin, which refers to the original cell or group of cells from which the tumor arose. TICs have been isolated from several cancers including brain tumors such as GBM and medulloblastoma (Galli et al. 2004; Singh et al. 2004; Taylor et al. 2005). BTICs are able to propagate in an undifferentiated manner and are able to recapitulate the tumor when injected in low numbers compared with non-purified tumor cells (Clarke et al. 2006; Galli et al. 2004; Singh et al. 2004; Taylor et al. 2005). BTICs candidates express markers including CD133, Nestin, SOX2, and can behave similar to normal NSCs and are isolated using several means. For more detailed review on CSC please refer to the following reviews highlighting emerging concepts and controversies (Dietrich et al. 2010; Lefranc et al. 2009; Munoz and Guha 2011; Park and Rich 2009; Sanchez-Martin 2008).

Fig. 3 Putative cells of origin in GBM. Normal CNS differentiations and CNS tumor formation. Neural stem cells (NSC) give rise to neural and glial progenitors that then differentiate into the major cell types of the CNS neurons, oligodendrocytes and astrocytes. BTIC have been theorized to come from terminally differentiated cells (1) which acquire genetic mutations which endow them with a proliferative advantage a slow accumulation of critical mutations result in its transformation. Normal NSCs give rise to progenitors with limited proliferative and self-replicative capacity. BTIC have been theorized to form as a result of NSC (2) or neural progenitor transformation GBM can also arise through differentiation of mature glial or neuronal lineages (3). Curved arrow represents ability to self renew

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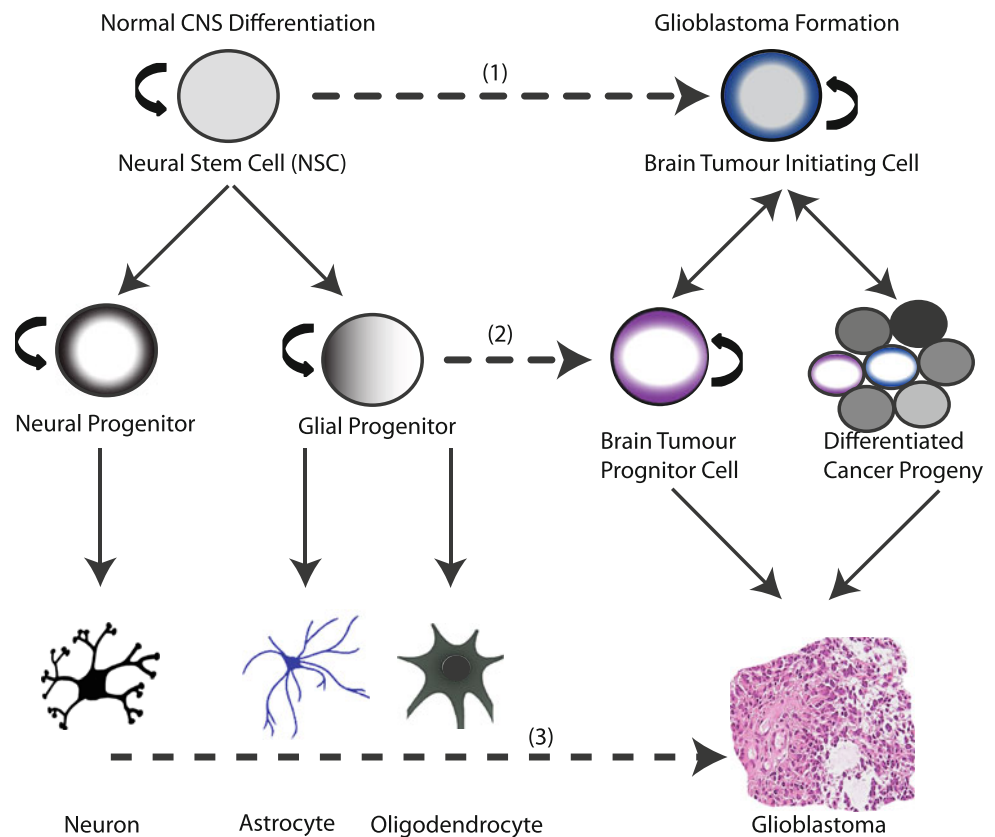
Genetically Engineered Mouse Models (GEMs)

The Need for GEMs in Glioblastoma Tumor Research

Recent novel therapies like immuno- and gene therapy have shown some promise in existing pre-clinical models, but have failed to demonstrate therapeutic benefit in patients. The reason(s) for such failures include our incomplete understanding of the molecular pathogenesis of these tumors and also the testing of novel biological therapies in less than ideal pre-clinical models. Transgenic mouse models offer an opportunity to develop and utilize an easily replenished, reproducible, manipulated spontaneous, and more accurate pre-clinical model of human cancers, which we can use to add to our molecular knowledge and test promising therapies. The recent completion of the sequencing of the human and mouse genomes have revealed a high homology between the two genomes; thus, mouse models that recapitulate human GBM may be an invaluable tool.

