1 Glioblastoma Genomics: A Very Complicated Story

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Abstract: Glioblastoma is a deadly disease that has not shown improvement despite the development of new diagnostic tools and innovative targeted therapies. This grim outcome is mainly related to a complex intra- and inter-individual heterogeneity resulting from severe genetic instability. Understanding glioblastoma biology may establish a foundation to improve prophylaxis, early diagnosis, prognosis, and treatment prediction, thus leading to a better outcome. Recent advances in technologies such as genomics, epigenomics, transcriptomics, and proteomics have led to unprecedented discoveries of potential prognostic and predictive markers. Several of these biomarkers are in deep need of validation to be used in clinical routine. In this chapter, we will discuss the most accomplished recent advances in the genomics of glioblastoma and insight into personalized medicine using validated, and not yet validated, biomarkers that may have great potential to improve patients' outcomes.

Key words: Glioblastoma; Heterogeneity; Prognosis; Subtypes; Targeted therapies

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Introduction

Glioblastoma (GBM) is the most frequent type of primary tumors of the central nervous system in adults, and its very poor prognosis has not significantly improved despite the development of innovative diagnostic strategies and new therapies (1, 2). Complex and poorly reproducible diagnoses and the inability to accurately predict sensitivity or resistance to chemotherapy regimens, as well as less than optimal CNS bioavailability, have contributed to the poor prognosis for patients with glioblastoma. Therefore, understanding the molecular mechanisms underlying its aggressive behavior may lead to better management, appropriate therapies, and good outcomes. Cancer progression is promoted by somatic evolution, a process in which an accumulation of mutations causes the genome of a cancer cell to deviate from that of a healthy cell. Some cancers, such as colon cancer, have a very well-defined sequence of events leading to their development. GBM development is however remarkable in that it occurs via a complex network of different genetic and molecular aberrations, leading to significant changes in major signaling pathways. In recent years, substantiated data have emerged and demonstrated that tumors are made of multiple populations of cancerous cells harboring specific genetic alterations in addition to the classical founder genetic abnormalities (3). This heterogeneity in tumors results from the characterized genetic instability and increased mutation rates that accompany all neoplasms and from a Darwinian selection of the fittest clones through genetic and epigenetic modifications (4). GBMs are lethal as they disperse extensively throughout the brain parenchyma, making maximal surgical resection unattainable and also because of a high level of vascularization. Thus, the need for tumor-specific drug targets and pharmacological agents to inhibit cell migration, dispersal, and angiogenesis is indeed immense. There are no inheritable traits that are predisposing to GBM development; therefore, all characterized genetic alterations are somatic and acquired aberrations. This chapter will discuss some of the most commonly affected signaling pathways and their relevance for possible use into a personalized medicine approach.

Pathogenesis of Glioblastoma

ONCOGENIC PATHWAYS

The most frequently altered pathway involves receptor tyrosine kinases (RTKs) (5–7). RTKs are cell-surface receptors that bind growth factors (GFs). GF binding occurs via cross-linking, inducing the dimerization of two adjacent receptors and a conformational shift. This shift activates the kinase function of the RTK allowing cross-phosphorylation of tyrosine residues in preparation for downstream signaling cascades (Figure 1A). Epidermal growth factor receptor (EGFR) signaling functions in the proliferation, migration, differentiation, and survival of all types of central nervous system cells (8). In GBM cells, EGFR signaling can be activated either through overexpression of the receptor or its ligand, amplification of the *EGFR* locus, and/or receptor mutation (9). It is important to note that any combination of these alterations may coexist within the same tumor. The oncogenic

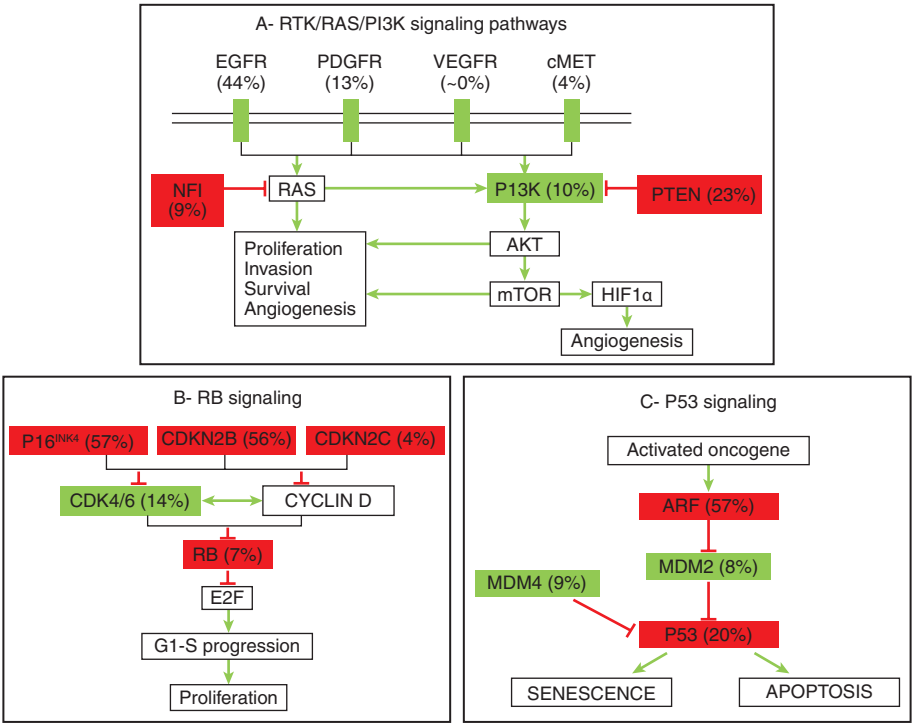


Figure 1 Genetic alterations in major key pathways altered in glioblastoma. Mutations, deletions, and amplifications in (A) RTK/RAS/PI3K, (B) RB, and (C) p53 signaling pathways are shown. Green boxes indicate activating mutation and amplifications. Red boxes indicate inactivating alterations such as mutations and deletions. Frequency of alterations are shown in each box. (Adapted from Ref. (7))

properties of EGFR are associated with constitutive activation and uncontrolled increases in phosphorylation activity. The majority of GBMs that overexpress EGFR also have mutation of the *EGFR* gene. The most common mutation is the EGFRvIII, which corresponds to the loss of exons 2–7, leading to a deletion of 267 amino-acids in the extracellular domain making the receptor ligand independent and constitutively active. This mutation is never observed in healthy tissues and secondary GBM (10).

