2 Molecular Genetics of Secondary Glioblastoma

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Abstract: Glioblastoma (GBM, WHO grade IV astrocytoma) is among the most common adult brain tumors and one that is invariably fatal. GBM is classified as either primary (de novo) or secondary in origin. Secondary GBMs are derived from previously lower grade (WHO grades II or III) gliomas. While secondary GBMs present with similar clinical characteristics as their primary counterparts, the molecular pathways involved in their pathogenesis distinguish the two and have functional consequences for their behavior. Although a large number of histologic markers are routinely utilized to distinguish primary from secondary GBM, advances in genomic and bioinformatics techniques have drastically improved classification of high-grade gliomas and our understanding of the molecular pathways that influence tumor behavior and response to treatment. The significant influence of molecular identity on tumor behavior has been recognized by the most recent WHO classification of CNS tumors, wherein specific molecular markers have been integrated as part of tumor subtype identification process, as a supplement to traditional histological analysis. Indeed, the change heralds a new era for neuro-oncology, one that is moving toward targeted

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therapeutics as part of the standard of care. Thus, a comprehensive grasp of this diverse landscape is necessary. In this chapter, we provide an overview of our latest understanding of the molecular diversity of GBM, specifically as it pertains to primary and secondary GBMs, and how it influences prognostication and therapeutic decision-making.

Key words: Alpha thalassemia/mental retardation syndrome X-linked (ATRX); Isocitrate dehydrogenase (IDH); Low-grade glioma; Secondary glioblastoma

Introduction

Glioblastoma (GBM, WHO grade IV astrocytoma) is the most common malignant primary brain tumor among adults. Despite aggressive therapy, the current median survival is approximately 15 months (1). In addition to the diffusely infiltrative nature of these tumors, which prevents complete surgical resection, tumor recurrence and ultimate patient demise is also largely attributed to the significant molecular and cellular heterogeneity of these lesions, which inevitably results in treatment resistance and tumor recurrence. GBMs are further classified into primary (de novo) and secondary tumors that, while they present with similar clinical characteristics, are derived from previously lower grade (WHO grades II or III) gliomas. While both categories are diffuse in nature, the molecular pathways involved, along with functional tumor behavior, treatment strategy, and clinical outcomes are different (2, 3). Although clinical and imaging biomarkers can be used to distinguish primary from secondary GBM, advances in genomic and bioinformatics techniques have drastically improved classification of high-grade gliomas and our understanding of the molecular pathways that influence tumor behavior and response to treatment. The significant influence of molecular identity on tumor behavior has been recognized by the most recent WHO classification of CNS tumors, wherein specific molecular markers have been integrated as part of tumor subtype identification process, as a supplement to traditional histological analysis (4). Indeed, the change heralds a new era for neuro-oncology, one that is moving toward targeted therapeutics as part of the standard of care. Thus, a comprehensive grasp of this diverse landscape is necessary. In this chapter, we provide an overview of our latest understanding of the molecular diversity of GBM, specifically as it pertains to primary and secondary GBMs, and how it influences prognostication and therapeutic decision-making.

Distinguishing Primary and Secondary GBMs

Primary and secondary GBMs are histologically indistinguishable. Historically, the distinction between the two has been based on clinical history. With a more in-depth understanding of the genetic, epigenetic, and molecular profile of these tumors, however, the distinction has become clearer (Table 1) (5).

TABLE 1 **Key Characteristics of *IDH*-Wildtype and *IDH*-Mutant Glioblastomas (adapted from Ref. (5).)**

	IDH-WT GBM	*IDH*-mutant GBM
Synonym	Primary glioblastoma	Secondary glioblastoma
Precursor lesion	Identified de novo	Diffuse astrocytoma Anaplastic astrocytoma
Proportion of glioblastomas	~90%	~10%
Median age at diagnosis	~62 years	~44 years
M:F ratio	1.42:1	1.05:1
Median length of clinical history at diagnosis	4 months	15 months
Median overall survival Surgery + radiotherapy Surgery + RT + CTX	 9.9 months 15 months	 24 months 31 months
Location	Supratentorial	Preferentially frontal
Necrosis	Extensive	Limited
TERT promoter mutations	72%	26%
TP53 mutations	27%	81%
ATRX mutations	Exceptional	71%
EGFR amplification	35%	Exceptional
PTEN mutations	24%	Exceptional

ATRX, adult thalassemia mental retardation x-linked; CTX, chemotherapy; EGFR, epidermal growth factor receptor; GBM, Glioblastoma multiforme; *IDH*, Isocitrate dehydrogenase; PTEN, phosphatase and tensin homolog; TERT, telomerase reverse transcriptase; TP53, tumor protein 53; RT, radiotherapy.

