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Review article ■

New Insights Into Molecular Basis of Glioblastoma Multiforme and Associated Immunosuppression

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SUMMARY

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor in adults and carries the poorest prognosis despite aggressive multimodal therapy. The majority of GBMs develop de novo (primary) with a short clinical history, while secondary GBMs develop through progression from preexisting lower-grade precursor gliomas and show distinct genetic and expression profiles including the high frequency of isocitrate dehydrogenase 1 (IDH1) mutations, already present in precursor lesions.

Large-scale integrative genomic studies provided the new view that GBMs are remarkable molecularly heterogeneous tumors and identified distinct molecular entities that may lead to different therapeutic approaches. Although being restricted to the intracranial compartment, GBMs are associated with global immunosuppression. Better understanding of the immune response to GBMs growing in the immunologically distinct microenvironment in the brain and mechanisms by which they may escape the response and even suppress it will accelerate the development of more effective immunotherapies. This review summarizes the current knowledge regarding genetic alterations and signaling pathways critical to the biology of GBMs, few mechanisms of developing local and systemic GBM-induced immunosuppression, and the role of GBM stem cells.

Key words: **glioblastoma multiforme, genetics, markers, immunosuppression, glioma stem cells**

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INTRODUCTION

Malignant gliomas represent the most common primary tumors of the central nervous system in adults and carry a poor prognosis due to their propensity to infiltrate diffusely throughout the brain. The most biologically aggressive of these is glioblastoma "multiforme" (GBM) (World Health Organization /WHO/ grade IV) (1). GBM is characterized by rapid growth, widespread invasiveness, and intense resistance to radiotherapy and chemotherapy. Despite advances in surgical management, followed by radiotherapy and concomitant and adjuvant chemotherapy with temozolomide, the median survival for GBM patients is 14.6 months (2).

Molecular approaches during the past two decades greatly improved our understanding of the genetics and biology of these aggressive tumors (3, 4), but this has failed to provide required advance in effective therapy. Chemoradiotherapy is standard of the care for GBM, but there has been a growing interest in applying targeted molecular therapies, particularly focused on inhibitors of angiogenesis and growth factor receptors. Simultaneously, large-scale integrative genomic studies of GBMs uncovered new genetic alterations and signaling pathways which provided novel targets that may be used for diagnostic, prognostic, or therapeutic purposes (5, 6) and further stimulated the molecular classification of GBMs into four subtypes (7) that may lead to different treatment regimens (7, 8). Immunotherapy for GBM is another area of increasing interest. Despite being confined to the intracranial compartment, malignant gliomas appear to induce systemic and profound depression of cellular immunity that is more severe than seen with other cancers (9). Better understanding the mechanisms that GBMs evolve to evade the immune system and even suppress it may improve the efficacy of immunotherapeutic strategy. This review summarizes recent advances regarding genetic alterations and signaling pathways critical to the biology of GBMs, few mechanisms underlying GBM-induced local and systemic immunosuppression and the role of GBM stem cells.

Classification and grading of diffuse gliomas

Diffuse, infiltrative gliomas are the most common brain tumors of adults. These gliomas are classified histologically as astrocytomas, oligodendrogliomas, or tumors with morphological features of both lineages, termed oligoastrocytomas. Using the WHO criteria, they are further graded on a scale from II to IV according to degree of malignancy (1). Oligodendrogliomas and oligoastrocytomas are divided into grade II (low-grade) and anaplastic, grade III lesions. Astrocytic tumors, the most frequent type of diffuse gliomas, encompass diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III) and GBM (grade IV), the most malignant and deadly tumor (1). Low-grade diffuse astrocytoma has an inherent tendency for malignant progression to anaplastic astrocytoma and eventually secondary GBM (1). Increased cellularity, distinct nuclear atypia, and mitotic activity are distinguishing features of anaplastic astrocytoma, while, GBM along with malignant features contains areas of microvascular proliferation and /or necrosis (1).

GBMs have been subdivided into the primary and secondary GBM subtypes on the basis of clinical presentation (1, 3). The majority of GBMs develop rapidly in older patients (mean, 62 years) after a short clinical history (usually less than 3 months), and arise de novo (primary GBM) without any evidence of a less-malignant precursor lesion (1, 10). Secondary GBMs affect younger patients (mean, 45 years), and develop through progression from low-grade diffuse astrocytoma or anaplastic astrocytoma (1, 3, 10). The time to progression from low-grade astrocytoma to secondary GBM varies considerably (mean 4-5 years) (1). In a population-based study, the mean time of progression from anaplastic astrocytoma to GBM was approximately 2 years (4). Although histologically largely indistinguishable, primary and secondary GBMs develop through different genetic pathways (4, 10), show different RNA and protein expression profiles, and may differ in their response to radiotherapy and chemotherapy (4, 11). For example, primary GBMs are characterized by frequent epidermal growth factor receptor (EGFR) amplification, the phosphatase and tensin homolog (PTEN) mutations and typical loss of the entire chromosome 10 (10p and 10q) (3-5, 10), while secondary GBMs are characterized by more frequent TP53 and isocitrate dehydrogenase 1 (IDH1) mutations, as well as by loss of heterozygosity (LOH) on 10q but rarely on 10p (3, 4, 6, 12, 13).

The Cancer Genome Atlas (TCGA) project aims to profile a large numbers of GBMs at the DNA, mRNA, micro RNA and epigenetic (DNA methylation) levels (5). Integration of multidimensional genomic data has further stimulated the identification of molecular subtypes of GBM designated as classical, mesenchymal, proneural and neural (7). These molecular subtypes have potential implications for patient prognosis and response to treatment (7, 8). For example, 97% of tumors in the "classical" subtype demonstrated high-level EGFR amplification (7), while tumors in the "proneural" subtype affected younger patients and were characterized by frequent IDH1 mutations and high expression levels of genes relevant in neuronal development, including oligodendrocytic and proneural development genes (7, 14). Notably, IDH1 mutations (and to a lesser extent IDH2 mutations) were found to be very frequent in grade II-III diffuse gliomas and secondary GBMs (>80%) (12, 13, 15, 16), suggesting that secondary GBM belong to proneural subtype (6, 7, 13).

GENETIC ALTERATIONS AND SIGNALING PATHWAYS IN GBMs

This section focuses primarily on the molecular alterations underlying the development of GBMs and are relevant to the biology of these the most malignant gliomas (Figures 1, 2) (3-6, 11). The genetic alterations are linked to the activation of the receptor tyrosine kinase pathways and inactivation of the p53 and retinoblastoma (RB) tumor suppressor pathways, the key molecular pathways that altered in the majority of GBMs, suggesting that they are critical for GBM pathogenesis (3, 5).

