Review Article

Evolving Molecular Genetics of Glioblastoma

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Abstract

Objective: To summary the recent advances in molecular research of glioblastoma (GBM) and current trends in personalized therapy of this disease.
Data Sources: Data cited in this review were obtained mainly from PubMed in English up to 2015, with keywords "molecular", "genetics", "GBM", "isocitrate dehydrogenase", "telomerase reverse transcriptase", "epidermal growth factor receptor", "PTPRZ1-MET", and "clinical treatment".
Study Selection: Articles regarding the morphological pathology of GBM, the epidemiology of GBM, genetic alteration of GBM, and the development of treatment for GBM patients were identified, retrieved, and reviewed.
Results: There is a large amount of data supporting the view that these recurrent genetic aberrations occur in a specific context of cellular origin, co-oncogenic hits and are present in distinct patient populations. Primary and secondary GBMs are distinct disease entities that affect different age groups of patients and develop through distinct genetic aberrations. These differences are important, especially because they may affect sensitivity to radio- and chemo-therapy and should thus be considered in the identification of targets for novel therapeutic approaches.
Conclusion: This review highlights the molecular and genetic alterations of GBM, indicating that they are of potential value in the diagnosis and treatment for patients with GBM.

Key words: Epidermal Growth Factor Receptor; Genetics; Glioblastomas; Isocitrate Dehydrogenase; Molecular; PTPRZ1-MET; Telomerase Reverse Transcriptase

Introduction

Glioblastoma (GBM) is the most frequent and aggressive malignant primary brain tumor with only about 12% of patients surviving beyond 36 months (long-term survivors).[1,2] According to the latest Central Brain Tumor Registry of the USA statistical report, the age-adjusted incidence rate for GBM is 3.19/100,000. The incidence of GBM increases with age and peaks at 75–84 years (14.93/100,000), being more common in males (3.97/100,000).[3]

The current treatment strategy for GBM patients combines maximal surgical resection, followed by radiotherapy (RT) with concomitant and adjuvant temozolomide (TMZ).[4,5] Complete surgical resection is virtually impossible due to the infiltrative nature of these tumors, yet gross total resection is still a positive prognostic marker. Concurrent adjuvant RT in combination with TMZ represents the standard of care for patients with newly diagnosed GBM, but still <5% of patients survive for longer than 5 years after diagnosis.[6-8]

Decades of molecular studies have identified key genetic abnormalities in human GBMs, including the following: (1) dysregulation of growth factor signaling pathways via amplification and mutational activation of receptor tyrosine kinase (RTK) genes; (2) activation of the phosphatidylinositol-3-OH kinase (PI3K) pathway; and (3) inactivation of the p53 and retinoblastoma tumor suppressor

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pathways.[9] During recent years, large-scale research efforts – spearheaded by The Cancer Genome Atlas (TCGA) and Chinese Glioma Genome Atlas (CGGA) – have made rapid advances in understanding GBM tumor genetics. The discovery of new genetic alterations and their mapping against clinical outcome will trigger an avalanche of novel perceptions of the genomic and epigenomic landscape, biological subgroups and putative cells of origin of GBM, which has encouraged hopes for more effective treatment strategies in the near future. This review mainly discusses the recent advances in GBM molecular research and current trends in personalized therapy of this disease.

Morphological Diagnosis

Malignant gliomas are histologically heterogeneous and invasive tumors that are derived from glia. The World Health Organization (WHO) classification system groups gliomas into 4 histological grades defined by increasing degrees of undifferentiation, anaplasia, and aggressiveness.[10,11] Malignant gliomas, the most common form of gliomas, consist of WHO grade IV tumors (GBM) and grade III tumors (anaplastic astrocytoma, oligodendroglioma, and oligoastrocytoma).[12,13] GBMs account for approximately 60–70% of malignant gliomas and is characterized histologically by considerable cellularity and mitotic activity, microvascular proliferation, necrosis and they are also recalcitrant to radio/chemotherapy.[12,14] Primary (*de novo*, approximately 95% of cases) GBMs manifest rapidly, without evidence of less malignant precursor lesions, after a short clinical history. Secondary GBMs (approximately 5% of cases) develop more slowly by progression from low-grade diffuse astrocytoma and anaplastic astrocytoma.[15,16] GBM and other malignant gliomas are highly invasive, infiltrating surrounding brain parenchyma, yet they are typically confined to the central nervous system (CNS) and do not metastasize.[17] Unfortunately, WHO morphological classification is based on subjective criteria, lacks reproducibility, and remains imperfect in its ability to predict individual outcomes.[18,19]