Another commonly modified pathway in GBM is the Ras pathway. Increases in Ras pathway activity are seen in nearly all GBMs; however, Ras mutations are rare in this population (11). In the absence of mutated Ras, these high levels can be attributed to increased activation of upstream factors, such as the EGFR (Figure 1A). Ras is a guanosine-binding protein (G protein) that cycles between an inactive state when bound to GDP and an active form when bound to GTP. Active Ras (Ras-GTP) promotes progression through the cell cycle, survival, and migration through a cascade of downstream effectors. The Phosphatidylinositol-4,5-Bisphosphate 3-Kinase/Phosphatase and Tensin Homolog/serine threonine kinase Akt (PI3K/PTEN/Akt) pathway is also initiated by growth factor–receptor

interactions (Figure 1A). Upon growth factor receptor activation, PI3K is drawn to the cell membrane, resulting in the generation of the secondary messenger phosphatidylinositol (3,4,5)-trisphosphate (PIP3) (12). Akt is a downstream effector of PIP3 that leads to cell proliferation and inhibition of apoptosis. PTEN normally acts as a negative regulator of PI3K and terminates the PIP3 signal. In GBM, the tumor suppressor function of PTEN is frequently inactivated, either by loss of heterozygosity (LOH) or mutation-induced constitutive activation of PI3K, resulting in increased PI3K availability. Unopposed PI3K-mediated signaling has been implicated in GBM pathogenesis (13).

The retinoblastoma (RB) pathway plays a key role in the cell cycle. In cells that are dormant, or nonproliferating, RB is hypo-phosphorylated and actively binds to the transcription factor E2F. RB binding to E2F prevents the transcription of genes that are necessary for mitosis and the cell cycle is halted at the G1/S checkpoint. In proliferating cells, GFs induce Cyclin D1 formation and activation of cyclin-dependent kinase-cyclin (CDK/cyclin) complexes. Active CDK/cyclin complexes phosphorylate RB, resulting in the release of E2F. Free E2F induces transcription of genes that promote DNA synthesis and cell proliferation occurs (14). Negative regulation of the RB pathway can be accomplished by cyclin-dependent kinase inhibitor proteins (CDKN) belonging to the INK family. One example is the CDKN2A-p16^{INK4a}, which competes with cyclins for CDK binding to prevent RB phosphorylation (15). Certain GBM cells can override this negative regulation via methylation of the RB promoter and gene silencing. Alteration of the RB pathway leads to substantial cell cycle imbalances (Figure 1B). The TP53 pathway also functions in cell cycle control, DNA damage response, cell death, and differentiation. When DNA damage occurs, the cell becomes stressed and activates the TP53 pathway. To allow time for DNA repair to occur, TP53 increases transcription of p21, a CDKN that binds cyclin proteins and inhibits their functions to halt progression through the G1 phase of the cell cycle (16). If there is more damage than can be repaired quickly, TP53 will induce cell death to prevent division of cells containing mutated or damaged DNA. The TP53 pathway has negative feedback loops. TP53 induces transcription of MDM2, a proto-oncogene, which leads to the degradation of TP53 and prevention of DNA repair. To maintain TP53 activity, the CDKN2A-p14^{ARF} inactivates MDM2 via degradation. MDM4, a regulator of TP53, can inactivate TP53 via binding of the transcriptional activation domain (17). In human gliomas, TP53 mutations are often missense mutations that target exons crucial for DNA binding. Other alterations seen in GBMs are MDM2 amplification, MDM4 amplification, and CDKN2A-p14^{ARF} deletion (7) (Figure 1C). Currently, there are no defined sequence of events that definitively lead to GBM development. Any number or combination of these pathways may contribute to GBM formation. Although these pathways are well defined, the complexity of GBM is enhanced by high levels of variability both between different tumors, as well as within a single tumor.

INTRATUMOR HETEROGENEITY

Intratumor heterogeneity is defined as the presence of multiple different cell subpopulations within a single tumor from one patient (18). Tumor heterogeneity allows a tumor to respond to selective pressures, thus contributing to tumor

aggressiveness, growth, and treatment failure (19). Heterogeneity poses a significant challenge to the design of effective new drug therapies. There are currently two proposed mechanisms for the development of intratumor heterogeneity: cancer stem cells that may possess varying degrees of stemness, the ability to self-renew and differentiate into different tumor cell types, and clonal evolution that may enhance genetic diversity within the affected tissues (20, 21). Intratumor heterogeneity is spatially defined from the core of the tumor to the periphery. The core of a GBM tumor is an area of high proliferation and inflammation. The core is comprisesd of a zone of necrosis surrounded by the tumor zone. The margin between the tumor tissue and brain parenchyma is called the interface. Tumor cell density decreases throughout the interface as distance from the core increases (Figure 2) (22). The outermost area is known as the peripheral brain zone (PBZ), and it consists of mainly brain parenchymal tissue with isolated infiltrates (23). These isolated infiltrates dispersed throughout normal brain tissues in the PBZ help to explain why total surgical resection is impossible and recurrence is nearly inevitable. Studies have shown that biopsies taken from the core and interface zones had much higher levels of genomic alterations compared to biopsies of tissues from the PBZ, suggesting that changes in gene expression are dependent upon tumor area. These results are clear evidence that tumor fragments from the same patient may be classified into different molecular subtypes (23). Tumor recurrence in the primary site or in surrounding brain parenchyma is all too often a great challenge despite new therapies and interventions. This is related to astrocytic tumor diffusion and invasion properties that are linked to the migrating glioma stem cells (24).