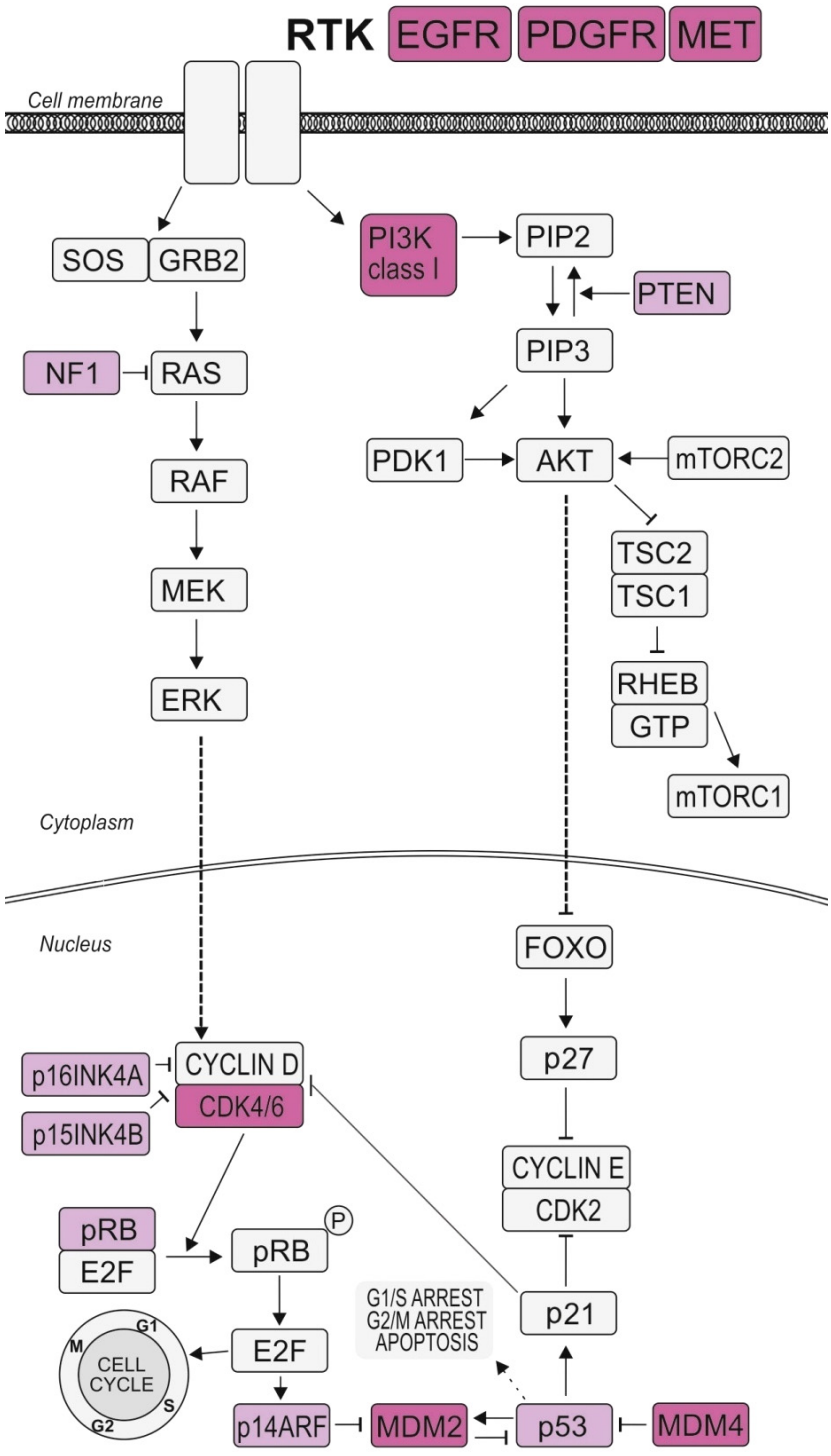

Figure 1. *Genetic alterations in glioblastomas multiforme occur frequently in three major signaling pathways: the RTK/RAS/PI3K, p53 and RB pathways (see the text for details). Dark red-violet indicates activating genetic alterations. Inactivating genetic alterations are shown in light-violet. RTK, receptor tyrosine kinase; EGFR, epidermal growth factor receptor; PDGFR, platelet-derived growth factor receptor-α; MET, mesenchymal-epithelial transition factor.*

RTK/RAS/PI3K Pathway

The EGFR and the PDGFR, members of the receptor tyrosine kinase (RTK) family, and their ligands play important roles in both central nervous system (CNS) development and gliomagenesis, and targeted therapy against growth factor receptors and intracellular signaling molecules is currently under extensive basic and clinical investigations (3, 8).

Epidermal growth factor receptor

The EGFR gene (at 7p12) encodes a 170 kDa protein, which is a transmembrane receptor with intrinsic tyrosine kinase activity. EGFR is the most frequently amplified gene in GBMs and is characteristic of primary (de novo) subtype. Amplification of the EGFR gene occurs in approximately 40% of primary GBMs, but rarely in secondary GBMs (1, 10, 17, 18). All primary GBMs with EGFR amplification showed EGFR overexpression, and most of those with EGFR overexpression (70-90%) had gene amplification (4, 19). GBMs with EGFR amplification also often harbour EGFR mutations (~40%) (17, 19-21). These display several EGFR variants, and variant III (EGFRvIII) with loss of the extracellular, ligand-binding domain, resulting from deletion of exons 2-7, is the most common. EGFRvIII is structurally and functionally similar to the v-erbB oncogene, and is constitutively activated in a ligand-independent manner, that activates persistent downstream Ras/ mitogen-activated protein kinase (MAPK) growth and phosphatidylinositol-3-kinase (PI3K) survival signaling (20). Neither EGFR amplification nor the presence of EGFRvIII can predict patient outcome in the conventionally treated GBM (22, 23). However, in patients surviving one year or longer after diagnosis, the expression of EGFRvIII was found to be an independent negative prognostic indicator (22). Thus, EGFR has been one of the most attractive targets for therapeutic intervention in GBM with the small molecule EGFR tyrosine kinase inhibitors or antibody-based immunotherapy (3, 8, 23, 24).

Platelet-derived growth factor receptor

PDGFRα and its ligands (PDGF-A and PDGF-B) are expressed in gliomas, particularly in high-grade tumors, whereas strong expression of PDGFRβ occurs in proliferating endothelial cells in GBMs (3). Notably, PDGRα overexpressin at the mRNA and protein levels and its ligands has been observed in astrocytomas of all grades closely with p53 mutations, but amplification of the PDGFRα gene (at 4q12) was only detected in a small fraction (16%) of GBMs (25), suggesting that PDGF signaling plays a role in both early stages of gliomagenesis and tumor progression. In contrast to EGFR, a relatively rare deletion mutatant of PDGFRα (loss of exons 8 and 9) that is similar to EGFRvIII in activity has been described (26). Given the co-expression of PDGF ligands and receptors in malignant gliomas, it suggests that both autocrine and paracrine loops stimulate cell proliferation and tumor growth (3, 27). The important role of PDGF in gliomagenesis has been demonstrated using retroviral PDGF-driven glioma models (27). In cell culture-based studies, PDGF has been shown to exert mitogenic efect on glioma cells and promote proliferation and migration of glial progenitors, maintaining of their undifferentiated phenotype (27, 28). Interestingly, the retroviral PDGF glioma models have not only demonstrated paracrine signaling via blood vessel recruitment but also provided evidence for the existence of paracrine signaling between PDGF-expressing tumor cells and PDGFRα + glial progenitors, which drive glial progenitors to proliferate and significantly contribute to tumor growth (27). Taken together, these findings suggest that combination therapy will be necessary to cure the human disease. The PDGFR inhibitor imatinib mesylate was reported to exert antitumor activity both in vitro and in glioma models (29). However, when used alone, the drug has demonstrated minimal activity in malignant gliomas (30).

PI3K/PTEN/AKT/mTOR Pathway

The class I PI3Ks and its major downstream effector, AKT (protein kinase B) are strongly implicated in glioma initiation and progression (31). The EGFR and other growth factor receptors, such as PDGFR, become activated upon binding of growth factors (EGF, TGF-α, PDGF) to their extracellular domain, followed by receptor dimerization and autophosphorylation of the intracellular tyrosine kinase domains, which results in recruitment of PI3K to the cell membrane (Figure 1). The EGFR and the PDGFR are the most common RTKs with intrinsic tyrosine kinase activity that are aberrantly expressed in GBMs. The class IA PI3Ks are heterodimeric complex consisting of regulatory and catalytic subunits that activated by RTKs are highly implicated in tumor cell survival (32). In GBMs, mutations in both the PIK3CA and PIK 3R1 genes which encode the catalytically active (p110α) and regulatory (p85α) subunits of PI3K complex were found to confer with increased PI3K activity (5). In addition to p85α binding, the p110α catalytic subunit can also be activated by binding to GTP-bound Ras (33). PI3K at the cell membrane converts phosphatidylinositol-4,5-bisphosphate (PIP2) to the respective phosphatidylinositol -3,4,5-trisphosphate (PIP3), which binds to phosphoinositide-dependent kinase I (PDK1) and AKT allowing their translocation to the cell membrane and subsequent activation (34). PDK1 and the mammalian target of rapamycin (mTOR), acting in the rapamycin-insensitive mTORC2 complex, activate AKT through phosphorylation of two key residues, Thr-308 and Ser-473, respectively (35). The mTORC2 also phosphorylates AKT at the turn motif site (Ser-450), that stabilizes AKT (36). PI3K is antagonized by PTEN through dephosphorylation