EPIDEMIOLOGY OF SECONDARY GBM

The incidence of secondary GBMs based on clinical and imaging criteria is somewhat lower than that estimated by isocitrate dehydrogenase (IDH) status (5% vs. 6–13%, respectively) (2, 6, 7). Furthermore, patients with a clinical diagnosis of secondary GBM are on average 17 years younger than those with primary GBM (2, 7); this bias toward a younger patient cohort correlates very closely with *IDH1* status, as patients with *IDH* mutations are substantially younger (8, 9). The clinical course is substantially longer in patients with *IDH*-mutant GBM, indicative of a less aggressive behavior (2, 6, 8).

ANATOMIC PREVALENCE OF SECONDARY GBM

Interestingly, *IDH*-mutant GBM has a predilection for the frontal lobe and typically present with seizure rather than neurological deficit. The same has been demonstrated for *IDH*-mutant Grade II astrocytomas and oligodendrogliomas, including tumor with 1p/19q co-deletion (10). These findings support a hypothesis that the precursor cell of origin among *IDH*-mutant tumor subtypes is shared,

and suggest that these tumors may arise from mutations within a cell population that is independent of the cell populations at risk during development of de novo GBM (11).

MOLECULAR LANDSCAPE OF SECONDARY GBM

Amplification of the *EGFR* gene and activating mutations of its protein product are hallmarks of primary GBM and appear to be exclusive of *TP53* mutations (12). *PTEN* amplification and loss of chromosome 10 are additional features of primary GBMs (3, 13). Both primary and secondary GBMs have in common loss of heterozygosity (LOH) at chromosome 10q (14–16); although *PTEN* is also located on chromosome 10, mutations in this gene are only observed in primary GBM. Therefore, additional genetic events must be responsible for oncogenesis of high-grade gliomas that is shared among both primary and secondary tumors.

One of the earliest events, if not the initial event, in gliomagenesis is mutation of the *IDH1* or *IDH2* gene. Mutations in the promoter of the telomerase reverse transcriptase (*TERT*) gene lead to enhanced telomerase activity, which results in maintenance of telomere length and promotion of cell survival. Interestingly, *TERT* mutation is shared among both primary and secondary GBMs, potentially rendering this mutation as an early event in the process of tumorigenesis (17). In addition to these mutations, secondary GBM originating from a lower grade astrocytoma will frequently display mutations in the *TP53* and *ATRX* (adult thalassemia mental retardation x-linked) genes, while anaplastic tumors arising from a lower grade oligodendroglioma lineage will have co-deletions of 1p and19q (2, 3, 18). There are several key signaling pathways involved in this transformation as well, and knowledge of mutations in genes involved in these processes and pathways is critical for an in-depth understanding of the biology of secondary GBM and in working toward targeted therapeutics. We will review these pathways in detail below.

Molecular Classification of GBMs Based on Gene Expression

In 2010, Verhaak and colleagues analyzed somatic mutations, DNA copy-number alterations, and gene expression profiling to group GBMs into discrete categories (19). Through this work, they were able to establish four subtypes of GBMs (Classic, Proneural, Neural, and Mesenchymal) based on the specific clustering of molecular and gene expression profiles. The Classic category demonstrated a greater preponderance of EGFR amplification, decreased rates of *TP53* mutation, along with *p16INK4A* and *p14ARF* deletion. Histologically, the Classic subtype demonstrated features more consistent with astrocytes. The Proneural category was found to have a greater rate of PDGFR amplification, *TP53* mutation, LOH, and *IDH1* mutation. These tumors had histological features most consistent with oligodendrocytes. Moreover, patients harboring the Proneural subtypes were younger and responded better to therapy. The Neural subtype was found to have a greater degree of neuronal marker expression and the histology was consistent with a combination of oligodendroglial, astrocytoic, and neuronal features. The Mesenchymal subtype was found to have a greater degree of *NF1* mutations,

along with alterations of *PTEN* and *Akt*. Histologically, these tumors demonstrated a greater degree of necrosis and inflammatory features. Furthermore, astroglial and microglial cell signatures were commonly noted. This landmark study established the concept of differential behavior of GBMs that may be similar histologically but differ substantially from a molecular and gene expression perspective.