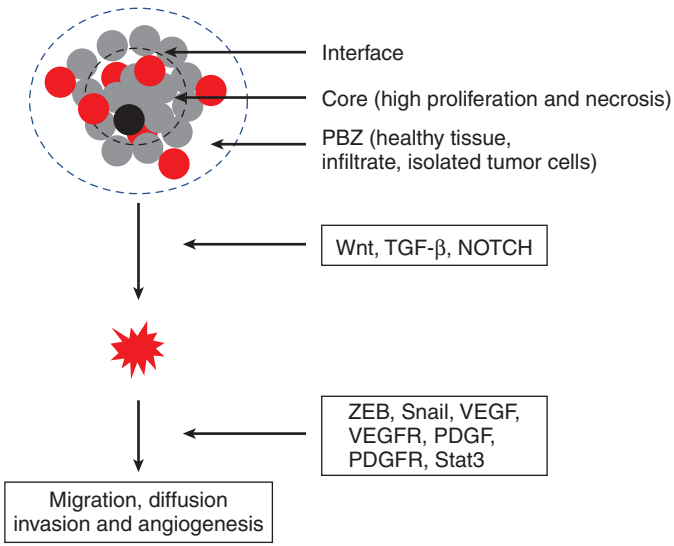


Figure 2 Pathogenesis of epithelial to mesenchymal transition (EMT). EMT is a programmed pathway for clonal outgrowth of localized tumors to colonize surrounding areas and promote angiogenesis. This process is a cross talk between glioblastoma stem cells (red circles), clonal cancer cells (gray and black circles [necrotic]), and epithelial cells via genetic reprograming, implicating several genes and transcription factors. (Adapted from Ref. (22).)

EPITHELIAL TO MESENCHYMAL TRANSITION

The epithelial to mesenchymal transition (EMT) is a programmed event in which epithelial cells, through a genetic reprograming or selection, acquire a mesenchymal phenotype. This process results from alterations in cell architecture and behaviors following cell–cell and cell–extracellular matrix interactions (25), leading to clonal outgrowth of localized tumors to promote a mesenchymal phenotype, conferring an unusual property for the cell to colonize surrounding areas and activate angiogenesis (26). It has been demonstrated that tumors with high EMT activation are associated with hyper-vascularization and worse outcomes. Aberrant activation of several signaling pathways and EMT regulators can lead to oncogenic EMT and cancer progression (Figure 2). Wnt, TGF-β, and NOTCH pathways among other signaling pathways have been shown to play major roles in EMT (27). They act via modulating several EMT key transcription factors such as Snai1, Slug, ZEB1, ZEB2, Twist1, and Twist2 (27). Specifically, positive correlation has been found between activation of NOTCH signaling pathway and the expression of EMT markers such as Snail in GBM specimens (28). Further studies have revealed that NOTCH acts upstream of Snai1 to confer invasive ability and mesenchymal phenotype to glioma cells (28). Moreover, recent transcriptomic studies have shown that among many cancer signature genes, mesenchymal genes are overexpressed at the expenditure of proneural genes in several GBM biopsies from patients with poor prognosis (29). Specifically, C/EBPb and STAT3 have been shown to act as mesenchymal driving genes of prognostic value (29). Patients with tumors that are double-positive for C/EBPb and STAT3 have shorter survival when compared to patients with tumors that are single- or double-negative (29). This confirms that these two genes are global regulators of mesenchymal transformation in stem cells and that they are necessary in the maintenance of the aggressive mesenchymal phenotype in glioma cells both *in vitro* and *in vivo* (29), and highlights potential cross talk between glioblastoma stem cell (GSC) theory and the EMT process.

EMT can generate cancer cells with stem-like properties (30). Indeed, upon acquisition of EMT phenotype, GSCs acquire both stemness and mesenchymal properties. Unlike tumors that metastasize, this double property may explain tumor invasion that is one of the hallmarks of recurrent GBM. It has been shown that high expression of Slug (EMT marker) correlates with higher grade glioma and is associated with high levels of the GSC marker, CD44, which also has been reported to promote glioblastoma cells migration, invasion, and angiogenesis (31, 32).

GBM tumors are extensively vascularized resulting from an overactivated angiogenesis, a process of forming new blood vessels which is a critical step for supplying oxygen for tumor growth (33). However, it is often an inefficient process, leading to tumors with areas of hypoxia, necrosis, and edema (34). Mechanisms of new blood vessel formation include differentiation of GSC into vascular endothelium in addition to the generation of new vessels that involves recruitment of endothelial progenitor cells (35). In response to hypoxia, the hypoxia inducible factor-1 (HIF-1α) is frequently activated in GBM (36) and induces VEGF expression (36) (Figure 1A). There is increasing evidence that

GSCs are maintained with a vascular niche which in turn is maintained with VEGF secreted by GSCs and acting through VEGFR-2/KDR (37). This shows that VEGF pathway might be the rate-limiting step of angiogenesis expansion. VEGFRs and PDGFRs are structurally and functionally related growth factor receptors that function in the promotion of angiogenesis and are well-known targets of cancer cells. The angiogenesis transition is believed to be a balance between pro- and anti-angiogenesis factors (38). Several other mediators have been shown to play roles in GBM angiogenesis. Such factors are represented by NOTCH, angiopoietins, PDGF, FGF, integrins, ephrins, and IL-8 (39–41). Conversely, many endogenous inhibitors such as angiostatin, thrombospondins, endostatin, tumstatin, and interferons oppose the action of these mediators (38). Several angiogenesis inhibitor drugs are used in recent clinical trials, most commonly targeting VEGF, VEGFR, PDGF and PDGFR, the key players in the angiogenesis pathway.