of PIP3, thereby preventing activation of both PDK1 and AKT (37,38). The loss of PTEN results in the upregulation of the PI3K/AKT pathway (3, 38,39). AKT phoshorylates many proteins involved in the regulation of various cellular functions including cell metabolism, growth, proliferation and survival/inhibition of apoptosis. Investigation of the phosphorylation status of two key residues of AKT is a reliable method for monitoring activity of PI3K pathway in cell lines and GBM samples, 85% of which have been reported to possess activated AKT that was highly correlated with activated NFκB (39). Several downstream targets of AKT include GSK3β, p21, p27, NFκB (40), MDM2 (41), FOXO, TSC2, BAD, and caspase 9, along with others (42), and activation of AKT plays a crucial role in gliomagenesis as shown in mouse models (43).

One of the major downstream effectors of AKT is mTOR. Notably, mTOR exists in two distinct multiprotein complexes (mTORC1 and mTORC2) (44). Activation of mTORC2 is associated directly with AKT phosphorylation. The mTORC2 is sensitive to growth factors but not nutriens, and is associated with the rapamycin-insensitive companion of mTOR (RICTOR) in addition to other proteins (44). In contrast, the mTORC1 is nutrient and growth factor sensitive, and is composed of proteins such as regulatory associated protein of mTOR (RAPTOR), which is rapamycin-sensitive. Function of mTORC1 is regulated by PI3K/AKT pathway through the function of tuberous sclerosis 2 (TSC2) within TSC1-TSC2 complex (34, 40, 42, 44). Activated AKT phosphorylates and inactivates TSC2, which acts as a GTPase-activating protein (GAP) for RHEB (Ras homolog enriched in brain). Inactivation of TSC2 allows RHEB to accumulate in the GTP-bound state, directly activating the mTORC1 (Figure 1). The mTORC1 via its downstream targets, 4E-binding protein 1(4E-BP1) and ribosomal protein S6 kinase 1 (S6K1), regulates translation of mRNAs of many cell cycle regulators such as MYC, cyclin D1, and hypoxia inducible factor-1α (HIF-1α), subsequently leading to proliferation and angiogenesis (40, 45, 46). Downstream effectors of mTOR thus promote protein translation and hypoxic adaptation. Levels of p70S6K have been shown to correlate with reduced overall survival time in GBM patients (47). Other studies have reported that p70S6K was associated with decreased overall survival time on univariate, but not multivariate analysis (48). Since aberrant PI3K/AKT/mTOR signaling is highly implicated in gliomagenesis, the mTOR has served as attractive therapeutic target. Inhibitors of mTOR, such as rapamycin or its analogs have been evaluated for treatment of GBMs, but as single agents, they provided limited clinical benefit (49, 50). The mTORC1 effector S6K1 via inhibitory phosphorylation of the insulin receptor substrate-1 (IRS-1) inhibits AKT function (46,51). Conversely, inhibition of the mTORC1 disrupts the negative feedback loop via IRS-1, resulting in AKT activation (44, 51). Additionally, prolonged suppression of the mTORC1 activates the mTORC2, via the AKT pathway, and can activate the MAPK pathway (52). Altogether, these findings provide the basis for combined inhibitors in GBM treatment (53).

PTEN functions as an important tumor suppressor that negatively regulates PI3K/AKT pathway by dephosphorylating the lipid second messenger PIP3 to PIP2 (37), and loss of PTEN function results in increased levels of PIP3, leading to enhanced activation of AKT (38, 54). Recently, in the mouse astrocytoma model ($NF1^{loxP/+}$; $p53^{-/+}$) was shown that PTEN haploinsufficiency accelerated the formation of high-grade tumors (grade III astrocytomas), whereas loss of the remaining PTEN allele and AKT activation produced grade IV tumors (55), suggesting a role of PTEN deficiency in gliomagenesis and tumor progression. Several studies have shown that losses on chromosome 10 (loss of the PTEN locus) or enhanced PI3K/AKT signaling are associated with poor outcome in GBM (14, 47, 48, 56). Somatic nucleotide substitutions in the PIK3CA gene were detected in 6 out of the 91 sequenced samples of GBMs (5). Some of detected deletions imposed spatial constrains that may result in PI3K constitutive activation (5). The TCGA project identified somatic mutations in the PIK3R1 gene, encoding regulatory p85α subunit of PI3K, in 9 out of the 91 sequenced GBMs (5). Importantly, in these GBMs, the PIK3CA and PIK3R1 mutations were mutually exclusive, suggesting a functional redundancy of these mutations as they both activate PI3K. PIK3CA and PIK3R1 gene alterations were independently reported in 8-10% of GBMs (6). The frequency of PIK3CA mutations was reported to be similar in primary and secondary GBMs (57). Amplification of the PIK3CA gene was found to be rare and generally mutually exclusive with gene mutations (57). While AKT3 amplification was recently detected in low frequency of GBM samples (2%) (5), a somatic mutations in the AKT1 gene could not be identified (5, 58), indicating that AKT activation in most GBMs mediated through aberrant PI3K signaling and by other possible mechanisms, but not by AKT activating mutations.

PTEN - multi-modal tumor suppressor

The PTEN gene (at 10q23.3) encodes a protein that is a phosphatase with dual lipid and protein specificity. The central domain of PTEN is homologous to the catalytic region of protein tyrosine phosphatases and has been demonstrated to possess both protein phosphatase and 3-phosphoinositol phosphatase activities (28, 37). The N-terminal domain of PTEN, with homology to tensin and auxilin, is important in regulating cell migration and invasion by directly dephosphorylating focal adhesion kinase (FAK) (59).

PTEN is frequently inactivated in malignant gliomas, particularly GBMs, which has been associated with increased invasion, tumor cell survival and proliferation through increased activation of the PI3K/AKT pathway (3, 31, 34, 56) and with increased angiogenesis, confir-

med in a number experimental studies *in vitro* and *in vivo* (45, 55, 60). Substantiating these data, studies have shown that the key glioma relevant mutations, including those that affect the PTEN and EGFR genes, may act as an "angiogenic switch" by stabilizing HIF-1α or one of its downstream targets, vascular endothelial growth factor (VEGF) (31). Several studies have pointed to additional levels at which PTEN actions appear to be relevant for suppression of tumor progression. PTEN have been reported to bind directly to p53 resulting in stabilization and stimulation its transcriptional activity, most likely by enhancing p53 acetylation (31, 54), a post-translational modification that promotes the p53-mediated transcription (61). Additionally, PTEN appears to play an important role in the maintenance of chromosomal integrity and DNA repair (62), suggesting that loss of PTEN can cause genomic instability.

PTEN mutations have been reported in 15-40% of primary GBMs, but rare in secondary GBMs (4, 10). Interestingly, most of missense PTEN mutations are located in the region homologous to tensin, auxilin, and dual-specificity phosphatases (10). In contrast, secondary GBMs display a high incidence of PTEN promoter methylation (63). This epigenetic alteration, associated with increased AKT phosphorylation, is also present in low-grade gliomas, and appears to correlate with the time and incidence of progression of these tumors (31, 63). PTEN promoter methylation is a rare occurrence in primary GBMs (63). A recent genomewide mutation analysis of GBMs revealed that PTEN gene is frequently altered (30%), in addition to EGFR (37%) gene (6). Alterations in at least one of EGFR, PTEN or PIK3CA genes were observed in about two-thirds of primary and one-third of secondary GBMs (57).