Genetics Variation of Glioblastoma

Isocitrate dehydrogenase mutations

The first genome-wide exon sequencing effort for glioma identified heterozygous hotspot mutations at codon 132 (most commonly R132H) in isocitrate dehydrogenase 1 (IDH1) in 12% of GBM.[20] These mutations change the enzymatic activity of the cytoplasmic and peroxisomal IDH1. The same holds true for codon 172 mutations in the mitochondrial IDH2 gene. These homologous enzymes decarboxylate isocitrate to α-ketoglutarate (αKG), and this "neomorphic" mutation renders the IDH enzyme to reduce αKG into 2-hydroxyglutarate in the nicotinamide adenine dinucleotide phosphate-dependent manner.[21] Mutant IDH1 or IDH2 are correlated with increased histone methylation, causing epigenetic alterations in both DNA and histones, altering gene expression and promoting oncogenic transformation.[22] Nowadays, mutations in IDH1 are commonly established as a hallmark molecular feature of secondary GBM (~70% of secondary GBM, compared with 5–20% in primary GBM) who have predominant localization of GBM in the frontal and temporal lobes.[23-25] Since primary GBM is a clinically defined entity and the presence of IDH1/2 mutations has been shown to be inversely related to or even mutually exclusive of epidermal growth factor receptor (EGFR) and phosphatase and tensin homolog (PTEN) abnormalities,[26] which are hallmarks of primary GBM, IDH-mutated GBM lesions may represent genetically "secondary" GBM tumors.[25,27] Moreover, the IDH mutation status is stable during the progression of lower-grade gliomas to secondary GBMs.[16,26,28] Mutations in the IDH genes are thought to cause glioma-CpG island methylator phenotype (G-CIMP) within the proneural GBM subgroup. IDH mutations seem to require cooperating mutations in TP53 and ATRX,[29-31] and they are less frequently detected in primary GBMs.

O(6)-Methylguanine-DNA methyltransferase promoter methylation

The O(6)-methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme, preventing errors during DNA replication. Abnormal methylation of the MGMT promoter caused its silencing, a reduction of the MGMT enzyme expression, and subsequently to less repair activity of DNA damage, including that induced by TMZ.[32] MGMT promoter methylation in GBM is a prognostic and predictive biomarker indicating a response to chemoradiation.[33] The frequency of MGMT promoter methylation ranged from 30% to 60% in GBM.[33] The trial of the effect of TMZ on newly diagnosed GBM showed that MGMT promoter methylation was an independent favorable prognostic factor. Patients with tumors with methylated MGMT promoter had a survival benefit when treated with TMZ and RT, compared to those who received RT only factor.[6,34] A recent report from the neuro-oncology working group (NOA) of the German Cancer Society confirmed a predictive value of MGMT methylation for benefit from chemotherapy in patients with a wild-type IDH, independent of tumor grade.[35]

Telomerase reverse transcriptase promoter mutations

Recently, novel somatic mutations in the promoter region of telomerase reverse transcriptase (TERT) have been identified in malignant melanomas,[36,37] as well as being associated with increased telomerase expression and activity.[38] The tumors derived from cell populations with low self-renewal capacity generally depend on alterations that keep telomerase activity, while epigenetic alteration maintains telomerase activity in tumor types arisen from self-renewing stem cells.[30] The two most common mutations are located at C228T and C250T, with identical hotspots also found in gliomas.[30] The highest incidence was identified among most tumors harboring 1p/19q co-deletion and IDH mutations (98%), as well as IDH wild-type (IDH wt) tumors with EGFR amplification (92%).[16,39] The former corresponds to oligodendroglioma, while the latter corresponds to primary GBMs.[40] The frequency of TERT mutations is relatively

low in diffuse and anaplastic astrocytomas (19% and 25%, respectively).[38] In the study by Killela *et al*.,[30] patients with TERT promoter mutations alone (i.e., no IDH mutation) had the poorest overall survival (OS) (median 11.3 months), patients with tumors without TERT or IDH1/2 mutations had a slightly better survival (median 16.6 months), while patients with only IDH mutant GBM had the best survival (median 42.3 months). Although another study with 358 patients found no significant difference in OS between TERT mutant and TERT wild-type (IDH wt) GBM,[16] the role of TERT promoter mutations may provide a tool to identify non-IDH mutant GBMs.