GEM Models of Glioma

Molecular progression of gliomas, like many tumors, involves the accumulation of genetic and epigenetic alterations that result in the loss of tumor suppressor gene function (PTEN, TP53, INK4A/p16, ARF/p19, Rb) or activation of oncogenic pathways (p21-Ras, PI3-kinase, EGFR, CDK4, MDM2) (Cancer Genome Atlas Research Network 2008; Furnari et al. 2007; Parsons et al. 2008). Here, we briefly highlight some seminal observations and results using mouse models of known GBM modifier genes. The importance of the p53 pathway signaling pathway early in gliomagenesis is also demonstrated by the small incidence of gliomas in mice null for p19$^{\text{Arf}}$, a protein involved in regulating the stability of p53 through its negative regulation of MDM2 (King et al. 2002). Alternatively, overexpression of relevant oncogenic receptors or downstream signaling pathways has also been employed in developing mouse glioma models. These have been either alone or in combination with mice harboring specific knockouts of relevant cell cycle regulatory proteins. For example, the S100 glial precursor promoter regulated v-ERBB (an activated member of the EGFR family) transgenic mice develop oligodendrogliomas, which are potentiated in terms of shorter latency and increased malignancy when undertaken in mice deficient for both p16 and p19 (Ink4a/ARF null mice) (Weiss et al. 2003). Since wild-type EGFR and mutant EGFRvIII are the most common gain of function alterations in malignant human astrocytomas, our group has recently created mice expressing these proteins under regulation of the Glial fibrillary acidic protein (GFAP) promoter (Ding et al.

2003). Mice with GFAP regulated expression of EGFR and EGFRvIII; both did not result in gliomas, suggesting that like in human gliomas, these are not initiation factors. However, when these mice were mated with a mouse that is prone to develop gliomas, such as the GFAP regulated activated p21-Ras mouse described below, the EGFRvIII but not the EGFR mice potentiated glioma formation. In addition, expression of EGFRvIII also altered the glioma sub-type, with the double transgenic mice (activated p21-Ras + EGFRvIII) developing oligodendrogliomas and mixed oligo-astrocytomas tumors, compared with the mainly astrocytic lineage tumors in the single transgenic activated p21-Ras mouse.

There are several examples of aberrant expression of relevant downstream signaling pathways in mouse glioma modeling. These include astrocytomas of varying grades resulting from GFAP regulated expression of v-src (Weissenberger et al. 1997). Our laboratory has utilized our initial observation of aberrant activation of the p21-Ras signaling pathway in astrocytomas to develop glioma models using ES transgenesis (Ding et al. 2001, 2003). GFAP regulated oncogenic/activated p21-Ras led to multifocal astrocytomas of varying grades, in a p21-Ras activity dose-dependent manner. Mice with extremely high levels of p21-Ras activity in the brain died as chimeras with GBM like tumors. Moderate levels of p21-Ras activation led to germline transmission, with mice that are born normally but start to develop and die from their multi-focal astrocytomas of varying grades commencing at ~12–14 weeks. Of interest, expression of activated p21-Ras in adult astrocytes leads to senescence, so the developmental expression of p21-Ras in early glial progenitor cells is of importance. Furthermore, expression of activated p21-Ras in the early glial progenitors leads to genetic instability, but is not sufficient alone for transformation, since the astrocytomas occur after a period of development post birth. When the GBM-like tumors are examined in these mice, additional genetic alterations such as those found in human GBMs (over-expression of EGFR, CDK4, MDM2; decreased expression of p16, p19, p53, PTEN) (Holland 2001) are present, which we hypothesize are facilitated by the genetic instability created by activated p21-Ras. As described above, experiments involving mating these p21-Ras mice to other genetically defined mice such as EGFRvIII have already commenced and are continuing, with the potential of these mouse models not only serving as useful pre-clinical reagents, but also to increase our understanding of the in vivo interactions of these molecular alterations in the pathogenesis of gliomas. Table 1 summarizes currently used and relevant glioma mouse models which recapitulate hallmarks of human GBM.

Virally transduced expression of relevant gain of function alterations, in combination with transgenic mouse

Table 1 Transgenic or knockout mouse models

Tumor classification	Transgene	Knockout	Study
Transgenic and knockout GEMs			
Low-grade astrocytoma	GFAP V12-Ha Ras		Ding et al. (2001)
	Src tg		Weissenberger et al. (1997)
Anaplastic astrocytoma	GFAP V12-Ha Ras	Flox *Nf1, p53* ko	Ding et al. (2001)
	GFAP-Cre		Reilley et al. (2000)
	Src tg		Weissenberger et al. (1997)
Glioblastoma	GFAP-Cre	Flox *Nf1* + *p53* ko	Zhu et al. (2005)
	GFAP-T121 tg	Pten$^{+/-}$	Xiao et al. (2002)
	NESTIN-Cre^{ER}	Flox *NF1*, Flox *PTEN*, Flox *p53*	Alcantara Llaguno et al. (2009)
		Arf ko	Kamijo et al. (1999)
Low-grade oligodendroglioma	S100b-vErbB tg		Weiss et al. (2003)
High-grade oligodendroglioma	GFAP V12-Ha Ras +		Ding et al. (2001)
	GFAP-EGFRvIII		
	S100b-v-erbB tg	Inka/Arf ko	Weiss et al. (2003)