PI3K and MAPK pathways interactions

The PI3K/AKT signaling pathway cross-talks with the MAPK pathway via Ras, which activation induced by RTKs is a common feature of GBMs (28, 64). RTK activation results in receptor dimerization and autophosphorylation, creating binding sites for adapter protein complex such as Grb2 and factor SOS, which subsequently activates Ras. EGFRvIII binds to the Grb2 and Shc proteins constitutively (23). Ras-GTP in turn binds to and phosphorylates Raf kinase and initiates the MAP-kinase phosporylation cascade via MEK and ERK (Figure 1). ERK1 and ERK2 kinases phosphorylate a several cytoplasmic and nuclear proteins such as S6Ks and transcription factors ELK-1 and ETS-2 (65). The Ras signaling pathway governs cell survival and proliferation by inducing the expression of genes promoting cell cycle progression through activating MAPK phosporylation cascade and the AP-1 transcription factor (66). Proliferative and survival signaling can be transduced by the MAPK and PI3K/AKT pathways by RTKs and integrins, membrane-bound extracellular matrix receptors (3, 45). In GBMs, a few Ras mutations have been identified (2%) (5), although high levels of active Ras-GTP are found (67), suggesting that activation of Ras and its downstream signaling effects is a consequence of aberrant tyrosine kinase receptor activation (EGFR, PDGFR, MET, etc.), which are frequent events in GBM (65). Inactivation of NF1, a negative regulator of Ras activity, also results in increased MAPK signaling (68, 69). Additionally, YKL-40, a secreted protein that is a product of one of the most expressed genes in GBMs and a prognostic marker in these tumors, is a possible candidate for signaling via Ras (48). Elevated expression of activated MAPK was found to be associated with increased radiation resistance, indicating that it stands for an independent prognostic factor in GBMs (48). Ras/MAPK and PI3K/AKT signaling pathways are critical in the malignant phenotype of GBM, and have been reported to promote cell proliferation, survival, invasiveness, and resistance to radiation (3, 47, 48, 56). Therefore, Ras and its down-stream effectors provide as attractive targets for novel therapeutic strategies.

NF1 - GBM suppressor gene

The NF1 gene (at 17q11.2) encodes a protein, termed neurofibromin, that acts primarily as a negative regulator of Ras (Figure 1), but also plays a role in adenylate cyclase and AKT/mTOR mediated pathways (68). This protein contains a region with homology to GTPase-activating proteins of the Ras family, which facilitate conversion of Ras from an active to an inactive state. Inactivation of NF1 permits unopposed Ras function, thereby promoting cell growth. Neurofibromatosis type 1 is one of the most common familial tumor syndromes affecting the nervous system, with an incidence of 1 in 3000. Patients with NF1 have increased risk of developing pilocytic astrocytomas (WHO grade I), particularly of the optic nerve, and also diffuse astrocytomas and GBMs, albeit with lower frequency (69). Recently, inactivating mutations or deletions in the NF1 gene were identified in sporadic human GBMs (5). Neurofibromin thus acts as an important tumor suppressor in GBMs.

The TCGA project identified additional genetic alterations in (mostly primary) GBMs, including PIK3R1 mutations (10%), NF1 mutations /homozygous deletions (18%) and ERBB2 mutations (8%), with the overall frequency of alterations in the RTK/RAS/PI3K signaling pathway in 88% of GBMs (5). In addition to EGFR mutations/amplification (45%), PDGFRA and MET showed frequent amplifications, 13% and 4%, respectively, as well as PTEN mutations/ homozygous deletions (36%) (5). Additionally, PI3K (class IA) mutations (15%), AKT amplification (2%), RAS mutations (2%) and FOXO mutations (1%) were identified (5).

The p53 Pathway (p53/MDM2/MDM4/p14ARF)

The TP53 gene (at 17p13.1) encodes the multimodally acting tumor suppressor p53. The p53 plays an important role in many cellular processes, including the cell cycle, response to DNA damage, apoptosis, and cell differentiation (70), as well as in cell motility/invasion, glycolysis, angiogenesis, development and aging (71). Following cellular stress, such as DNA damage, p53 ("the guardian of the genome") is stabilized acting primarily as transcription factor that induces the expression of target genes, predominantly mediating in cell cycle arrest, DNA repair, and apoptosis (72). Several notable p53 target genes include the cell cycle regulators CDKN1A (also known as p21WAF1/CIP1) and GADD45, and pro-apoptotic factors PUMA and NOXA, among others (71-74). The cell-cycle arrest caused by the p53-induced transcription of the cyclin-dependent kinase (CDK) inhibitor p21 occurs in the G1 phase. If the DNA damage cannot be repaired, the activity of p53 is directed to induction of apoptosis, preventing the propagation of DNA mutations in cells. It should be noted that p53-induced PUMA down regulates p21WAF1/CIP1 (74). Although somatic mutations in this gene have not been found in gliomas, its expression is frequently abrogated by p53 functional inactivity and also by mitogenic signaling via the MAPK and PI3K/AKT pathways (3). The p53 protein regulates the expression of many target genes, including those encoding anti-proliferative, pro-apoptotic, and anti-angiogenic proteins, and causes cell-cycle arest in the G1/S and/or G2/M phase through distinct downstream targets (71). Many post-translational modifications of p53 have been described, of which, for example, phosphorylation and acetylation promote the expression of p53 transcriptional targets, whereas others, such as ubiquitination, sumoylation, and neddylation associated with the suppression of p53-mediated transcription and p53 nuclear export (61). The p53 is actually a member of the family proteins. This p53 protein family consists of three transcription factors: p53, p63, and p73, which share significant structural and functional similarities (71). The p63 and p73 proteins share 63% identity with p53 in the DNA-binding domain, and can cause cell-cycle arrest and induce apoptosis. All members of p53 family were reported to have several isoforms (71). The exact role of each p53 isoform in the regulation of p53 function remains to be elucidated.

The p53 pathway is crucial for effective tumor suppression, and mutations in TP53 that compromise p53 function occur in more than 50% of human tumors. TP53 mutations are genetic hallmarks of secondary GBM (65%), and in similar frequency they are already present in precursor low-grade astrocytomas (4, 10, 18), suggesting that loss of p53 is an early event in gliomagenesis. The mutation frequency in primary GBM is lower (28 %) (4, 10). In secondary GBMs, 57% of mutations were found to be located in hotspot codons 248 and 273, whereas in primary GBMs, mutations were more evently distributed (10). G: C >A: T mutations at CpG sites were significantly more frequent in secondary than in primary GBMs, suggesting that the acquisition of TP53 mutations in GBM subtypes may occur through different molecular mechanisms (10). TP53 mutations are clustered in the DNA-binding domain, thus affecting the ability of p53 to activate transcription. In tumor cells lacking activities of wild-type (wt) p53, genomic instability facilitates the accumulation of additional molecular alterations enhancing the neoplastic phenotype. The importance of p53 in gliomagenesis is also underscored by the occurrence of astrocytomas in Li-Fraumeni syndrome, a familial tumor syndrome associated with TP53 germline mutation (75).

MDM2 and MDM4 - negative regulators of p53

The important regulator of p53 is a RING finger domain containing oncoprotein MDM2 (76, 77). The MDM2 gene at 12q14.3-15 encoding a 54 kDa protein contains a p53 DNA-binding site, and the transcription of MDM2 is induced by wt p53. MDM2 binds to mutant and wt p53 proteins, thereby inhibiting the ability of wt p53 to activate transcription (76). In normal cells, this autoregulatory feedback loop regulates both the activity of p53 protein and the expression of the MDM2. This protein is a potent inhibitor of p53 by binding to the transactivation domain and also promotes the rapid degradation of p53 through ubiquitination and subsequent proteasome-mediated degradation (76, 78, 79). MDM2, that is a p53-specific and exhibits E3 ubiquitin-protein ligase activity, was reported to be the major negative regulator of p53 (76, 77). In addition, MDM2 may oppose the function of p53 indirectly through interaction with the E2F1 and RB family proteins p107 and RB1 (76, 78, 79). Thus, amplification of MDM2 constitutes an alternative mechanism for inactivation of the p53 pathway, which occurs in <15% of primary GBMs that lack a TP53 mutation (11). In the p53-MDM2 regulatory network also implicated MDM4 and tumor suppressor p14 ARF (76, 80-82).