Mechanisms of Gliomagenesis

Gliomagenesis is a multicomponent process involving several genetic mutations affecting numerous molecular pathways (Figure 1). When considering tumor phylogeny, *IDH* mutation is critical to deciphering whether the identified tumor is a primary GBM or a GBM arising from secondary progression of a lower grade glioma. It is now established that while *IDH* mutations are early events in the process of gliomagenesis in secondary GBM, additional genes and their end products are altered during this process and these include *ATRX* mutation, loss of tumor suppressor genes such as *TP53* and *RB1*, and mutations in the promoter of *TERT* (5). Alterations of chromosomes 1, 7, 10, and 19, each harboring a distinct subset of tumor suppressor/promoter genes, are pivotal as well. Distinct pathways that have been identified as part of the core drivers of gliomagenesis include the *EGFR/PTEN/Akt/mTOR*, *TP53/MDM2/p14ARF*, and the *p16INK4a/RB1* pathways, which will be elaborated upon in the subsequent sections.

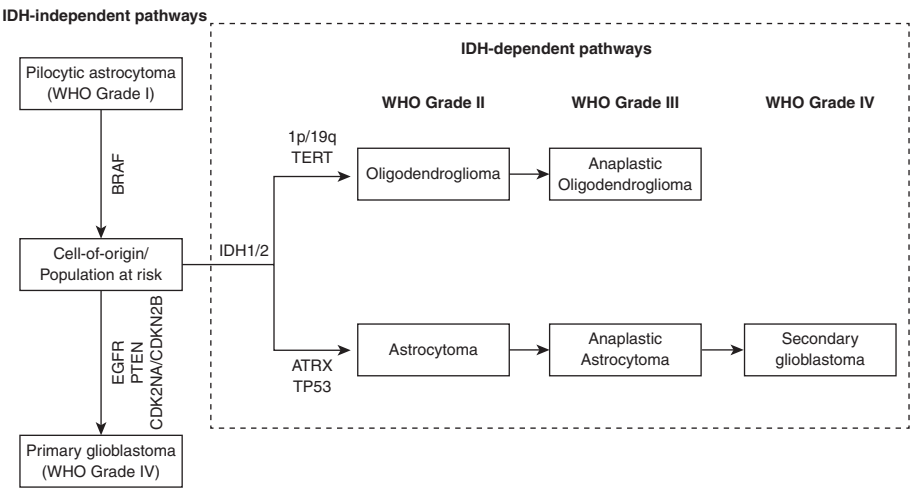

Figure 1 Molecular pathways to gliomagenesis. While the cell of origin in glioma is yet to be identified, large-scale expression and copy-number analyses have determined multiple molecular processes that result in glioma formation. Primary glioblastomas (and most Grade I gliomas) arise via an *IDH*-independent pathway. Conversely, *IDH* mutation is an early if not initiating event in the development of of low-grade astrocytomas and oligodendrogliomas. By definition, secondary glioblastomas arise from malignant degeneration of an *IDH*-mutant lower grade tumor.

IDH AND GLIOMA INITIATION

First reported by Parsons and colleagues in 2008, a number of recent studies have since confirmed recurrent somatic mutations in the *IDH1* and *IDH2* genes (R132H and R172K as the canonical mutations in these genes, respectively) in a significant proportion of patients with gliomas. Further, patients who harbored tumors with an *IDH* mutation exhibit distinct disease characteristics relative to patients with a glioma with wild-type (WT) *IDH*. In 615 WHO grade II/III gliomas, *IDH* mutations were identified in 79% of the patient tumors (17). In another series of 457 WHO grade II/III gliomas, 80.7% of the patients were found to harbor an *IDH* mutation (20). The Cancer Genome Atlas Research Network found an *IDH* mutation in 226 (80.1%) of 282 WHO grade II/III gliomas (21). Based on these results, the WHO now recognizes *IDH* mutation as a critical biomarker in the classification of gliomas (4).