Classification of Glioblastoma Based on Genetic Markers

GENOMIC ABNORMALITIES OF PRIMARY AND SECONDARY GBM

Most GBMs are primary tumors that arise in the absence of prior disease. Primary GBMs are aggressive, highly invasive neoplasms that are more commonly seen in the elderly. Secondary GBMs are much less common and typically affect people below the age of 45. Secondary GBMs develop from low-grade astrocytoma and are associated with better prognosis. Primary and secondary GBMs are histologically indistinguishable, yet they evolve from different genetic precursors and show distinctive genetic alterations that can allow for differentiation (42, 43) (Table 1). The alterations seen most frequently in primary GBM are EGFR amplification or mutation, PTEN deletion or mutation, and CDKN2A-p16^{INK4a} deletion (44). Amplification or mutation of EGFR results in constitutive activity, increased proliferation, and survival of mutated cells. PTEN deletions or mutations are almost exclusively seen in the advanced stages of disease in primary GBM. CDKN2A-p16^{INK4a} deletions can be found in both primary and secondary GBMs, although it is more common in primary GBMs. The clinical relevance of CDKN2A-p16^{INK4a} deletions is yet to be determined. Genetic alterations common to secondary GBM include TP53 mutations and Isocitrate Dehydrogenase 1/2 (IDH1/2) mutations (42, 45). TP53 mutations are detectable in the early stages of disease in secondary GBM. IDH1/2 mutations rarely occur in primary GBMs, and have recently been identified as alterations that frequently occur in low-grade gliomas and in the pathway to secondary GBMs. IDH1 mutations are considered the most reliable indicator to differentiate primary from secondary GBM (45). Platelet-derived growth factor receptor (PDGFR) gene amplification is also known to occur in secondary GBM. Even though much time and effort has gone into developing a standard for the classification of GBM, there are still some alterations that cannot be limited to one subclass over another. A more comprehensive list of commonly seen alterations in primary versus secondary GBMs can be found in Table 1, although the list is not all inclusive.

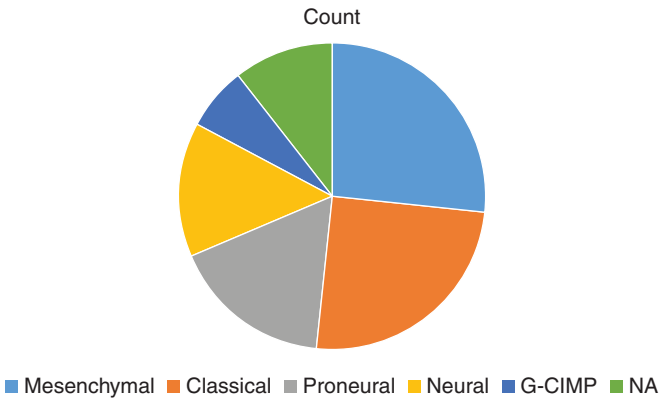
TABLE 1 Major Genomic, Epigenomic, Transcriptomic, and Proteomic Differences between Primary and Secondary GBM

	Primary	Secondary
Genetic alterations	EGFR Amplification CDKN2A-p16^{INK4a} deletion LOH[a] of chromosome 10 PTEN mutation	IDH1/2 mutation LOH of 22q, 13q, 19q TP53 mutation
Gene/protein expression profiles	Centrosome-associated protein 350 Enolase 1 Fas IGFBP2[b] MMP-9[c] Survivin Tenascin-X-precursor VEGF[d] VEGF fms-related TK	ADAMTS-19[e] ASCL1[f] Cadherin-related tumor suppressor homolog precursor DUOX2[g] ERCC6[h] HNRPA3[i] Loss of TIMP-3$_j$ PDGFR TP53 WNT-11$_k$ protein precursor
Promoter methylation	–	CDKN2A-p14^{ARF} CDKN2A-p16^{INK4a} MGMT$_l$ RB TIMP-3

[a]Loss of heterozygosity; [b]insulin-like growth factor binding protein 2; [c]matrix metallopeptidase 9; [d]vascular endothelial growth factor; [e]a disintegrin and metalloproteinase with thrombospondin motifs 19; [f]Achaete-Scute Family BHLH Transcription Factor 1; [g]dual oxidase 2; [h]excision repair cross-complementation group 6; [i]heterogenous nuclear ribonucleoprotein A3; [j]tissue inhibitor of metalloproteinases 3; [k]Wnt family member 11; [l]O-6-Methylguanine-DNA Methyltransferase.

GENOME-, EPIGENOME-, AND TRANSCRIPTOME-BASED CLASSIFICATION

Initiation and progression of GBM are linked to genetic and epigenetic aberrations. Genetic subgroups of GBM have unique gene expression profiles. Based on these profiles, GBMs can be stratified into four clusters: mesenchymal, classical (or proliferative), proneural, and neural (Figure 3). These molecular subtypes are also associated with different spatial zones of a GBM tumor. Mesenchymal GBMs have overexpression of mesenchymal and astrocytic markers in addition to neurofibromin 1 (NF1) deletion. NF1 normally functions as a negative regulator of the Ras pathway. The classical subtype displays high-level proliferation and is associated with EGFR amplification, Chr.10 monosomy, and CDKN2A-p16^{INK4a} deletion. Proneural subtype GBMs present with alterations in TP53, PDGFRA, PIK3C, and IDH1. These GBMs are seen most in younger



Expression Subtype	Frequency (%)
Mesenchymal	26.7
Classical	25
Proneural	16.9
Neural	14.3
G-CIMP	6.7
NA	10.3

Figure 3 Summary of GBM subtypes based on transcriptomics and methylation status analyses. Unsupervised clustering of GBMs delineates several molecular subtypes. These include proneural, neural, proliferative (or classical), mesenchymal, and G-CIMP. Their frequency is shown. (Adapted from ATLAS-TCGA.) NA: Not analyzed.

patients and are associated with favorable outcomes. Neural subtype GBMs show a strong composition of genes involved in nervous system development and function (46). The mesenchymal and classical subtypes are typically associated with more aggressive high-grade gliomas, while the proneural subtype represents less aggressive high-grade gliomas. Despite this fact, mesenchymal, classical, and proneural subtypes are all associated with tumor tissue. The neural subtype is associated with the interface and PBZ and is classified as a nonenhancing region (23). Another cluster of tumors has been recently identified based on the CpG island methylator phenotype, or G-CIMP tumors (Figure 3). These tumors have distinct copy number alterations, DNA methylation patterns, and transcriptomic profiles compared to the other four subsets of GBMs and are associated with a very favorable outcome (Table 2). The disease process of GBM is characterized by unique sets of molecular changes in cells and their microenvironment. It is increasingly evident that these processes not only differ from patient to patient but also differ between subtypes within the same tumor. These differences shed light on the difficulties seen when trying to develop new targeted drug therapies.