The MDM2-related gene (at 1q32), MDM4 (also called MDMX) encodes a MDM4 RING finger domain containing protein which also contains an N-terminal p53 -binding domain and regulates p53 activity (76, 77, 82). MDM4 functions by directly inhibiting p53-transcriptional activity and enhancing the ubiquitin ligase activity of MDM2 (76, 77, 82). MDM2 and MDM4 are structurally similar proteins, and both interacting through their RING domains to form heterodimers, however, the MD M4 RING domain does not have E3 ubiquitin ligase activity (61, 77, 82). Also, unlike MDM2, transcription of the MDM4 gene is not induced after DNA damage, and its promoter apparently lacks p53-responsive elements (76). MDM4 amplification is infrequent, occurring in 4% of

those GBMs with neither TP53 mutation nor MDM2 amplification (3). Given the major role of MDM2 in p53 regulation, targeting the p53-MDM2 interaction with small molecule inhibitors of MDM2 and p53 activators to restore activity of p53 provides a promising therapeutic strategy (83). The combined use of MDM2 and MDM4 antagonists in tumor cells expressing wt p53 should lead to more potent activation of p53 (77).

p14ARF - positive regulator of p53

The p14/ARF (at the CDKN2A locus, 9p21) gene encodes a protein which blocks the degradation of p53 through direct binding to MDM2, thereby stabilizing p53 (80, 81, 84). This tumor suppressor antagonizes the E3 ubiquitin ligase activity of MDM2 oncogene leading to protective responses that depend on the p53 functions as a transcriptional factor. In turn, expression of p14ARF is negatively regulated by p53 (80, 84). In response to oncogenic stress induced p14ARF acts as an upstream regulator of the p53/MDM2 feedback loop; it binds to MDM2 leading to activation the p53-dependent expression of p21 and cell-cycle arrest (84). The p14ARF negatively regulates MDM2 function by promoting its degradation or sequestering it into the nucleolus, or by both mechanisms (71). Promoter methylation of p14ARF was reported to be frequent in secondary GBMs, and was already present in one third of precursor low-grade astrocytomas (85). This epigenetic alteration is more frequent in secondary than primary GBMs. Loss of p14 ARF expression due to homozygous deletion or promoter methylation is frequent in primary GBMs (50%) (85). Thus, loss of p14ARF presents an additional mechanism for p53 functional inactivation. Additionally, functional mapping of 1p36.22-32 identified the gene for the chromodomain helicase DNA-binding domain 5 (CHD5), which has been shown to maintain p53 levels by facilitating expression of p14ARF, presenting a positive regulator of p53-mediated pathway (86).

Therefore, inactivation of the p53 pathway may result from altered expression of any of the TP53, MDM2, MDM4 or p14/ARF genes. In fact that both MDM2 amplification and p14ARF homozygous deletion induce the functional inactivation of p53 and are therefore functionally redundant, probably explains why it has been found that both alterations were mutually exclusive (87). Alterations at least in one of TP53, MDM2, or p14/ARF genes in the p53 pathway was found in 53 % of primary GBMs, and in 71 % of secondary GBMs (88). The TCGA project (mostly primary GBMs) showed that the overall frequency of genetic alterations in the p53 signaling pathway in GBMs was 87%: TP53 mutation or homozygous deletion (35%), MDM2 amplification (14%), MDM4 amplification (7%), CDKN2A (p14/ARF) homozygous deletion or mutation (49%) (5). Among 91 sequenced GBM samples, genetic alterations in TP53 were mutually exclusive of those in MDM2 or MDM4, but not of those in CDKN2A (p14/ARF) (5), and this finding was independently confirmed in another study (6).

The RB1 Pathway (p16INK4A/CDK4/CDK6/RB1)

The retinoblastoma (RB1) gene (at 13q14) encodes the107 kDa protein that plays a key role in regulating the cell cycle. In quiescent cells, hypophosphorylated RB1 is bound to E2F (E2F is a family of transcription factors), preventing the transcription of genes essential for progression through the G1/S restriction point (84, 89). Upon mitogenic stimulation, the activation of the MAPK pathway leads to the induction of cyclin D1, which subsequently complexes with the CDK4 and CDK6. The activated cyclin D/CDK complexes phosphorylate the RB1 protein, allowing release of the E2F transcriptional factor that activates target genes involved in the G1 → S cell-cycle transition (73, 89). The activated cyclin E/CDK2 complex further phosphorylates RB1 protein, enabling cell entry into S-phase and the cell-cycle progression. Negative regulation of the cyclin/CDK complexes activity is accomplished by CDK inhibitors such as p21 and p27 (Figure 1). Somatic mutations in the p27KIP1 gene have not been reported in gliomas, but the activation of PI3K/AKT pathway leads to down-regulation of p27. Activated AKT by phosphorylation of the FOXO transcription factors promotes their exclusion from the nucleus, thereby reducing the expression of target genes, including CDK inhibitors p21WAF1/CIP1 and p27KIP1, and the RB family member p130 (3, 42, 90).

The CDKN2A locus encodes two proteins, p16 INK4A and p14ARF (alternative reading frame), which block the cell cycle and act as tumor suppressors. The p16INK4A protein acts as inhibitor of both CDK4 and CDK6 (73, 84). By antagonizing the activities of G1 CDKs, p16INK4A blocks the phosphorylation of RB1, causing cell-cycle arrest at late G1 phase. The characterization of the region on chromosome 9p harbouring CDKN2A revealed the presence CDKN2B gene, that encodes p15INK4B, a closely related CDK inhibitor (28, 84). Two additional INK4 proteins designated as INK4C and INK4D also act as inhibitors of CDKs (84).

Therefore, the loss of normal RB1 function may result from altered expression of any of the CDKN2A (p16/INK4A), CDKs, or RB1 genes. Promoter methylation of the RB1 gene was found to be significantly more frequent in secondary (43%) than in primary (14%) GBMs, and correlated with loss of RB1 expression (91). LOH on 13q (including the RB1 locus) was reported in 12% of primary and 38% secondary GBMs (4). Amplification of the CDK4 gene (at 12q13-14) accounts for the functional inactivation of RB1 in about 15% of high-grade gliomas, while CDK6 (at 7q21-22) amplification occurs at a low frequency in those gliomas without CDK4 amplification or loss of RB1 (28, 92). Loss of RB1 function is also frequent through the inactivation of p16/INK4A by

homozygous deletion or promoter methylation (3, 28, 85, 87).

Homozygous p16/INK4A deletion is more frequent in primary than secondary GBMs, and there is a positive correlation between this genetic alteration and EGFR amplification (4, 10). In the study of 220 primary GBMs, CDKN2A deletion was found in 68 of 188 (36%) GBM samples (87). A recent integrated genome analysis revealed that CDKN2A was one of the most frequently altered genes (altered in 50% of GBMs) (6). The deletion of CDKN2A locus inactivates not only p16/INK4A but also the p14/ARF gene, resulting in disruption of both p16INK4A/CDK4/RB1 and p53/MDM2/p14ARF pathways (81, 87). Homozygous p16/INK4A deletion, CDK4 amplification, and loss of RB1 were largely mutually exclusive, and these alterations was reported to be common in GBMs at an overall frequency of 50% in primary and about 40% in secondary GBMs (11). The TCGA project (mostly primary GBMs) showed that the overall frequency of genetic alterations in the RB signaling pathway was 78%: CDKN2A (p16/INK4A) homozygous deletion or mutation (52%), CDKN2B (p15/INK4B) homozygous deletion (47%), CDKN2C (p18/INK4C) homozygous deletion (2%), CDK4 amplification (18%), CDK6 amplification (1%), CCND2 (CYCLIN D2) amplification (2%), RB1 homozygous deletion or mutation (11%) (5). A frequent codeletion of the genes two closely related INK4 family members, CDKN2A and CDKN2C were recently detected in GBM cell lines and tumor samples (93).