The *IDH* enzymes catalyze the oxidative conversion of isocitrate to α-ketoglutarate (α-KG). *IDH* mutations confer a gain-of-function neomorphic activity, converting α-KG to R-2-hydroxyglutarate (R-2-HG), instead of its racemic enantiomer S-2-HG. Although 2-HG is a trace metabolic product in normal cells, it is markedly elevated in *IDH*-mutant gliomas and in other malignancies, such as acute myeloid leukemia (22–24). The oncogenic effect of *IDH* mutation is thought to be twofold. First, 2-HG is considered an oncometabolite that may play a role in the process of glioma development, and progression or resistance to treatment. Although the exact role of *IDH1* mutation in gliomagenesis had initially been hampered by difficulties in establishing *in vitro* cultures with *IDH1* mutations (25), recent reports have demonstrated that increased levels of 2-HG result in increased activity of HIF-1-α and increased levels of its downstream targets such as VEGF. In addition, 2HG also affects collagen maturation, resulting in defective basement membranes that are potentially pivotal to glioma progression (25). Second, *IDH* mutation results in decreased production of α-KG, which impairs the function of many α-KG-dependent dioxygenases, including but not limited to histone demethylases (e.g., collagen prolyl-4-hydroxylase, prolyl hydroxylases, and the ten-eleven translocation (TET) family of DNA hydroxylases) (26). Change in histone methylation is thought to also interfere with the terminal differentiation of cells and may predispose cells harboring mutant *IDH* to malignant transformation (27). Based on the above evidence, *IDH1/2* mutations have been termed as lineage markers by some authors (11), and it is now accepted as a more definitive marker of secondary GBM than any other clinical or pathological criterion (28).

ATRX, TP53, AND 1p/19q

The great majority of low-grade astrocytomas carry a *p53* mutation while most oligodendrogliomas demonstrate loss of chromosomes 1p and 19q (26, 29–33). Biopsy-based studies suggest that the *IDH1* mutation occurs prior to either *p53* mutation or 1p and 19q loss (26, 33). Following *IDH*-mediated oncogenesis, acquisition of *p53* and *ATRX* mutations occurs in the setting of development of an astrocytoma (34, 35), while loss of chromosomes 1p and 19q occurs in the setting of development of an oligodendroglioma. While both subgroups are capable with time of undergoing further malignant degeneration, the current WHO

classification system only considers progression to secondary GBM as an endpoint of astrocytoma progression. It is conceivable that all GBMs that harbor an *IDH* mutation are secondary tumors. In one study, the small subgroup of patients with primary GBM carrying an *IDH* mutation (3.4%) was younger than noncensored primary GBM patients and harbored frequent *p53* mutations and an absence of EGFR amplification, features consistent with secondary GBMs (8). These findings suggest that these tumors could represent cases of a rapidly progressive secondary GBM, rather than a true primary GBM. Conversely, it can be argued that all GBMs harboring a WT *IDH* are biologically primary GBMs: cases of secondary GBM without an *IDH* mutation likely represent a progression from an undergraded, lower grade, or anaplastic glioma (8). These assumptions are borne out by recent data that show that gliomas lacking mutation in *IDH* or having chromosomal loss at 1p and 19q cluster by expression analysis and DNA copy-number profiling (21) and portend a severe prognosis (17). With an increased understanding of molecular markers and their incorporation into clinical trials, the disparity between molecular markers and histopathology-based diagnostics methods becomes more evident. For now, the current WHO classification system posits that, despite histopathological features such as neo-vascularity and necrosis, a high-grade glioma with *IDH1* mutation and 1p/19q co-deletion should be considered an anaplastic oligodendroglioma. Conversely, from a biological perspective, a histological anaplastic astrocytoma with WT *IDH* is now considered a GBM (36). These modifications in the classification system have been corroborated by outcomes data emerging from clinical trials. Together, these findings confirm the integral role of *IDH* and 1p/19q status in determining patient survival.