Selected mouse models that recapitulate hallmarks of human gliomas

GEMs genetically engineered mouse models, *tg* transgene, *ko* knockout

technology, allows one to model such somatic alterations at a later stage in life, though it does not lead to germline colonies. Although the link between a viral etiology and human gliomas is weak, retroviruses that have been engineered to express relevant gain of function genes have been used to create glioma models in mice and other mammals (Holland et al. 2000; Uhrbom et al. 1998, 2002). This includes members of the Rous sarcoma virus family and simian sarcoma virus, whose transforming properties are due to over expression of the viral oncogene *v-sis*, the cellular counterpart of which is c-sis or PDGF-B. Over expression of PDGF and activation of PDGF-receptors are well recognized in gliomagenesis (Uhrbom et al. 1998, 2002). Retroviruses carrying *v-sis* (PDGF-B), when injected into normal mice, have yielded astrocytic tumors, with varying glioma types when injected in Ink4a/ARF null mice (Uhrbom et al. 1998). The frequency of gliomas using this retroviral strategy was between 40 and 80 %, with the most frequent histology being high-grade gliomas that have characteristics similar to GBMs. One of the best examples of coupling retroviruses to express somatically defined gain of function genes in varying cell lineages and genetic backgrounds to model gliomas is the RCAS-*tva* system, detailed previously. This system develops focal gliomas, the sub-type and grade of which varies with the injected retroviral transduced gene (i.e. PDGF-B, EGFRvIII, activated p21-Ras, activated Akt), the lineage of the cell expressing the *tva* receptor (GFAP, Nestin) and underlying genetic cell cycle alterations in the mice (null for Ink4a/ARF, p53, etc.). For example, retroviral transduced expression of *v-sis* or PDGF-B in GFAP-*tva* mice developed oligodendroglioma or mixed oligo-astrocytoma in

40 % of the mice, with 60 % developing similar gliomas in Nestin-*tva* mice (Dai et al. 2001). When these experiments were undertaken in Ink4a/ARF null mice, the gliomas formed with a shorter latency and were of higher grade. Another example with the RCAS-*tva* system are experiments with retroviral transduction of activated p21-Ras and Akt, representing the two most implicated aberrant signaling pathways in gliomas, as previously discussed. Neither of these activated signaling molecules by itself formed gliomas, but when expressed together in more primitive Nestin+'ve precursor glial cells, astrocytomas developed.

Similar results with EGFRvIII have also been obtained by our ES transgenesis model (Ding et al. 2003) and those with the RCAS-*tva* system (Holland et al. 1998). In both, expression of EGFRvIII by itself is not transforming, as it is a progression and not an initiation factor. In the RCAS-*tva* system, retroviral transduction of both EGFRvIII and CDK4 in mice null for p53 was required for formation of mixed gliomas. In addition to the use of retroviruses or adeno-cre virus injections and lentiviruses may also be used to initiate glioma formation. Briefly, the injection of adenovirus containing the EGFRvIII mutant into mice harboring activated Ras lead to efficient formation of GBM (Wei et al. 2006). Lentiviruses expressing oncogenes such as H-Ras or AKT were efficiently introduced into mice expressing Cre-recobinase under varying promoters such as GFAP. These constructs contained a locus of cross over (lox)-stop-lox sequence in front of the oncogene. Upon integration of the lentivirus, Cre-excision of the lox-stop-lox codon would allow for activation of the oncogenes. GBM tumors were efficiently formed when lentiviruses

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harboring activated Ras and AKT were injected into mice expressing GFAP-Cre on a TP53 heterozygous background (Ikawa et al. 2003; Marumoto et al. 2009). The advent of inducible transgenic systems will also allow for temporal and spatial regulation of gene-expression. With relevance to glioma and CNS biology two genetically engineered mice strains express Cre recombinase fused to the estrogen receptor ligand-biding domain. Upon administration of Tamoxifen, the Cre recombinase is active, translocates to the nucleus, and excises DNA flanked by locus of cross over (Flox) sites as detailed earlier. With the generation of GFAP-Cre[ER], Nestin-Cre[ER] mice gene activation/inactivation will allow to be regulated not only spatially as dictated by the promoter but also temporally as dictated by administration of tamoxifen (Casper et al. 2007; Chen et al. 2009; Chow et al. 2008). Current studies have allowed for determining that gliomagenic potential of mice is greater at a younger age with excision of GBM-relevant genes such as PTEN, NF1, and TP53 (Alcantara Llaguno et al. 2009). Currently, inducible tetrocycline systems are being generated for regulation of glioma and GBM genes under cell specific manners by our lab and others, which may be of potential promise. Last, utilization of orthotopic xenograft models for gene discovery or as preclinical models is also of use (Carlson et al. 2009; Pandita et al. 2004; Sarkaria et al. 2006; Verhaak et al. 2010). They retain hallmark features of GBM such as invasion and necrosis that cell models lack. In addition, serial passaging tumors in mice better retain genetic hallmarks of GBM such as EGFRvIII which is lost in culture or IDH1 which is also lost in culture (Piaskowski et al. 2011; Schulte et al. 2012). Last, several xenograft models developed by our lab and others have GBM molecular profiles similar to the human molecular GBM subtypes recently identified (Piaskowski et al. 2011).

Current Treatment Paradigms

The standard therapy for newly diagnosed malignant gliomas involves maximal surgical resection, radiotherapy, and chemotherapy, yet this does little to improve life expectancy. The surgical incurability of most GBMs is underscored by the invasive, diffuse, and ill-defined borders of these tumors. Following surgery, intensity-modulated or image-guided radiotherapy is delivered and enhances median survival from 3 to 12 months. However, tumor recurrence occurs from invasive tumor cells that have escaped surgical removal and dodged lethal radiation exposure and inevitably, 90 % of tumors recur at the site of surgery (Berens and Giese 1999). Although the prognosis remains poor, recent changes to standard of care treatment strategies have significantly improved the median survival of patients with GBMs. A clinical study carried out by