IDH1 and IDH2 mutations

The IDH1 gene (at 2q33) encodes NADP-dependent isocitrate dehydrogenase 1 (IDH1), which catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG), resulting in the production of NADPH. Of the three IDH isoforms, IDH1 is localized within the cytoplasm and peroxisomes, whereas IDH2 and IDH3 are localized to the mitochondria. In a recent genomewide analysis of GBMs, somatic mutations at codon 132 of the IDH1 gene were first identified in a fraction of tumors, most frequently in secondary GBMs (6). Subsequent studies demonstrated that IDH1 mutations (and to a lesser extent IDH2 mutations) are very frequent (>80%) in grade II-III diffuse gliomas and secondary GBMs (12, 15, 16). Mutations in IDH1 (R132) and IDH2 (R172) are located in the active site of enzymes, and both decrease enzyme activities in vitro (15). Forced expression of mutant IDH1 in cultured cells reduced the formation of α-KG and increased the levels of HIF-1α, a transcription factor that facilitates tumor growth (94). However, a more recent study has not found that HIF-1α target genes were upregulated in IDH1 mutant gliomas when compared with wt IDH1 gliomas (13). One study showed that the gain-of-function ability of IDH1 mutant to catalize the NADPH-dependent reduction of α-KG to R(-)-2-hydroxyglutarate (2HG) led to the accumulation of 2HG, which potentially contributed to formation and malignant progression of gliomas (95).

Several studies reported the high frequency of IDH1/2 mutations in secondary GBMs as well as in diffuse astrocytic, oligodendroglial, and oligoastrocytic gliomas of grades II to III (12, 13, 15, 16, 96-98). In addition to IDH1/2 mutations, >60% of low-grade diffuse astrocytomas harbour a TP53 mutation, and about 70% of oligodendrogliomas show the loss of 1p/19q, whereas, oligoastrocytomas harbour either a TP53 mutation (40%) or loss of 1p/19q (45%) (1, 16, 98, 99). IDH1/2 mutations in diffuse gliomas (grade II) occur at a very early stage, likely before TP53 mutations or 1p/l9q loss, suggesting that these tumors share a common progenitor cell population (16, 98, 99), or these gliomas may derive from a stem cell that can give rise to both astrocytic and oligodendroglial lineages (15).

In contrast to secondary GBMs, only 3% to 7% of primary GBMs harbor IDH1 mutations (12, 15, 16, 97). The small fraction of primary GBMs with IDH1 mutations occurred in significantly younger patients in comparison to those with primary GBMs harbouring wt IDH1 (12, 13, 15, 97). Interestingly, these mutations are very rare or absent in other tumors of the CNS, such as pilocytic astrocytomas and ependymomas (12, 15, 16). The IDH1 /2 mutations are reliable molecular markers for secondary GBMs, and using the presence of IDH1/2 mutations as a diagnostic criterion, secondary GBMs account for approximately 10% of all GBMs (12, 15, 97, 99). In addition, IDH1/2 mutations have important clinical implications as predict more favorable outcome of GBM patients (6, 15, 97, 100, 101) and patients with anaplastic astrocytomas (15, 101). It has been suggested that secondary GBMs share a common progenitor cell population with diffuse gliomas, while primary GBMs may have a different cell of origin; the simmilar histological phenotype of both GBM subtypes may reflect in common genetic alterations, including the loss of tumor suppressor genes on chromosome 10q (99).

Loss of heterozygosity (LOH)

The most frequent genetic alteration in GBMs is LOH on chromosome 10q, occurring in about 70% of primary and >60% secondary GBMs (4, 10, 87). LOH 10q also typically co-presents with any of the other genetic alterations (4, 10) (Figure 2). LOH 10p occurs almost exclusively in primary GBMs, and loss of the entire chromosome 10 is typical for this subtype, while LOH 10p is very rare occurrence in secondary GBMs (4). The LOH studies identified three commonly deleted loci, i.e. 10p14-15, 10q23-24 (PTEN), and 10q25-qter, suggesting the presence of several tumor suppressor genes that may play roles in the pathogenesis of GBMs (4). LOH at 10q25-qter was reported to be associated with histologically recognized progression from low-grade or anaplastic astrocytoma to GBM (4), suggesting that the tumor suppressor gene(s) in this region may be important in

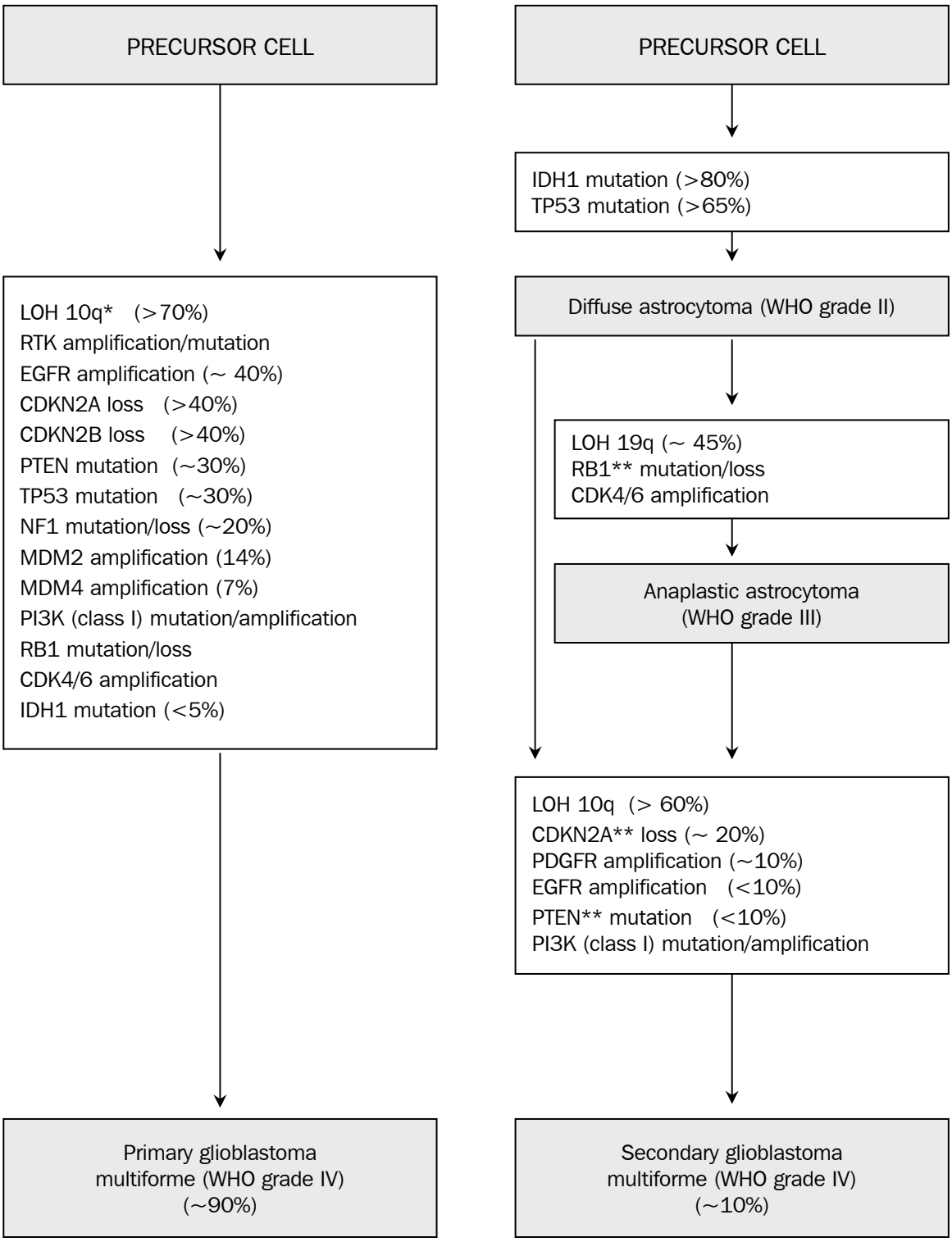

Figure 2. *Genetic, epigenetic and chromosomal alterations involved in the development of primary and secondary glioblastomas multiforme (GBMs). Both GBM subtypes arise from precursor cells that may be distinct. LOH, loss of heterozygosity. LOH 10q* in primary GBM typically with LOH 10p (see the text for details). **Promoter methylation of RB1, PTEN, p16/INK4A and p14/ARF (CDKN2A locus) is significantly more frequent in secondary than in primary GBMs.*