TABLE 2 Summary of Glioblastoma Subtypes Based on Genomics Data (adapted from Refs. (7, 45) and ATLAS-TCGA)

Gene	Proneural/Neural	Classical	Mesenchymal	G-CIMP
Age	Young	Old	Old	Young
Prognosis	Good	Poor	Poor	Good
Active process	Neurogenesis	Proliferation	Angiogenesis	Neurogenesis
Cell marker	Neuroblast	Stem cell	Stem cell	Neuroblast and nonneuroblast
Chromosomal aberration	Normal Chrs.7 and 10	Gain of Chr.7 Loss of Chr.10	Gain of Chr.7 Loss of Chr.10	Gain of Chrs.8 and 10 IDH1 mutations
EGFR/PTEN loci	Normal EGFR Intact PTEN	EGFR amplified Loss of PTEN	EGFR amplified Loss of PTEN	Normal EGFR Intact PTEN
Altered pathway	NOTCH, TP53, PDGFRA, PIK3C, IDH	AKT, CDKN2A	Met, NF1	MYC

Genomic Landscape of Glioblastoma

COMMON SOMATIC MUTATION ABERRATIONS

Somatic aberrations are nonheritable mutations that can arise spontaneously in somatic cells due to errors that occur in DNA replication or from exposure to environmental mutagens. The resulting changes from these mutations can lead to cellular transformation and cancer progression. Many researchers have focused their efforts on identifying genes relevant to GBM progression by targeting genes with the highest density of missense mutations. A challenge to this method is that higher missense mutations counts may also be associated with higher silent mutation counts and thus be indicative of relaxed purifying selection rather than positive selection (47). One approach to determining which genes are under positive selection in GBM is to identify parallel mutations. Parallel, or recurrent, mutations are identical nucleotide substitutions found at the same site in tumors from different patients. Parallel mutations provide powerful evidence of positive selection on GBM genes because independent random fixation of the same mutation in different patients is highly improbable (47). Genes that are significantly mutated and that display parallelism include EGFR, TP53, PTEN, RB, and IDH1 (Table 3). The advantage of using parallelism is the ability to identify sites under positive selection in GBM when the overall mutation count is not statistically significant. For example, PDGFRA is a known oncogene that shows parallelism, but it is not significantly mutated. Research focusing solely on mutation counts would not classify PDGFRA as a significant mutation in GBM pathogenesis, which could preclude PDGFRA from further investigation (48).

TABLE 3 Most Frequently Mutated Genes Observed in TCGA Glioblastoma Database

Genes	Number of mutations	Number of patients	Frequency (%)
PTEN	69	131	23.02
EGFR	73	117	20.62
TP53	69	115	20.27
PIK3R1	32	60	10.65
NF1; PIK3CA; SPTA1	28	51	8.93
FLG; PCLO	24	47	8.25
RYR2	21	39	6.87
RB1	20	39	6.87
HMCN1	19	35	6.19
AHNAK2; MUC17	18	33	5.84
IDH1	15	29	5.15
SYNE1; TCHH	14	27	4.81
OBSCN	13	23	4.12
RELN	12	23	4.12
KEL	11	21	3.78
FBN3; GABRA6; MROH2B	10	19	3.44
LZTR1; SEMA3C	9	18	3.09
PDGFRA	10	18	3.09
CNTNAP2; DMD; RBM47	9	18	3.09
BCOR; KMT2C; RPL5; STAG2; TAF1L	8	16	2.75
GRIN2A; HCN1; MYH2	8	14	2.41
ABCB1; ADAMTS16; AFF2; FGD5; GRM3; KIF2B; LRFN5; MYH8; NLRP5; OR8K3; PCDHA1; PCDHA3	7	14	2.41

COMMON COPY NUMBER ABERRATIONS

Copy number aberrations (CNAs) are somatic changes to chromosome structure that result in either a gain or loss of copies in sections of DNA. CNAs are different from copy number variations (CNVs) in that CNAs occur in somatic tissues, whereas CNVs occur in germline tissues and are present in all cells of the organism, not solely in the tumor tissue. The most common CNAs seen in GBM include loss, or partial loss, of chromosomes 9 and 10; polysomy of chromosomes 7, 19, and 20; focal deletion of CDKN2A/B locus (9p21.3); and focal high-level amplifications of EGFR locus (7p11.2) (5, 7) (Figure 4 and Table 4). CNAs targeting chromosomes 7 and 10 are some of the earliest events in GBM tumor evolution.