Epidermal growth factor receptor aberrations

The range of high-amplitude focal copy-number aberrations in adult GBM highlights a key role of EGFR amplifications (43% of cases)[27] which co-occurred with EGFR intragenic deletions and/or point mutations.[41] EGFR mutations were accompanied by regional DNA amplification in the majority of cases, leading to a wide range of mutation allelic frequencies.[42] The prominent intragenic deletions in GBM target parts of the gene encoding either the extracellular domain of EGFR (exons 2–7, the deletion of which forms EGFR variant III) or the carboxyl terminus,[43] and these deletions are always correlated with amplification and co-expression of the wild-type EGFR.[44] EGFR was recently shown to be activated by recurrent translocations in 7% of GBM samples: It was most frequently fused in-frame to septin 14 or phosphoserine phosphatase as the 3′ gene segment.[27,45] Overall, 57% of GBM showed evidence of mutation, rearrangement, altered splicing, and/or focal amplification of EGFR.[27]

PTEN alterations

Loss of heterozygosity (LOH) at chromosome 10q23 occurs at high frequency in a variety of human tumors.[46] LOH at 10q23 occurs in ~70% of GBMs.[47] Mutations of PTEN were detected in 31–44% of GBM.[48,49] PTEN is a negative regulator of the phosphoinositide 3-kinase pathway, a major signaling pathway that stimulates cellular proliferation in response to growth factor stimulation.[50] PTEN deletions were more common in GBM, except classical grade II/III gliomas. PTEN deletions were fairly common across all gene expressions subtypes, but absent in IDH1 mutant tumors.[51] PTEN loss and deletion were associated with incremental increases in AKT pathway activity.[27] Several studies demonstrated that patients with loss of function mutations of PTEN generally had shorter survival than patients with PTEN retention.[52-54] However, PTEN loss was not associated with worse OS in newly diagnosed GBM patients of the TMZ era.[55]

Other novel genetic aberrations

In a smaller fraction of primary GBMs (about 3%), a fusion of the tyrosine kinase coding region of fibroblast growth factor receptor 1 (FGFR1) to the transforming acidic coiled-coil (TACC) coding domain of TACC1 (or fusion of FGFR3 to TACC3) results in constitutive kinase activity.[56,57] In transcriptome profiling of 272 gliomas from CGGA, 67 in-frame fusion transcripts were identified, including three recurrent fusion transcripts: FGFR3-TACC3, RNF213-SLC26A11, and PTPRZ1-MET (fusion transcript involving the protein tyrosine phosphatase, receptor-type, Z polypeptide 1 gene and the MET proto-oncogene, ZM). ZM fusion was found in three of 20 (15%) specimens. Exogenous expression of the ZM fusion in the U87MG GBM line enhanced cell migration and invasion. Clinically, patients afflicted with ZM fusion harboring GBMs survived poorly relative to those afflicted with non-ZM-harboring. Therefore, recurrent fusion events that involve RTK-encoding genes might be a promising therapeutic target and provide a strong rationale for the inclusion of these patients in future stratified clinical trials using different RTK inhibitors. Table 1 summarizes all of the above described and other genetic alterations and related altered signaling pathways in primary versus secondary GBM.[9,15,16,23,27,30,31,56,58-62]

Molecular Classification

The phenotype of a tumor is the result of the genotype and the influence of the tumor's environment on the tumor. One would expect that molecular diagnostics will contribute to a better classification of brain tumors [Tables 2–4].[17,19,20,63-65] Phillips described three subclasses of high-grade gliomas (termed proneural, mesenchymal, and proliferative) associated with different outcomes; specifically, prolonged survival of the proneural subclass. Similar classification of GBMs

Table 1: Genetic abnormalities and the major signaling pathways involved in the pathogenesis of GBM