GBM phenotype of both subtypes (99). LOH 10q is also frequent in anaplastic astrocytomas (35-60%) (1). Several candidate tumor suppressor genes are located on 10q distal to the PTEN gene, including DMBT1, MXI1, LGI1, WDRI1, FGFR2 and others (4, 13). The DMBT1 (deleted in malignant brain tumors 1) gene (at 10q 25.3-26.1), encoding a member of the SRCR superfamily that may play a role in the evolution of chromosomal instability, is homozygously deleted in 13-38% of GBMs (1, 102). Homozygous deletions of the FGFR2 gene at 10q26.13 was recently found in 2 primary GBMs, with reduced FGFR2 mRNA levels being a frequent finding in primary GBMs and linked to poor outcome (13). The molecular mechanisms leading to the frequently reduced expression of this gene in GBMs remain to be elucidated.

Deletion of chromosomal region at 9p23-24.1 is frequent in GBMs, where the tumor suppressor PTPRD gene is located. This gene encodes a receptor protein thyrosine phosphatase, which is also mutated in 6% of GBMs and frequently inactivated (37%) by promoter methylation (103, 104). LOH 19q is more frequent in secondary (54%) than in primary (6%) GBMs, while LOH 1p is rare in both primary (12%) and secondary (15%) GBMs (4), but is associated with longer survival (105). In study of 220 primary GBMs, LOH on 19q and 1p were found to be more frequent, 29% and 19%, respectively (87). Recent studies revealed that the loss of the NOTCH2 gene maped on 1p11 is a predictor of longer survival in subtypes of oligodendroglioma and GBM, suggesting involvement/engagement of less aggressive Notch2-independent pathways (106). Conversely, gain of Notch2 was associated with less differentiated and more malignant forms of astrocytomas and GBMs, indicating dual functions for NOTCH2 in gliomagenesis (106).

LOH 22q is more frequent in secondary (82%) than in primary (41%) GBMs (107). In primary GBMs have been identified two minimally deleted regions at 22q12.3-13.2 and 22q13.31, while in 22 of 23 secondary GBMs the same small deletion at 22q12.3 was present, a region in which the TIMP-3 (tissue inhibitor of metalloproteinases-3) is located (107). TIMP-3 promoter methylation was also more frequent in secondary than in primary GBMs and correlated with loss of TIMP-3 expression (107). LOH on 22q also occurs in low-grade and anaplastic astrocytomas (20-30%), suggesting the presence of tumor suppressor gene that plays a role in the early stages of astrocytoma progression (1).

GBM - ASSOCIATED IMMUNOSUPPRESSION

Despite being confined to the intracranial compartment, malignant gliomas such as GBMs appear to induce systemic depression of cellular immunity in patients that is notably severe (9). The features of the CNS may be more appropriate considered as immunologically distinct rather than an immune-privileged site. Thus, the brain represents a unique tumor microenvironment. Malignant gliomas, especially GBMs develop diverse strategies to escape or evade the immune system and even suppress it. For example, GBM cells produce several "classic" immunosuppressive cytokines, such as TGF-β that exerts effect on multiple and complex inhibitory functions. Brain tumor-infiltrating lymphocytes (TILs) do not yield consistent correlation with clinical outcome. In addition, tumor-associated macrophages (TAMs) within the GBM microenvironment have been found to release proangiogenic factors and other factors that facilitate tumor growth and invasion, angiogenesis, and suppress antitumor immune activities (108).

Mechanisms of GBM immune escape

The impairments in immunity of GBM patients is manifested as cutaneous anergy, lymphopenia, decreased T-cell responsiveness, increased fraction of regulatory T cells (Tregs) in the peripheral blood of patients (and also within GBM TILs) and by the inappropriate or dysregulated cytokine secretion (9, 109, 110). There is a multitude of potential immune escape mechanisms in GBMs, including the production of immunosuppressive factors such as TGF-β, IL-10, prostaglandin E2 (PGE2), and gangliosides by tumor cells, the induction of Tregs, the "eduction" of TAMs to exhibit protumoral and immunosuppressive activities and impaired function of antigen presenting cells leading to loss of T-cell effector function (108-110). Defective T-cell function represents one of the major mechanisms of tumor escape and one of the critical factors limiting the success of tumor vaccines in patients.

The downregulation of MHC expression, impaired ability to process and present MHC-compatible antigen, or upregulation of immunosuppressive molecules may contribute to the GBM immune escape (9, 110). GBM cells may avoid T-cell recognition by downregulation of MHC class I expression or by their impaired ability to antigen presentation, which may concomitantly inhibit natural killer (NK) cell activation. Interestingly, both downregulation of MHC class I and antigen-processing machinery (APM) molecules were demonstrated in GBM specimens (111, 112). The altered expression of the B7 family molecules is also often involved in malignant glioma immune evasion. One study showed that GBM cells express elevated levels of MHC but lack expression of B7 costimulatory molecules (113). Other authors have demonstrated that malignant gliomas express the immunosuppressive protein B7 homolog 1 (B7-H1), also known as the programmed death receptor ligand-1 (PD-L1) that through interaction with PD-1 receptor expressed on tumor-specific T cells may contribute to immunoresistance. Specifically, T cells undergo apoptosis upon interaction of PD-1 with its PD-L1/B7-H1 ligand. The expression of B7-H1 has been associated with genetic

alteration in glioma (114). The loss of PTEN, a common alteration in GBMs (3-6, 10), increases B7-H1 expression through activation of the PI3K pathway (114). B7-H1 expression can be upregulated by IFN-γ, and this could explain, at least partially, why IFN-γ as a therapeutic agent has not been effective for most cancers (109).

The activating receptor NKG2D, expressed by NK cells and some CD8+ T cells, has a role in the killing of NKG2D ligand-expressing tumor cells (115). Specifically, NKG2D can induce cytotoxic function mediated by NK cells, and provides costimulatory signals to TCR-mediated activation CD8+T cells. Ligands for NKG2D include MHC class I-chain-related molecules A and B (MICA and MICB) and the UL16-binding proteins (ULBP) 1-4, the expression of which is induced by cellular stress. Recent data indicate that malignant glioma cells express MICA/B and ULBP1-3 ligands (115, 116). The levels of MICA and ULBP2 expression was demonstrated to correlate negatively with increasing WHO tumor grade (116). Glioma cells that concomitantly express of MHC I appear to be protected from NKG2D-mediated lysis (9). Additionally, TGF-β produced by GBMs appears to downregulate NKG2D on NK cells and CD8+T cells, rendering them less efficient at tumor cell killing. Furthermore, soluble MICA/B ligands that are detectable in the serum of GBM patients can bind NKG2D and cause receptor internalization, decreasing its surface expression (115).

Of the major immunosuppressive cytokines, TGF-β and IL-10, that are produced by tumor cells and immunosuppressive cells (109, 115), TGF-β appears to be particularly implicated in GBM-related immune-escape mechanisms. TGF-β (TGF-β1 and TGF-β2) has an array inhibitory functions including suppression NK and T-cell proliferation and antitumor function, suppression of the IL-2-dependent generation of cytotoxic T lymphocytes (CTLs) from peripheral blood lymphocytes and TILs, inhibition of IL-2 receptor expression on T-cells, reduction in production of IFN-γ, downregulation of MHC II expression and antigen presentation, and suppression of Th1-type cytokine production (9, 110).