TERT PROMOTER MUTATION

Mutations in the *TERT* gene are thought to prevent cell senescence through increased telomere length, thus promoting tumorigenesis in several cancers, including GBM (37). The contribution of *TERT* mutation to tumor aggressiveness however is not clear. Focusing on a sample of GBM cases, Mosrati et al. found that *TERT* promoter mutation was associated with a shorter overall survival (37). Interestingly, this mutation was found in both primary and secondary tumors. More recently, Eckel-Passow et al. found that, while GBMs had a higher proportion of *TERT* mutations in isolation (74% of cases) or had neither *TERT* or *IDH* mutations or loss of chromosome 1p and 19q (what they termed "triple negative" tumors, making up17% of cases), lower grade gliomas were much less likely to be "triple negative" (7% of cases) or harbor a *TERT* mutation in isolation (10% of cases) (16). These findings suggest that while *TERT* promoter mutation is integral to tumorigenesis and may contribute to the overall aggressiveness of the tumor, its role is modified by other key mutations.

THE G-CIMP PHENOTYPE

Methylation of the promoter region of the *MGMT* gene is more frequently found in secondary GBMs compared to primary GBMs (75% vs. 36%) (38), and it is frequently associated with mutations in *IDH1/2* and *TP53* and utilized as a strong predictive marker for response to chemotherapy in GBM patients. In fact,

IDH mutation has been shown to mediate widespread changes in chromosome structure and remodeling of the DNA methylome, resulting in the establishment of the glioma CpG island methylator phenotype (G-CIMP). Introduction of mutant *IDH1* into primary human astrocytes was found to be sufficient to alter specific histone methylation marks and induce extensive DNA hypermethylation in a manner that resembles the changes observed in G-CIMP+ lower grade gliomas. Furthermore, the epigenomic alterations resulting from mutant *IDH1* activate specific gene expression programs that are associated with G-CIMP+ proneural glioblastoma, but not other glioblastoma subtypes, and are associated with longer survival. Based on these data, *IDH* mutation is likely the molecular basis of G-CIMP in gliomas, highlighting the interplay between genomic and epigenomic changes in cancers including GBM.

In GBM, the proneural subtype is predominantly associated with *IDH1/2* mutations and these are further subclassified as either CIMP+ or CIMP- (of which the G-CIMP+ shows better prognosis). The proneural subtype by itself, however, appears to bear little prognostic significance unless considered in association with the *IDH1/2* mutation status (39). In fact, Turcan et al. have demonstrated that the *IDH1* mutation alone is capable of remodeling the genomic methylation profile of the tumor, thus promoting the CIMP+ profile (40). Interestingly, WT *IDH1* status promoted hypomethylation at numerous foci and CIMP- low-grade gliomas lacked *IDH1* mutation. In addition, decreased expression of *ATRX* is associated with downregulation of MGMT expression via promoter hypermethylation (41). Therefore, *ATRX* mutation status not only predicts cell of origin but also has a significant prognostic role as well (34, 42, 43).

Genetics of Glioma Progression

EGFR/PTEN/AKT/MTOR PATHWAY

Activation of the *PI3K/Akt* pathway results in increased cell proliferation via downregulation of p27, thereby influencing cell-cycle progression (44), inactivation of pro-apoptotic genes (45), and increased transcription of pro-survival genes under the influence of NFkB (46). PI3K is recruited to the cell surface and activated through EGFR. Once phosphorylated, PI3K activates PIP3 via phosphorylation, which induces activation of downstream molecules such as Akt—a serine/threonine kinase (47)—promoting cell survival and proliferation (48).

EGFR is a tyrosine kinase growth factor receptor situated in the cell membrane. Amplification of the *EGFR* gene and mutation of the protein product are key contributors to the activation of this receptor tyrosine kinase (RTK) pathway in primary GBM. The most common of the *EGFR*-activating mutants is the EGFRvIII variant, in which gene mutation results in a truncated protein product that is constitutively active. Mutations in Akt itself, however, are not common in gliomas (49).