Genetic abnormalities	Frequency (%)	Major altered signaling pathways
Secondary GBM		
IDH mutation	60–80[23,31]	Metabolism
ATRX mutation or loss	57[58]	Genome integrity
TP53 mutation	65[15]	p53 pathway
RB1 loss	43[59]	Rb pathway
CDKN2A loss	19[15]	Rb pathway
PTEN loss	4[15]	PI3K signaling
PTPRZ1-MET fusion	15[60]	RTK signaling
Primary GBM		
TERT promoter mutation	60–80[16,30]	Telomere maintenance
NF1 loss	10–18[9,27]	MAPK signaling
PTEN loss	36–41[9,27]	PI3K signaling
PI3K mutation	15–25[9,27]	PI3K signaling
TP53 mutation	28–35[9,27]	p53 pathway
EGFR vIII	25–50[61]	RTK signaling
EGFR ampl.	36–60[15]	RTK signaling
PDGFRA ampl.	10–13[9,27]	RTK signaling
RB1 loss	14[59]	Rb pathway
CDKN2A loss	31–78[15]	Rb pathway
FGFR3-TACC3 fusion	3[56,62]	RTK signaling

IDH: Isocitrate dehydrogenase; CDKN2A: Cyclin-dependent kinase inhibitor 2A; PTEN: Phosphatase and tensin homolog; NF1: Neurofibromatosis 1; RB1: Retinoblastoma 1; TERT: Telomerase reverse transcriptase; ampl.: Amplification; EGFR: Epidermal growth factor receptor; PDGFRA: Platelet-derived growth factor receptor alpha; FGFR3: Fibroblast growth factor receptor 3; TACC3: Transforming acidic coiled-coil 3; RTK: Receptor tyrosine kinase; GBM: Glioblastoma; MAPK: Mitogen-activated protein kinase.

Table 2: Phillips classifications of GBM based on transcription profiling

Classifications	Subgroups		
	Proneural	Proliferative	Mesenchymal
Patient age (years)	Younger (~40)	Older (~50)	Older (~50)
Biological process	Neurogenesis	Proliferation	Angiogenesis
Chromosome alterations	None	Gain of 7 and loss of 10 or 10q	
EGFR/PTEN	EGFR normal/PTEN intact	PTEN loss	PTEN loss

EGFR: Epidermal growth factor receptor; PTEN: Phosphatase and tensin homolog; GBM: Glioblastoma.

Table 3: TCGA classifications of GBM based on transcription and methylation profiling

Classifications	Subgroups				
	Proneural		Neural	Classical	Mesenchymal
	G-CIMP+	G-CIMP−			
Genetic alteration	IDH/TP53/ATRX	4q ampl.		7p ampl.	NF1/RB1
Phenotype	Oligodendrocytic		Neuron	Astrocytic	Culture astroglial
Prognosis	Best	Worst	Middle		
Chemotherapy	Resistant		Response	Response	Response

TCGA: The cancer genome atlas; GBM: Glioblastoma; G-CIMP: Glioma-CpG island methylator phenotype; ampl.: Amplification; IDH: Isocitrate dehydrogenase; NF1: Neurofibromatosis 1; RB1: Retinoblastoma 1.

Table 4: DKFZ classifications of GBM based on methylation profiling

Classifications	Subgroups			
	IDH	RTK I "PDGFRA"	RTK II "classic"	Mesenchymal
Median age (years)	40	36	58	47
Genetic alteration	IDH	PDGFRA ampl.	EGFR ampl.	
Tumor location	Frontal and temporal	Hemispheric	Hemispheric	Hemispheric
Prognosis	Favorable	Poor		

DKFZ: Deutsches Krebsforschungszentrum (German Cancer Research Center); GBM: Glioblastoma; RTK: Receptor tyrosine kinase; PDGFRA: Platelet-derived growth factor receptor alpha; EGFR: Epidermal growth factor receptor; IDH: Isocitrate dehydrogenase; Ampl.: Amplification.

was also detected in a larger cohort of mixed gliomas.[66] In 2010, unsupervised clustering of gene expression data from adult GBM samples from the TCGA identified four different molecular subtypes: Proneural, neural, classical, and mesenchymal.[41] Proneural GBMs were subdivided into G-CIMP-positive and G-CIMP-negative GBM subsets on the basis of characteristic DNA methylation patterns that strongly correspond with IDH1 mutation status.[27,67] Another later study, which compared DNA methylation patterns across both pediatric and adult patients with GBM, found a similar clustering in tumors from adult patients and further identified three more distinct clusters that predominantly consisted of children and adolescents.[68] Recently, Liu *et al*. profiled the genetic features of multifocal GBM and found that M-GBMs had no IDH1, ATRX, or PDGFRA mutations, significantly associated with the mesenchymal subtype. They also identified the CYB5R2 gene to be hypomethylated and overexpressed in M-GBMs.[69]