Stupp and colleagues found that concomitant treatment with radiation and the alkylating agent temozolomide (TMZ) followed by adjuvant TMZ increased median overall survival from 12.1 to 14.6 months compared with that of patients receiving radiation alone (Stupp et al. 2005). The 2-year survival rate increased significantly from 10.4 % in those receiving radiation alone to 26.5 % in those receiving concurrent treatment. Furthermore, a subgroup of patients who responded better to concurrent TMZ treatment was identified; patients who had silencing of the DNA repair enzyme O^6-methylguanine DNA methyltransferase (MGMT) through promoter methylation and underwent concurrent treatment as described above, had a median overall survival of 21.7 months compared with 12.7 months in those patients receiving a similar treatment but with no *MGMT* promoter methylation (Hegi et al. 2005). The two-year survival rate in patients with *MGMT* promoter methylation and receiving concomitant treatment was 46 % compared with 13.8 % for patients receiving concurrent treatment but lacking *MGMT* promoter methylation. However, there is still a significant proportion of patients who do not benefit from TMZ and in whom MGMT promoter methylation fails to predict response. Emerging evidence has suggested that the mismatch repair pathway and base excision repair pathway also promote resistance to TMZ. In particular, a recent study identified that the DNA base excision repair enzyme, alkyl purine DNA glycosylase (APNG), mediated resistance to TMZ in vitro and in vivo. APNG directly repairs alkylated bases at N7 guanine and N3 adenine, the later cytotoxic (Elder et al. 1998; Smith and Engelward 2000). Interestingly, patients with elevated levels of APNG also had poorer overall survival compared with pateints that were APNG negative (Agnihotri et al. 2011, 2012).

Novel and Investigational Therapies

The dismal prognosis of patients diagnosed with GBMs is attributed to the highly infiltrative nature of these tumors, as well as the heterogeneous molecular profiles of seemingly histologically similar GBMs. The objective of many investigational therapies is to (1) target cellular pathways or specific molecules implicated in pathogenesis and (2) identify molecular features of the tumor that predict a response, so that patients who are most likely to benefit can be selected for a particular treatment. The number and types of current experimental therapies are too numerous to describe here, but suffice it to say that inhibition of all features of tumor function, survival, proliferation, apoptosis, invasion, and angiogenesis, are under investigation. Table 2 summarizes current clinical trials underway in GBM. Current experimental strategies to increase the effectiveness of targeted molecular therapies include the

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Table 2 Summary of clinical and investigational trials in GBM

Pathway	Inhibitor target	Mono-therapy	Combined therapy
Receptor tyrosine kinases (RTK)	EGFR	Erlotinib	Erlotinib + radiation
			Erlotinib + temozolomide
			Erlotinib + sorafenib
	EGFRvIII and amplified wt EGFR	mAb 806	
	PDGFR/cKit/ABL	Imatinib (Gleevec)	Imatinib + temozolomide
			Imatinib + hydroxyurea
			Imatinib + vatalanib
	VEGFR and multi RTKs	AZD2171 (VEGFR, PDGFR, c-Kit)	
		Vatalanib (VEGFR, PDGFR, c-Kit)	
		AEE788 (EGFR, VEGFR1/2)	
		ZD6474 (EGFR, VEGFR2/3)	Sorafenib + erlotinib
		Lapatinib (EGFR, HER2)	
		Sorafenib (RAF, VEGFR2/3, PDGFR, c-KIT)	Pazopanib + lapatanib
		Tandutinib (FLT3, PDGFR)	
		Pazopanib (VEGFR, PDGFR, c-Kit)	
Ligand	VEGF	Bevacizumab	
		VEGF-trap	
Signal transduction	HIF1a	2-methoxyestradiol	
	AKT	Perifosine	
	PKC	Tamoxifen	
	mTOR	AP23573	
		Everolimus	Everolimus + temozolomide
		Serolimus	
		Temsirolimus	Temsirolimus + temozlomide + radiation
Protein modification	HDAC	Suberoylanilide hydoxamic acid (SAHA, vorinostat)	SAHA + temozolomide
	Farnesyl transferase	Tipifarnib, lonafarnib	Lonafarnib + temozolomide
Other	αvβ3 Integrin	Cilingitide	Cilingitide + radiation
	Proteosome	Velcade	
	SP1 (transcription factor)	Tetra-*O*-methyl nordihydroguaiaretic acid	

Various molecules expressed in GBM have become targets of inhibitors, alkylating agents, and antibodies. These are just a representation of several hundred clinical trials open and closed that can be viewed at the National Institutes of Health (NIH) clinical trial website: http://www.clinicaltrials.gov

SAHA suberoylanilide hydoxamic acid; *RTK* receptor tyrosine kinase

use of a single agent targeted against several kinases (e.g. ZD6474), combinations of agents that inhibit complementary targets such as EGFR and mTOR (e.g. erlotinib + temsirolimus), and targeted agents combined with radiotherapy and chemotherapy (e.g. Cetuximab + TMZ + radiation) (Furnari et al. 2007).

Targeting Angiogenesis as a Therapeutic Strategy

It is well established that angiogenesis plays an important role in tumor progression, especially in the case of GBMs which due to the rapidity of their progression have a high metabolic demand for efficient oxygen delivery and waste removal (Carmeliet and Jain 2000; Darland 2001; Folkman 1990; Holash et al. 1999; Kargiotis et al. 2006; Klein and Weinhouse 1974; Risau 1991; Risau and Flamme 1995). The end result of uncontrolled vessel formation is a highly torturous and dilated physiological abnormal vascular network that provides an insufficient blood supply creating increased edema, and hypoxic conditions (Voest 2004), in turn, trigger a continuous cycle of dysfunctional vessel formation (Bergers and Benjamin 2003; Carmeliet and Jain