PTEN is the second most commonly mutated tumor suppressor gene in all cancers after *p53* (50), and *PTEN* mutation is found in approximately 40% of GBMs, predominantly in the primary form (51). PTEN is a tumor suppressor and one of its functions is dephosphorylation of PIP3, thus preventing activation of Akt and mTOR (47). Through this role, PTEN is central in inhibiting cell

proliferation and regulating the ability of cells in migration and invasion (52). Loss of PTEN function, either through genetic or epigenetic modifications, is a common component of the *Akt/PI3K/mTOR* activation pathway in cancer.

TP53/MDM2/P14ARF PATHWAY

Although mutations of the *TP53* gene have been identified in both primary and secondary GBMs, its role appears to be predominantly related to the latter, where the mutation is an early event in gliomagenesis (2). While *p53* mutations in primary GBM appear to involve all exons indiscriminately, they are predominantly focused at codons 248 and 273, particularly involving CpG sites, in secondary GBM (2). This discrepancy suggests that *p53* mutation in secondary GBM is a specific and stereotyped event in secondary GBM ontology, while *p53* mutation in primary GBM is potentially a consequence of widespread genomic instability (3).

MDM2 amplification appears to be specific to primary GBMs that lack the *p53* mutation (53, 54). In normal cells, WT *p53* induces the expression of MDM2, which in turn inhibits the function of WT *p53*. Furthermore, WT *p53* inhibits the function of *p14*ARF, which would normally inhibit the downregulation of p53 by MDM2. This autoregulatory loop is disrupted when any of the above is dysfunctional, adversely affecting cell-cycle control, DNA damage repair, cell proliferation/differentiation, and neovascularization (55).

P16INK4A/RB1 PATHWAY

Either through homozygous deletion or promoter methylation, the alteration of p16INK4a is an important step in both primary and secondary GBMs (56). Conversely, methylation of the *RB1* promoter, correlating with decreased RB1 expression, is more specific to secondary GBM (57). The *p16INK4a/RB1* pathway is critical to cell-cycle control (58), as RB1 regulates the progression of the cell cycle from G1 to the S phase by preventing the release of the E2F transcription factor. The latter enables the transcription of genes required for cell-cycle progression, in addition to *p14*ARF. The phosphorylation of RB1, via the CDK4/cyclin D complex, inhibits this function enabling the progression of the cell cycle along with increased p53 expression via the activated *p14*ARF. WT *p16*INK4a serves as an additional checkpoint by binding to CDK4 and inhibiting the function of the CDK4/cyclin D complex. Therefore, altered expression of any of these genes results in an inability to control cell-cycle progression. The central role of cell-cycle regulation in the genesis of secondary GBM has also been confirmed with cDNA expression profile analysis (59).

Effect of Treatment on Glioma Transformation

By virtue of the inherent heterogeneity of these tumors, it is expected that not all of the cells within a glioma will respond to chemotherapy and radiation, inevitably resulting in tumor progression/recurrence. Further, recent evidence suggests

that chemotherapy and radiation may actually result in mutations that promote tumor cell survival. This pro-mutational ability has been most extensively studied in the setting of temozolamide (TMZ) and ionizing radiation.

TEMOZOLAMIDE AND LGG PROGRESSION

An alkylating agent, TMZ is an integral component of the standard treatment regimen for patients with GBM. Accumulating evidence from numerous studies suggests that acquired treatment resistance following TMZ administration is multifactorial and rooted in transcriptional, metabolomic, genomic, and epigenomic changes that lead to this phenotype (60–67).

Costello and colleagues undertook genome sequence analysis of 23 initial and matched recurrent human gliomas to address two questions: (i) What is the extent to which mutations in initial tumors differ from mutations in their subsequent recurrent tumors? (ii) How does chemotherapy with TMZ affect the mutational profile of recurrent tumors? The authors found an average of 33 somatic coding mutations in each initial tumor, of which an average of 54% were also detected at recurrence (shared mutations), including mutations in *IDH1, TP53*, and *ATRX*. All other somatic mutations were identified only in the initial tumor or only in the recurrent tumor from a given patient (private mutations), though overall, the initial and recurrent gliomas displayed a broad spectrum of genetic relatedness. Interestingly, in multiple patients, the recurrent tumors shared ≤25% of mutations detected in the initial tumors, suggesting that these tumors were seeded by cells derived from the initial tumor at an early stage of its evolution, and that tumor recurrence can occur as the result of either linear or branched evolution.