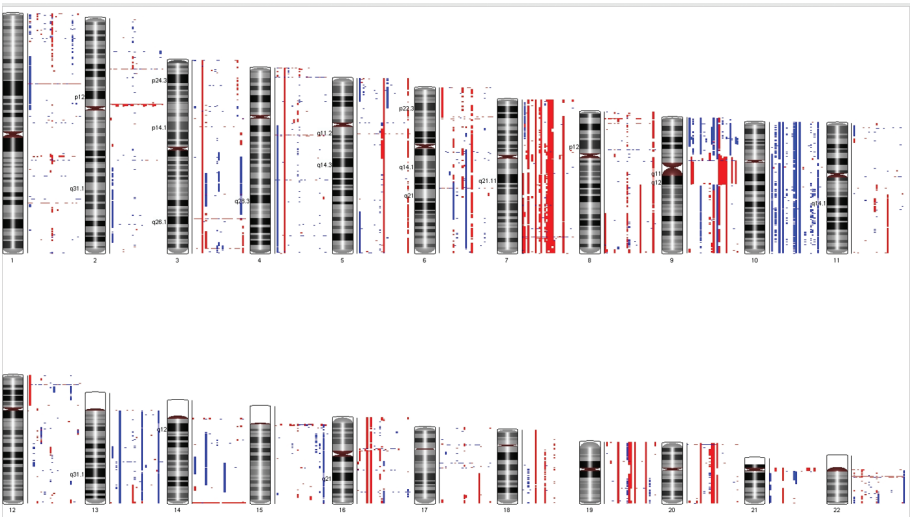


Figure 4 Genomic landscape of glioblastoma. Digital karyotype showing major CNA observed in glioblastoma. Major gain (red) and loss (blue) events are shown. (Adapted from Refs. (5, 7) and ATLAS-TCGA.) Clustering was performed using PartekGS software (Partek, St. Louis, MO).

TABLE 4 Most Frequent Copy Number Alterations (CNAs) and the Corresponding Genes Observed in TCGA Glioblastoma Database

Gene	Cytoband	CNA	Number of patients	Frequency (%)
CDKN2A	9p21	DEL	323	57.37
CDKN2B	9p21	DEL	315	55.95
EGFR	7p12	AMP	246	43.69
MTAP	9p21	DEL	239	42.45
CDK4	12q14	AMP	80	14.21
PDGFRA	4q12	AMP	72	12.79
MLLT3	9p22	DEL	67	11.90
CHIC2	4q11	AMP	66	11.72
KIT	4q12	AMP	52	9.24
MDM4	1q32	AMP	48	8.53
FIP1L1	4q12	AMP	48	8.53
MDM2	12q14.3-q15	AMP	47	8.35
DDIT3	12q13.1-q13.2	AMP	46	8.17
PTEN	10q23.3	DEL	41	7.28
GLI1	12q13.2-q13.3	AMP	37	6.57

TABLE 4 **Most Frequent Copy Number Alterations (CNAs) and the Corresponding Genes Observed in TCGA Glioblastoma Database** (Continued)

Gene	Cytoband	CNA	Number of patients	Frequency (%)
KDR	4q11-q12	AMP	35	6.22
TEK	9p21	DEL	34	6.04
LRIG3	12q14.1	AMP	22	3.91
SOX2	3q26.3-q27	AMP	21	3.73
CDKN2C	1p32	DEL	20	3.55
MET	7q31	AMP	19	3.37
CDK6	7q21-q22	AMP	19	3.37
IGFBP7	4q12	AMP	18	3.20
DCUN1D1	3q26.3	AMP	18	3.20
KLHL6	3q27.3	AMP	17	3.02
PIK3CA	3q26.3	AMP	16	2.84
AKAP9	7q21-q22	AMP	15	2.66
CCND2	12p13	AMP	15	2.66
FRS2	12q15	AMP	14	2.49
EPHB3	3q27.1	AMP	14	2.49
FGF6	12p13	AMP	14	2.49
FAS	10q24.1	DEL	13	2.31
IKZF1	7p12.2	AMP	13	2.31
MAGI2	7q21	AMP	13	2.31
SMO	7q32.3	AMP	13	2.31
PTPRD	9p23-p24.3	DEL	13	2.31
NFIB	9p24.1	DEL	13	2.31
FGF23	12p13.3	AMP	13	2.31
MYCN	2p24.3	AMP	12	2.13
KMT2C	7q36.1	AMP	12	2.13
XRCC2	7q36.1	AMP	12	2.13
SBDS	7q11.21	AMP	12	2.13
MAP3K13	3q27	AMP	12	2.13
HIP1	7q11.23	AMP	12	2.13
GRM3	7q21.1–q21.2	AMP	12	2.13
ABCB1	7q21.12	AMP	12	2.13
RB1	13q14.2	DEL	11	1.95
BTG2	1q32	AMP	11	1.95
JAZF1	7p15.2–p15.1	AMP	11	1.95

Aberrations in other known GBM drivers include focal amplification of PDGFRA, sex determining region Y-box (SOX2, involved in the determination of cell fate), MDM2, and MDM4. These aberrations can occur at different steps in the tumor development process (23).

Potential Biomarkers for Prognosis and New Therapeutic Prediction

Several clinical trials are evaluating efficacy of numerous new targeted therapies with or without a predictive biomarker (Table 5).

TABLE 5 Targeted Therapeutic Agents Currently Used in Several Ongoing Clinical Trials for Patients with Glioblastoma (Obtained from clinicaltrials.gov) and Their Official FDA Approval

Target	Class	Name	FDA approval
EGFR	Tyrosine kinase inhibitors	Panitumumab (Vectibix®)	For metastatic colorectal cancer, KRAS wild type
		Gefitinib (Iressa®)	For advanced nonsmall-cell lung cancer
		Erlotinib (Tarceva®)	For advanced nonsmall-cell lung cancer and pancreatic cancer
		Lapatinib (Tykerb®)	For breast cancer as combination therapy
		AEE788 (also a VEGFR inhibitor)	–
		Vandetanib (Caprelsa®, also a VEGFR and RET inhibitor)	For metastatic medullary thyroid cancer
	Monoclonal antibodies	Cetuximab (Erbitux®)	For KRAS wild-type metastatic colorectal cancer and squamous cell carcinoma of the head and neck
Ras	Farnesyltransferase inhibitors	Tipifarnib (Zarnestra®)	–
		Lonafarnib (Sarasar®)	–
Raf	Tyrosine kinase inhibitors	Sorafenib (Nexavar®, also a VEGFR and PDGFR inhibitor)	For advanced renal cell carcinoma and hepatocellular carcinoma
PDGFR	Tyrosine kinase inhibitors	Imatinib (Gleevec®)	For treatment of multiple cancers, most notably Philadelphia chromosome-positive chronic myelogenous leukemia