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2000; Darland 2001; Wilting et al. 1995). The initiation of vessel formation is reliant on the 'angiogenic switch', a balance of multiple pro- and anti-angiogenic factors secreted by both host and tumor cells, favoring the pro-angiogenic factors (Bergers and Benjamin 2003; Darland 2001). Multiple candidate factors that signal tumor cells to initiate the cascade of angiogenic factor signaling have been proposed (Carmeliet and Jain 2000; Cavallaro and Christofori 2000; Darland 2001; Durairaj et al. 2000; Kerbel 2000; Lutsenko et al. 2003). The main contributors are hypoxia, increased physical forces, and the products of oncogenes and mutated tumor suppressor genes (Carmeliet and Jain 2000; Cavallaro and Christofori 2000; Darland 2001; Durairaj et al. 2000; Kerbel 2000; Lutsenko et al. 2003). The molecular mechanisms of tumor angiogenesis recapitulates loosely those of normal physiological vessel development, which occurs via two processes, "vasculogenesis" and "angiogenesis" (Houck et al. 1991; Papetti and Herman 2002; Pardanaud et al. 1987; Patan 2000; Risau 1991, 1997; Risau and Flamme 1995). During angiogenesis new vessels are formed via sprouting and elongation of pre-existing vessels to create a stabilized vascular network (Hillen and Griffioen 2007; Jain and Carmeliet 2001; Risau 1991, 1997; Risau and Flamme 1995; Wilting et al. 1995; Wilting and Christ 1996; Yancopoulos et al. 2000). Vasculogenesis is primarily an embryonic process whereby angioblasts differentiate from the mesoderm into endothelial cells (ECs) (Risau and Flamme 1995), which in turn proliferate to form de novo primitive vascular networks (Risau 1991, 1997; Risau and Flamme 1995). Recent studies suggest circulating bone marrow-derived progenitor cells (BMDPCs) are the adult counterpart of embryonic angioblasts and demonstrate that vasculogenesis occurs in adulthood in response to pathological stimuli such as tumor growth (Asahara et al. 1999; Kioi et al. 2010; Ribatti 2004), whereby BMDPCs differentiate into ECs to produce de novo blood vessels (Aghi and Chiocca 2005; Ahn and Brown 2009; Ribatti 2004; Zhang et al. 2009). Other more indirect mechanisms facilitating access of tumor cells to the blood supply include "vessel co-option" where tumor cells physically migrate toward blood vessels in response to signals within the tumor microenvironment and grow alongside existing vessels. Alternatively, there have been suggestions that tumor cells themselves can form vascular channels to transport blood independently of EC lined vessels, a phenomenon referred to as "vascular mimicry" (El Hallani et al. 2010; Hillen and Griffioen 2007; Ribatti et al. 2003; Ricci-Vitiani et al. 2010; Wang et al. 2010). The challenges of studying the origins of vascularity come in understanding the dynamic contribution of progenitor cells to the tumor vasculature. In our recent work we have taken advantage of two-photon laser microscopy and green