Their findings regarding the effect of TMZ on tumor evolution and recurrence were as striking. Although the initial tumors and most of the recurrent tumors in their cohort had 0.2 to 4.5 mutations per megabase (Mb), 6 of the 10 patients treated with TMZ had recurrent tumors that were hypermutated; that is, they harbored 31.9 to 90.9 mutations per Mb. Overall, 97% of these were C>T/G>A transitions predominantly occurring at CpC and CpT dinucleotides, which is a signature of TMZ-induced mutagenesis distinct from nonhypermutated tumors. Further, acquisition of DNA mismatch repair (MMR) pathway dysfunction, which results in resistance to TMZ, appeared to exacerbate hypermutation in the face of continued TMZ therapy. The authors postulated that introduction of thousands of de novo mutations could drive the evolution of TMZ-resistant glioma cells to higher states of malignant potential. Indeed, all six recurrent tumors that showed evidence of TMZ-induced hypermutation underwent malignant progression to GBM. Many of these tumors developed mutations in pathways described as critical to gliomagenesis, including Akt-mTOR and the p16/RB. Treatment-induced somatic mutations were recently longitudinally studied in a patient with a 5-year survival period following initial diagnosis (68). Using whole exome sequencing, the investigators demonstrated that each successive therapy selected for resistant clones of tumor cells and that these had arisen via the process of chromothripsis. In addition, this approach enabled the provision of personalized therapy for this patient, based on the identification of target clonal populations sensitive to available treatment, which was critical for this long-term survival. Given the evidence derived from such analyses, it is clear that the genome of GBMs is dynamic and in

order to offer true personalized treatment, the genome of each successive tumor population must be investigated thoroughly.

Stepaneko et al. extended these findings with *in vitro* studies that demonstrated that long-term exposure of glioma cells to TMZ induces chromosomal instability, leading to alteration of cell growth, invasiveness, migration, and response to re-treatment (69). Among the TMZ-resistant cell lines, some responded to temsirolimus, an mTOR inhibitor. Interestingly, although TMZ has been shown to induce the transformation of glioma nonstem-like cells into glioma stem-like cells, the sensitivity of both differentiated and stem-like cells to TMZ was similar (70, 71). These findings further highlight the importance of the evolution of the genetic network that infers TMZ resistance in GBM.

EFFECT OF RADIATION ON GLIOMA BEHAVIOR

The introduction of radiation therapy to the armamentarium of therapy in patients with GBM has been a significant contribution. However, similar to TMZ, radiation is thought to promote malignant progression of gliomas as well. Based on transcription profiling of patient-derived radiation-resistant GBM cells, the mesenchymal subtype was the most commonly identified (72). *In vitro* studies have also demonstrated a proneural to mesenchymal transition among oligodendroglioma cell cultures that were irradiated (73). The authors proposed that the activation of the STAT3 transcription factor following radiation was contributory, given that its inhibition prevented this transition. Furthermore, Jak2 inhibition in mice undergoing radiation prolonged their survival. Alternative mechanisms such as activation of the *TNF-α/NFkB* pathway may also be involved (72). Other post-translational effects of radiation exposure, such as the stabilization of HIF-1α, promoting angiogenesis, have been proposed (74). Therefore, a combination of intrinsic cell changes and modifications to the tumor microenvironment may be responsible for the radiation-induced malignant progression noted in gliomas.

Conclusion

The recent publication of the modified WHO classification for CNS tumors, integrating molecular signatures into histological-based classifications, is timely and reflects the field's evolution. Based on our understanding of the vast intratumoral heterogeneity among GBMs, the logical next step is to establish biomarkers that would be predictive of treatment response, identify clonal populations that are potentially resistant to therapy, and develop combination therapies tailored to the specific pathways involved within the entirety of the tumor. Analysis of initial and recurrent tumor samples may be helpful for better clonal evolution analysis.

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