TABLE 5 **Targeted Therapeutic Agents Currently Used in Several Ongoing Clinical Trials for Patients with Glioblastoma (Obtained from clinicaltrials.gov) and Their Official FDA Approval** (Continued)

Target	Class	Name	FDA approval
		Dasatinib (Sprycel®)	For chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia
		Sunitinib (Sutent®, also a VEGFR inhibitor)	Mainly for treatment of renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumors
	Small molecule	Crenolanib	–
VEGFR	Tyrosine kinase inhibitors	Vatalanib (also a PDGFR inhibitor)	–
		Cediranib (Recentin®)	–
		Axitinib (Inlyta®)	For advanced renal cell carcinoma
VEGFR	Small molecule	Carboxyamidotriazole	–
		Pazopanib (Votrient®)	For advanced renal cell carcinoma and advanced soft tissue sarcoma
		Lenvatinib (Lenvima®)	–
IL-2	Monoclonal antibodies	Basiliximab (Simulect®)	For the prophylaxis of acute rejection for renal transplant
		Daclizumab (Zenapax®)	For relapsing multiple sclerosis
PD-1	Monoclonal antibody	Nivolumab (Opdivo®)	For squamous cell head and neck cancer, Hodgkin lymphoma, metastatic melanoma, nonsmall-cell lung cancer, advanced renal cancer, and urothelial carcinoma
PD-L1	Monoclonal antibody	Durvalumab	–
NF-κB	Proteasome inhibitor	Bortezomib (Velcade®)	For mantle cell lymphoma and multiple myeloma
TGF-β2	Antisense oligo-deoxynucleotide	Trabedersen	–
Tenascin	Monoclonal antibody	I^{131} 81C6 (Neuradiab®)	–
PARP	Small molecule	Olaparib (Lynparza®)	For advanced ovarian cancer

Table continued on following page

TABLE 5 Targeted Therapeutic Agents Currently Used in Several Ongoing Clinical Trials for Patients with Glioblastoma (Obtained from clinicaltrials.gov) and Their Official FDA Approval (Continued)

Target	Class	Name	FDA approval
FLT3	Tyrosine kinase inhibitor	Tandutinib, also inhibits c-KIT and PDGFR	–
Rb	Cyclin-dependent kinase inhibitor	Ribociclib (Kisqali®)	For advanced breast cancer
BRAF	Small molecule	Dabrafenib (Tafinlar®)	For metastatic melanoma in patients with BRAF mutations
mTOR	Small molecule	Sapanisertib	–

CLINICALLY RELEVANT ABERRATIONS (BIOMARKERS)

Although there appears to be a motif of common aberrations, only a select few have been associated with clinical relevance. Specifically, EGFR amplification, IDH1/2 mutations, and MGMT promoter methylation are currently regarded as having clinical significance. EGFR amplifications are associated with high-grade malignancy, poor prognosis, and shorter survival time (49). Currently, EGFR status can be used to predict patient response to EGFR-targeted therapies. Gefitinib and ertlotinib are small molecule tyrosine kinase inhibitors that act to prevent phosphorylation of tyrosine residues and block downstream signaling. Both gefitinib and erlotinib have been rigorously tested for use in GBM; however, they have not been proven effective for monotherapy (50). Furthermore, targeting the mutation EGFRvIII using vaccine alone or in combination with tyrosine kinases inhibitors and temozolomide has been shown to improve *in vitro* cytotoxicity, to significantly reduce tumor development in xenograft models and in clinical trial by eliminating EGFRvIII-expressing cells and targeting its downstream target genes (51).

IDH1 mutations have been shown to exhibit characteristics associated with better prognosis. IDH1 mutations are typically found in younger patients that have high frequencies of TP53 mutations, and are currently used as positive predictors of prognosis. Wild-type IDH1 functions to convert a-ketoglutarate to isocitrate; however, a mutated IDH1 results in the formation of 2-hydroxyglutarate (2HG) (52). The consequences associated with the formation of 2HG are yet to be determined and is currently thought to function as an oncogenic metabolite (53). Serum levels of 2HG are being used to identify IDH1 mutations in patients with acute myeloid Leukemia (AML). MGMT promoter methylation is one of the most relevant prognostic markers and can also be used to predict therapeutic response to alkylating agents such as carmustine and temozolomide. The normal function of MGMT is to repair DNA damage, which would counteract the apoptotic effects of temozolomide. Silencing MGMT would lead to enhanced cytotoxic activity of temozolomide. It has been shown that patients that have MGMT

promoter methylation have clinically significant increases in survival time when given temozolomide concurrently with radiation therapy (54). This is related to MGMT methylation that sensitizes tumor cells to alkylating agents, leading thus to increased survival time. One of the many challenges associated with glioblastoma is the lack of standardized testing for these prognostic markers. Per the guidelines published by the National Comprehensive Cancer Network (NCCN) for glioblastoma, patients under the age of 70 years are recommended to receive temozolomide therapy regardless of their methylation status, and there is no mention of IDH1 and/or 2HG testing. Even though this testing is noninvasive, it has not yet been implemented as part of a standardized protocol.

TEMOZOLOMIDE AND GLIADEL WAFER

Temozolomide (Temodar®) and carmustine (BCNU, Gliadel®) are chemotherapeutic alkylating agents that function as prodrugs and are noncell cycle specific. The Gliadel wafer is a polymer that contains 3.85% carmustine and is applied locally immediately following surgical resection of the GBM tumor (55). These agents exploit a weakness in mismatch repair function when given to patients with silenced MGMT. Although they fall under the same broad classification, their mechanisms of action differ. Temozolomide forms the active intermediate MTIC [(methyl-triazene-1-yl)-imidazole-4-carboxamide]. MTIC can methylate the 6-OH on guanine. This methylation causes guanine to mispair with thymine, resulting in DNA double-strand breaks and cellular apoptosis. Carmustine can be more specifically classified as a nitrosourea. Upon activation, it forms active metabolites that are capable of DNA alkylation, DNA and RNA strand cross-linking, and protein carbamylation. The cross-linking effects of carmustine result in inhibition of DNA synthesis, RNA production, and translation. Carbamylation of proteins may inhibit enzyme processes necessary for cell survival. Collectively, these actions contribute to its cytotoxic nature. Recent studies have shown that MGMT promoter methylation (MGMT inactive or silenced) in GBM patients treated with Gliadel, radiotherapy, and TMZ was associated with significantly improved overall survival and progression-free survival (PFS) compared to patients with active MGMT. Therefore, MGMT methylation status can be used as a predictive marker for these therapies.