fluorescent protein positive bone marrow (GFP + BM) chimeric mouse models to study the true origin of tumor vasculature through repeat intra-vital long-term imaging. Using this strategy, we have been able to study in real-time at a single-cell level the recruitment GFP + BMDPCs (bone marrow derived precursor cells) into the vasculature at the tumor site. We have demonstrated conclusively that BMDPCs have an intimate relationship with the newly forming vascular networks and so vasculogenesis does in fact occur in response to tumor (Fig. 4a, b). However, by highlighting the vasculature with a CD31 antibody we can also demonstrate that this relationship is not endothelial in nature, but instead, the BMDPCs appear to provide a more supportive role in the form of immuno-modulatory peri-vascular cells which in ex vivo tissue stain positively for MAC3, CD11b and IBA1 (Fig. 4c–e) (Burrell et al. 2012). Built around the knowledge that tumor angiogenesis was vital for tumor growth, anti-angiogenic treatment held much promise in arresting tumor growth and progression, maintaining patients in a stable asymptomatic state (Brem 1999; Durairaj et al. 2000; Muehlbauer 2003; Puduvalli and Sawaya 2000; Risau 1991; Sipos et al. 1994). Levels of vascularization observed in all cancers results in vascular networks which are readily accessible and contain altered and up-regulated EC receptors. This would allow for identification of exclusive therapeutic targets of aberrant tumour vasculature. Combinatorial therapy, using anti-angiogenic strategies concomitantly with radiation therapy, however, has become the primary focus, due to the concept of "vascular normalization" following anti-angiogenic therapy where abnormal tumor vasculature is somewhat corrected allowing a transiently more efficient tumor blood flow to the tumor, improving delivery of therapeutics and tumor oxygenation, and ultimately response to radiation (RT) and chemotherapy (Carmeliet and Jain 2011a, b; Jain 2001, 2005; Jain et al. 2007). There are some pre-clinical studies and early clinical trials that have explored the therapeutic benefits of combining anti-angiogenesis with RT, but results have been inconsistent (Abdollahi et al. 2003a, b; Browder et al. 2000; Bruns et al. 2002; Choy and Kim 2003; Geng et al. 2001; Gorski et al. 2003; Jain 2001; Kerbel and Folkman 2002; Kozin et al. 2001, 2007; Schueneman et al. 2003; Willett et al. 2006) and the optimal scheduling of these adjuvant treatments remains unknown. Moreover, individual tumor type plays a significant role in determining the correct combinatorial scheduling and very few studies have focused on brain tumors. Current anti-angiogenic drugs can take many forms including more traditional forms such as monoclonal antibodies and antibody fragments, as well as proteins and other small organic molecules. The drugs are designed to target the many pro-angiogenic factors involved in the initiation of tumor vascularization including PDGF, SDF1,
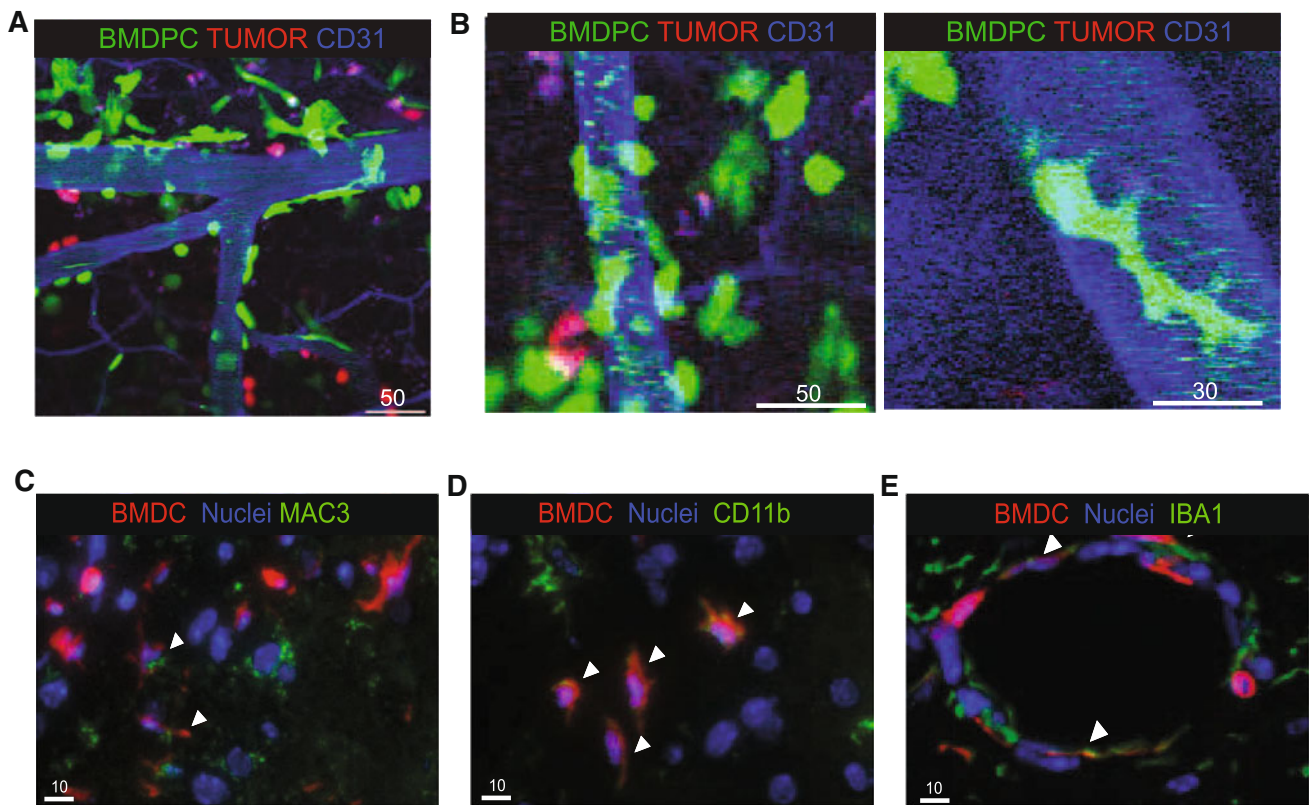
Fig. 4 Angiogenesis and bone marrow recruitment in GBM. **a** 2 Photon image demonstrating the recruitment of GFP + BMDPC to the RFP + tumor area. Vasculature is highlighted with a CD31APC tagged antibody magnification ×10. **b** Zoomed in 2 photon intravital images demonstrate GFP + BMDCs are intimately involved in the vasculature of the tumor but remain surrounding the vasculature and as such do not co-localize with the CD31 ×20 magnification and magnification ×40. **c** IF staining on ex vivo tissue confirms the relationship the RFP + BMDPCs have with vasculature but in the form of immune cells staining positively for MAC3, CD11b and IBA1 with 488 tagged secondaries. These immune markers suggest a macrophage/microglial cell fate and that the BMDPCs play more of a support role to newly forming vasculature magnification ×20

HIF1a, FDF, and EDFR, although many drugs at present target a single pathway, the pro-angiogenic signal vascular endothelial growth factor (VEGF), as it is thought to be the main driver of angiogenesis in tumor pathophysiology. Strategies include interfering with either the VEGF ligand and its receptor VEGFR2 (Batchelor et al. 2010; Vredenburgh et al. 2007) or its downstream signaling cascade. Bevacizumab (avastin) is an anti-VEGF antibody which is now used readily in a clinical setting for the treatment of recurrent GBM. Initial phase II trials of bevacizumab used concomitantly with irinotecan demonstrated a 60 % radiographic response and increased progression-free survival (Vredenburgh et al. 2007). Similarly, two follow-up phase II trials demonstrated a radiographic response and increased progression-free survival (Kreisl et al. 2009), as such phase III trials, including the RTOG-0825 study, are currently under way. The recent approval of bevacizumab as a therapeutic agent in the treatment of recurrent GBMs provides the foundation for future studies in this disease and the use of anti-VEGF angiogenics in the disease treatment paradigm. In the context of GBMs there

are other redundant factors which work in conjunction with VEGF to initiate the pro-angiogenic switch including PDGF, FGF, the ANG/TIE system, notch signaling, integrins, ephrins, interleukin 8, and SDF-1a (Bergers and Benjamin 2003; Carmeliet and Jain 2011a, b). As such therapeutics have also been targeted at the inhibition and blockade of these signaling pathways including the multiple tyrosine kinase receptor inhibitors such as cedirinab (AZD2171) and Sunitunib (sutent) both of which are able to pan block all tyrosine kinase receptors and as such sense both VEGF and PDGF signaling. Preliminary clinical trials, however, did not demonstrate as much promise as the VEGF inhibition exclusively and showed a greater effect in the cancer types than GBM suggesting as previously reported tumor stage and microenvironment play a great effect in what signals are initiating angiogenesis in tumor.