The TGF-β is multifunctional cytokine that directly suppresses NK and T-cell proliferation and antitumor functions, but also promotes other suppressive cells (110). TGF-β has been shown to selectively downregulates MICA and ULBP2 expression on malignant glioma cells (116), while upregulates the expression of lectin-like transcript-1 (LLT1), a ligand for the inhibitory NK cell receptor CD161 (117). Malignant gliomas also express HLA-E and HLA-G which can inhibit tumor cells lysis (109, 110). Thus, dysregulated antitumor immune response in GBM patients probably leads to a shift away from Th1-type cytokines to the production of Th2-type cytokines with antitumor immunosuppressive functions. It may be concluded that GBMs utilize several mechanisms to disrupt both innate and adaptive immune responses. The molecular mechanisms that mediate the initiation and propagation of local and systemic immunosuppression have not been fully established. The signal transducer and activator of transcription 3 (STAT3) activation in both tumor cells and tumor-infiltrating immune cells appears to be critical although not only factor, which will be discussed in detail later.

Immune suppression by STAT3 in GBM

The STAT3 is constitutively activated in a variety of tumors, including GBM, and is believed to play important role in the tumorigenesis (118). Activated by growth factors, multiple cytokines, or other exogenous stimuli, such as hypoxia, STAT3 contributes gliomagenesis through promoting tumor cell proliferation and survival/ preventing apoptosis, stimulating angiogenesis and invasion, as well as acting as one of the key regulators of immunosuppression (118-120).

Growth factors and cytokines, including EGF and IL-6, activate Janus kinase 2, which subsequently activates STAT3 by phosphorylation of the tyrosine residue at position 705. Phosphorylated STAT3 (STAT3-dimer) translocates to the nucleus and induces expression of a variety of target genes (118). STAT3 activation may induce production of VEGF and cytokines such as IL-10 and IL-6 by tumor cells, which in turn may activate a STAT3-mediated expression of genes in a variety of immune cells, leading to impaired cytotoxicity in both innate and adaptive immune responses through promotion of Treg function and inhibition of dendritic cell (DC) maturation/activation (9, 119). STAT3 is a potent regulator of anti-inflammatory responses by influencing macrophage activation, and STAT3 reduces the cytotoxicity of NK cells and neutrophils, and reduces the expression of MHC II, CD80, CD86, and IL-12 in DCs rendering them unable to stimulate T cells and generate antitumor immunity (121). Therefore, growth of GBMs is “privileged” by the immunocompromised microenvironments of the tumor (118). STAT3 also proved to be required for both TGF-β and IL-10 production by Tregs (122), which are highly involved in the GBM immunosuppression. The levels of activated (phosphorylated) STAT3 expression within malignant gliomas correlated with the degree of immune cell infiltration and with poor prognosis in patients with anaplastic astrocytomas (120). Constitutively activated STAT3 both in tumor cells and in immune cells within the tumor microenvironment thus mediates tumor-induced immune suppression at many levels.

Human GBM-infiltrating immune cells treated with WP1066, a STAT3 inhibitor, showed increased expression of activation molecules and production inflammatory cytokines, which stimulate T-cell effector functions (123). The systemic inhibition of STAT3 promotes antitumor activities of tumor-infiltrating immune cells, leading to enhanced survival in a mouse glioma model (124). These data clearly indicate an immunosuppressive function of activated STAT3 in GBMs. Thus, blockade of the STAT3 pathway appears to be a promising therapeutic approach.

Recent data have shown that STAT3 acting as an oncoprotein, may paradoxically acts as a tumor suppressor, suggesting the complexity of STAT3 functions in GBMs depending on the mutational profile of the tumor (125). According to this notion, in the loss of the PTEN function, STAT3 was found to act as a tumor suppressor, however, STAT3 forms a complex with EGFRvIII in the nucleus and acts as an oncoprotein in gliomagenesis (125). Additionally, activated STAT3 was shown to occupy the IL-8 promoter and suppress its activation, suggesting that inhibition of STAT3 may facilitate tumor growth and invasion via the upregulation of IL-8 (126). Thus, STAT3 appears to contribute gliomagenesis through a complex of molecular mechanisms, and has emerged as a promising target for therapy. However, for any therapeutic strategy, a genetic heterogeneity of GBMs should be taken into account, as well as important roles of STAT3 in many physiological processes.

GLIOMA STEM CELLS

There is increasing evidence that within solid tumors, including primary brain tumors, only a small population of cells are tumorigenic. These cells termed (however, not yet defined) cancer stem cells, brain tumor initiating cells or glioma stem cells (GSCs) are characterized by their ability for self-renewal, multilineage differentiation and tumor propagation. GSCs have been enriched by selection of the CD133 (Prominin-1) cell surface marker (127-131). However, the CD133- cells isolated from gliomas have been found to be also tumorigenic (132-134). The GBM-derived CD133+ and CD 133- population of cells showed differential growth characteristics and molecular profiles (132, 135, 136), suggesting that different types of GSC may lead to the formation of heterogeneous GBM (136). These cells *in vitro* showed to share some characteristics similar to those of neural stem cells (NSCs) including the expression of NSC markers (such as Nestin, CD133 and Sox2) (137, 138), the capacity for self-renewal, proliferation, and differentiation (137). Few signaling pathways (such as: PI3K, Olig2, Sonic hedgehod, Notch, Wnt and BMI-1) essential for the development and regulation of NSCs have been shown to be active in GSCs of GBMs and need to be considered as candidate targets (137, 139).

CONCLUSION

Better understanding of molecular pathogenesis of GBMs has lead to the identification of novel biomarkers and the development of molecular targeted therapies. The molecular heterogeneity of GBM, both within and across tumors provides a challenge to combinatorial therapeutic strategies. Identification of GSCs, a subset of tumorigenic cells of GBM, that mediate chemotherapy and radiation resistance opened many issues that need to be resolved in further investigations in this area which could lead to the development of effective therapeutic strategies that specifically target of GSCs. Further studies will be necessary to fully understand the molecular biology of GBMs, the complex and dynamic relationship between GBM and immune system in distinct tumor microenvironment and biology of GSCs that will allow rapid improvement in outcome of GBM patients.

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NOVI POGLEDI NA MOLEKULARNU OSNOVU GLIOBLASTOMA MULTIFORME I UDRUŽENU IMUNOSUPRESIJU

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Sažetak

Glioblastoma multiforme (GBM) je najčešći primarni maligni tumor mozga odraslih, sa najgorom prognozom, uprkos agresivnoj multimodalnoj terapiji. Većina GBM nastaje de novo (primarni) sa kratkom kliničkom istorijom, dok sekundarni GBM nastaju progresijom prethodno postojećih glioma nižeg gradusa i pokazuju različitu genetiku i profil ekspresije, uključujući visoku učestalost mutacija izocitrat dehidrogenaze 1 (IDH1), koje su već prisutne u prekursornim lezijama. Sveobuhvatne studije genoma dale su bolji uvid u izvanrednu molekularnu heterogenost GBM i identifikovale molekularne entitete, koji mogu zahtevati različite terapijske pristupe. Premda su lokalizovni intrakranijalno, GBM su udruženi sa globalnom imunosupresijom. Bolje razumevanje imunskog odgovora na GBM koji rastu u imunski posebnoj mikrosredini u mozgu i mehanizama putem kojih izbegavaju imunski odgovor, čak ga suprimiraju, ubrzaće razvoj efikasnijih imunoterapija. Ovaj rad sumira tekuća saznanja koja se odnose na genetske alteracije i signalne puteve od ključnog značaja za biologiju GBM, uz neke mehanizme razvoja lokalne i sistemske immunosupresije kod GBM i ulogu GBM matičnih ćelija.

Ključne reči: **glioblastoma multiforme, genetika, markeri, imunosupresija, glioma matične ćelije**