GROWTH FACTOR RECEPTOR INHIBITORS

There are many growth factor receptor inhibitors currently in use across several cancer types. Growth factor receptor inhibitors can be stratified into two main subclasses, monoclonal antibodies (mAbs) and small molecules. mAbs exert their effects extracellularly and can target either the ligand growth factor or the transmembrane tyrosine kinase receptor. Once bound, mAbs can inhibit signaling pathways and may induce cell death via apoptosis, complement activation, or effector cell activation. Small molecule growth factor receptor inhibitors were developed to penetrate the cell membrane and act on the cytoplasmic tyrosine kinase domain to inhibit its enzyme activity and disrupt signaling.

Recent clinical trials have attempted to translate the predictive qualities of EGFR status to GBM. Cetuximab is a mAb that targets the EGFR to prevent

receptor dimerization. Gefitinib and ertlotinib are small molecule EGFR tyrosine kinase inhibitors that act to prevent phosphorylation of tyrosine residues and block downstream signaling. Cetuximab, gefitinib, and erlotinib, although tested for use in GBM, have not been proven effective (50, 56). Aside from EGFR inhibitors, studies have also been done targeting growth factor receptor inhibitors that target angiogenic pathways. Several EGFR mutations have been discovered and some are associated with an oncogenic activity or have a predictive power. Specifically, the point mutations A289V, G598V, R108K, and T263P were shown to predict *in vitro* response to erlotinib (57). Their relevance is much less studied than the T790M mutation that was shown to be oncogenic and to predict response to several TK inhibitors drugs in lung cancers (58). Indeed, patients with this mutation have been shown to not respond to erlotinib, afatinib, and gefitinib (first-generation TK inhibitors) but respond remarkably to second-generation TK inhibitors such as osimertinib (59). However, the therapeutic relevance of these mutations is under investigation in several clinical trials or still needs to be studied in GBM.

ANGIOGENESIS INHIBITORS

Bevacizumab is a mAb that targets VEGF ligand to prevent its binding to VEGFR. It is the only mAb that has been approved for GBM treatment. Bevacizumab studies have shown a significant improvement in PFS over radiotherapy alone (60). Small molecule inhibitors of VEGFR and PDGFR, such as sorafenib and pazopanib, have been studied in GBM and have shown no significant clinical benefit. Apart from bevacizumab, most clinical trials testing targeted therapies for GBM have been unsuccessful. This lack of response may be attributed to the vast number of overlapping pathways, resulting in the development of GBM. Combination therapy design studies are ongoing (Table 5); however, they are not without challenge. A study combining the EGFR inhibitor erlotinib and the mammalian target of rapamycin (mTOR) inhibitor temsirolimus resulted in dose-limiting toxicity without showing any significant benefit (61). Several clinical trials evaluated bevacizumab and irinotecan combination in high-grade gliomas including GBM (62, 63). This combination significantly improved PFS and overall median survival (62, 63) despite development of severe side effects. However, long-term use of bevacizumab is associated with emergence of resistance, high recurrence, rapid disease progression, and failure to respond to other chemotherapy (64, 65). Thus, there is a necessity to combine therapies that target multiple pathways simultaneously.

MISCELLANEOUS AGENTS

All of the previously mentioned agents target well-known pathways in GBM, yet little progress has been made in developing effective treatments. Some researchers have shifted their focus away from these aberrations and have developed alternative approaches to determining potential therapies. One such approach was to determine subtype-specific drugs for each of the four accepted GBM subtypes. Candidate drugs were chosen based on their association to subtype-specific genes and predicted patient phenotypes. The drugs chosen for the classical subtype

included irinotecan, a topoisomerase poison, and paclitaxel, an anti-microtubule agent, to target CDK6. For the mesenchymal subtype, pravastatin, a cholesterol-lowering agent, was chosen to target the gene ITGB2, which encodes for integrin beta chain. Clomipramine, an antidepressant, was selected for the proneural subtype targeting the gene SLC1A1, a solute carrier transporter. Lastly, the GABA antagonist bicuculline was selected for the neural subtype based on its association with the gene CALM2, which encodes calmodulin. These subtype-specific drugs showed significant inhibitory effects on GBM cell clonogenicity and synergistically reversed temozolomide resistance in MGMT methylation negative patients. Further studies must be done to refine this approach, though it does show promise (66).

Conclusion

Omic-based personalized medicine encompasses the utilization of data gathered via genomics, transcriptomics, proteomics, and metabolomics to create patient-specific therapies and/or regimens for successful treatment of disease. There is a common expectation that with an understanding of the changes occurring in gene and protein expression, one would be able to establish the most effective pharmacotherapy for the patient in question. However, intratumor heterogeneity confounds current efforts to solidify molecular biomarkers. Genetic alterations are not common to all tumor tissues within the same patient and between patients, and thus cannot be effectively targeted using the same protocol and therefore need an individualized approach to implement a personalized medicine of this deadly disease. Utilizing Omic-based technologies, it is foreseeable that soon GBM might be treated much in the same way that HIV is currently treated. Upon diagnosis, HIV patients have resistance testing done for their specific strain of the virus. Based on that information, a practitioner has different combination therapies to choose from to suit each patient individually. Ultimately, the goal would be for a patient sample taken during tumor resection, before and after treatments, to be sequenced and analyzed by several omic technologies, and to design a regimen that includes a combination of therapies to target patient-specific aberrations and development of resistance. Combination therapies will require management of toxicities, drug interactions, and therapeutic response monitoring.

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