In addition to the micro-stage selectivity of angiogenic signals it will be important to account for the level of redundancy between angiogenic signals and any compensatory up-regulation in other signals upon inhibition of certain pathways, resulting in decreased drug effect

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(Batchelor et al. 2007; Du et al. 2008). Remaining concerns include the ability to measure the effect of drugs which have a dramatic effect on existing vasculature affecting the magnetic resonance imaging, the basis for looking at tumor volume in patients (Wen et al. 2010).

The lack of clinical trials means to-date the long-term effects of these drugs are not evident; however, preliminary data suggest that reoccurrences post anti-angiogenic treatment vary dramatically from the original tumor and are harder to treat. In addition, the invasiveness of tumor cells has been shown to increase when under angiogenic therapy possible as a result of oxygen starvation forcing them to home to other existing vessels (Mrugala 2009; Narayana et al. 2012). All of these remaining questions will be essential in optimizing the potential use of anti-angiogenics in therapy of GBMs.

Invasion of Glioblastoma Negatively Impacts Therapy and Survival

One of the hallmark features of glioma is their diffuse infiltrative nature. Both low- and high-grade astrocytomas grow as masses amongst normal brain, yet their borders are poorly defined. This invasive nature of GBM in particular contributes to the surgical incurability. This is in contrast to tumors that have metastasized to the brain, in which defined tumor borders are observed. Tumor cells, single or in groups can disseminate from the primary tumor and travel following migration routes that are defined by architecture of the brain and the related extra cellular matrix (ECM). Common routes of invasion and migration include dispersion along the white matter tracks, the basal lamina of brain blood vessels, or in between the glia limitans and the pia mater (Bellail et al. 2004). The ECM in the brain mainly consists of the polysaccharide hyaluronan and proteoglycan-based matrix, mostly hyaluronic acid-binding secreted chondroitin sulfate proteoglycans of the lectican family. This environment also lacks many ECM elements found in other organs, including most basal lamina matrix (collagens, fibronectin, and type I laminin) and supporting stromal tissue (Bellail et al. 2004). This unique brain parenchyma may explain why many tumors do not metastasize to the brain and also why primary brain tumors such as GBM do not metastasize outside the brain, perhaps due to lack of proper microenvironment. The intrinsic ability of glioma cells to deeply invade within normal brain structures poses serious clinical challenges because these cells are ultimately believed to be responsible for tumor recurrence after maximal surgery, radiation, and chemotherapy. Invasion of GBM likely involves sequential adhesion to the ECM, degradation of the ECM, and altered cell contractility/motility (Giese and Westphal 1996; Nakada et al. 2007). Glioma cells adhere to the ECM

using several methods. The immunoglobulin superfamily member CD44 and the hyaluronan-mediated motility receptor (HMMR or RHAMM) are receptors for hyaluronan and are expressed in GBM (Akiyama et al. 2001). CD44 is cleaved by both ADAM proteases (Murai et al. 2004) and matrix metalloproteinase (MMP)-9 (Chetty et al. 2012) that promotes motility. CD44 is repressed by p53 (Godar et al. 2008; Sohr and Engeland 2008), highly suggestive that cellular progression through cell cycle checkpoints and the ability to migrate/invade are intertwined. Integrins also contribute to cell invasion and migration, particularly the **a**v**b**3 and **a**v**b**5 heterodimers (Bello et al. 2001). Other factors that contribute to cell adhesion/migration and may be of therapeutic relevance include focal adhesion kinase (Riemenschneider et al. 2005; Rutka et al. 1999). MMP proteins have been implicated in altering the ECM that ultimately leads to both promotion of invasion and migration or alternatively loss of adhesion (Rao 2003). MMP-2 and MMP-9 also drive glioma invasion (Forsyth et al. 1999) and are regulated through several molecular cascades. Both enzymes promoted invasion when up-regulated via a CD95-mediated activation of AKT1 involving recruitment of Src family member Yes and p85 (Kleber et al. 2008). Collectively, a better understanding of invasion and the key mediators may allow for specific inhibitors that may reduce or prevent glioma invasion. Although in its infancy, clinical trials targeting the GBM invasion machinery are underway and may be of benefit. In particular, suberoylanilide hydoxamic acid has shown to have anti-invasion properties in vitro and in vivo through inhibition of MMP2 and MMP9 (An et al. 2010).

The Emerging Role of Tumor Metabolism

Altered glycolytic metabolism is one of most commonly altered biological processes in solid tumors. Cancer cells demonstrate a number of alterations in their metabolism to survive unfavorable and harsh microenvironments, while being able to proliferate and evade cell death (Vander Heiden et al. 2009). A classic metabolic adaptation of tumor cells is a shift to aerobic glycolysis as a main source of ATP rather than oxidative phosphorylation (OXPHOS), irrespective of oxygen availability, a phenomenon referred to as the "Warburg effect" (Warburg 1956). This phenotype may promote a state of apoptosis resistance (Kroemer and Pouyssegur 2008; Plas and Thompson 2002), the generation of biosynthetic precursors for proliferation (Vander Heiden et al. 2009), as well as increased invasive ability (Stern et al. 2002). Genetic and epigenetic alterations in key enzymes resulting in metabolic modification include primary mutations, altered isoform expression profile, and altered regulation/function secondary to

oncogenic signaling pathways or the tumor microenvironment (Vander Heiden et al. 2009). An example of alterations in isoform expression profile of metabolic enzymes is exemplified by a switch in splice isoforms from the adult pyruvate kinase M1 (PKM1) to the fetal PKM2, believed to promote aerobic glycolysis and tumor growth in lung cancer cell lines (Christofk et al. 2008). IDH1, a metabolic enzyme, was recently identified in GBM, the most common and lethal of all primary human CNS tumors (Mellinghoff et al. 2005; Stupp et al. 2005). High-throughput genomic screening of GBMs identified point mutation (predominantly R132H) in the *IDH1* gene in ~12 % of all GBMs (Parsons et al. 2008) and ~80 % of LGA or secondary GBMs that developed from their malignant progression (Watanabe et al. 2009; Yan et al. 2009). Mutation in *IDH1* results in neomorphic (not familiar with this word) activity producing a different metabolite, 2-hydroxyglutaric acid, while wild-type IDH1 normally converts isocitrate to α-ketoglutarate coupled with $NADP^+/NADPH$. The impact of this mutation and of the accumulation of the metabolite 2-hydroxyglutarate on GBM metabolism and glucose utilization and subsequent growth is still under investigation but may contribute to a hypermethylator phenotype in a subset of GBM (Dang et al. 2009; Noushmehr et al. 2010; Zhao et al. 2009). However, more than 90 % of GBMs are primary GBMs and the molecular basis of the Warburg effect in this subset of GBMs is under active investigation. An important role of the glycolytic enzyme HK2 was first demonstrated in hepatomas in which it was shown that the glucokinase isoform expressed in liver is substituted by HK2 (Mathupala et al. 2006). Similarly, several reports including TCGA dataset support up-regulation of HK2 in GBMs (Dong et al. 2005) and our own work with variable levels of HK1 expressed in normal brain. As the first enzyme of the glycolytic pathway, HK controls glucose flux into glycolysis or the PPP. HK2 is a highly regulated form of hexokinase, whose transcript is regulated by HIF1α, glucose, insulin, glucagon, cAMP, and p53, among others (Pedersen et al. 2002). Mitochondrial binding of HK2 to the outer membrane promotes its stability and reduces feedback inhibition from its product G6P (Bustamante and Pedersen 1977). HK2 interacts with voltage-dependent anion channel (VDAC) at the mitochondria and regulates the release of cytochrome *c* and intrinsic apoptosis, although the exact mechanisms of this association are not well understood (Majewski et al. 2004; Pastorino et al. 2002). Pyruvate dehydrogenase (PDH) is a mitochondrial multi-enzyme complex that catalyzes the oxidative decarboxylation of pyruvate, whose enzymatic activity is regulated by a phosphorylation/dephosphorylation cycle. The mitochondrial matrix protein Pyruvate dehyrogenase kinase (PDK1–4) is an important inhibitor of OXPHOS via

its phosphorylation of the E1 α subunit of PDH. HIF1 α transactivates PDK1 resulting in decreased conversion of pyruvate to acetyl-coA and compromising OXPHOS (Kim et al. 2006). Treatment of cancer cells with the small-molecule inhibitor of PDK dichloroacetate (DCA), currently employed for the treatment of congenital lactic acidosis, was found to activate OXPHOS and promote apoptosis in cancer cells (Bonnet et al. 2007). DCA is believed to sensitize cells to apoptosis via two mechanisms: (1) enhanced flux of electrons through the ETC resulting in greater depolarization of the mitochondrial membrane, which is generally hyperpolarized in tumor cells, and enhanced release of apoptotic cytochrome c; (2) return in OXPHOS function generating greater reactive oxygen species, up-regulating voltage-dependent K^+ channel leading to an efflux of K^+ and activation of caspases (Bonnet et al. 2007). DCA, which crosses the blood–brain barrier, has been administered in a small number of GBM patients with limited toxicity aside from a dose-dependent, reversible peripheral neuropathy (Michelakis et al. 2010). However, large, randomized controlled trials are warranted to ascertain its effectiveness in GBM patients. Furthermore, whether DCA can sensitize GBM cells to TMZ or radiation remains to be determined. The metabolic divergence between GBM and normal cells may provide novel therapeutic strategies to be exploited. Selective targeting of HK2 may have dual effects on cells by inhibiting glycolysis and promoting OXPHOS, thereby impacting proliferation as well as dissociating the anti-apoptotic interaction between HK2 and VDAC. Current agents targeting HK2 (e.g. 3-bromopyruvate, 2-deoxyglucose) have limited clinical potential in GBMs due to non-specificity (e.g. impact on HK1 or other metabolic proteins) and systemic toxicity. Newer agents currently under development are aimed at interfering more selectively the interaction between HK2 and VDAC (e.g. methyl jasmonate) (Goldin et al. 2008). However, to date, no specific inhibitor of HK2 has been developed. It also remains to be investigated whether combined targeting of metabolic enzymes, including inhibitors to HK2, PKM2, and DCA for PDK, will further enhance GBM cell death.

Future Directions and Conclusions

The past decade of research has been invaluable in increasing our understanding of GBM. However, several outstanding questions remain. First, tumor heterogeneity is still poorly characterized in GBM. GBM has both molecular and pathological heterogeneity and future studies will need to delineate the role of microenvironment and the influence it has on tumor regions. Second, based on recent evidence it is becoming apparent that there is no single cell

of origin in GBM. GBM can arise from stem cells, progenitor cells or through de-differentiation, and this may already be reflected by the observation that GBM comprises of distinct molecular subtypes. Last, overcoming therapeutic resistance is of great importance as GBM is a highly aggressive and resistant tumor. The fast pace of genomic, epigenetic, and proteomic discoveries in GBM will likely help integrate this molecular and histological data into clinical practice by improving diagnosis, prognosis, response to therapy, and ultimately delivery of more specialized and targeted therapy.

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