The recent reports published on the Nature Genetics and NEJM were comprehensively analyzed by whole-exome sequencing and/or targeted deep sequencing as well as array comparative genomic hybridization. In the Nature Genetics article,[70] grade II and III gliomas were divided into and exhausted by the genetically well-defined type I–III subtypes. Type III tumors represented the IDH wild-type grade II and III tumors in the current cohort, showing an OS rate more similar to that of GBM. Similarly, the report[71] from TCGA research network independently identified similar groups, using unsupervised clustering analyses of DNA mutation, RNA expression, DNA copy number, and DNA methylation data. The integration of genome-wide data from multiple platforms delineated three molecular classes of lower-grade gliomas (grade II/III gliomas) that were more concordant with IDH, 1p/19q, and TP53 status than with histologic class. This multi-platform approach yielded three groups similar to those initially described by Jiao's model.[58] The large majority of lower-grade gliomas without an IDH mutation had genomic aberrations and clinical behavior strikingly similar to those found in primary GBM.

The report[72] from Mayo Clinic and UCSF defined *a priori* groups that were based on the presence or absence of TERT promoter mutations, IDH mutations, and 1p/19q codeletion and found consistent associations between the molecular groups and age at diagnosis, survival, patterns of acquired alterations, and germline variants across the three data sets. The group with only TERT mutations has a high prevalence

of loss of chromosome 4 and acquired PIK3CA or PIK3R1 mutations. Gliomas with only TERT mutations are primarily grade IV gliomas. These tests (for IDH mutations, 1p/19q codeletion, and TERT promoter alterations) can be used to define five principal groups of gliomas with characteristic distributions of age at diagnosis, clinical behavior, acquired genetic alterations, and associated germline variants.

Application of Genetics Study in Clinical Practice

Over the past decade, insights into the molecular pathology of gliomas have significantly improved both our biological understanding of neoplasms as well as our abilities to diagnose tumors and estimate their prognosis and likelihood of response to specific therapies. To discuss the inclusion of molecular information into the next WHO classification of CNS tumors, a meeting under the sponsorship of the International Society of Neuropathology (ISN) has been held in Haarlem, the Netherlands.[73] According to the ISN-Haarlem consensus, "integrated" diagnosis was established through the usage of ATRX, IDH1-R132H IHC, 1p/19q analyses, and IDH sequencing in the diagnosis of diffuse gliomas.[74]

RT plus concomitant and adjuvant TMZ chemotherapy is the current standard of care for patients with GBM.[6,7] Several clinical trials have displayed that MGMT promoter methylation correlated with prolonged progression-free and OS in patients with GBM receiving alkylating drug chemotherapy.[34,7,75-78] In 2012, two independent randomized trials in elderly patients with GBM assessed RT alone versus TMZ chemotherapy alone as an initial treatment. Subgroup analyses of both trials showed better outcome for chemotherapy in patients with MGMT promoter methylated tumors but reduced survival in patients with unmethylated tumors.[79,80] Recently, the CGGA project delineated that patients with IDH wild-type GBM who underwent RT + TMZ exhibited significantly longer survival times compared to the patients who were assigned to the RT alone treatment. However, among patients with IDH mutation tumors, the survival pattern of patients undergoing RT + TMZ or RT was comparable.[81] These results strongly suggest that treatment strategies for elderly patients with GBM should be individualized dependent on IDH and MGMT.[61]

In addition, due to the high heterogeneity of GBM,[82] each of which may respond differently to one targeted therapy, there has been considerable interest in identifying molecular markers that predict response to a specific molecular targeted therapy. Bevacizumab, a monoclonal antibody against vascular endothelial growth factor, being granted approval by the US Food and Drug Administration for treating recurrent GBM in 2009.[83-85] However, it does not benefit OS in either recurrent GBM or newly diagnosed GBM.[86,87] The presence of EGFR overexpression and EGFR mutations in GBM could predict the activity of EGFR-targeted drugs in patients with these aberrations. However, these potential treatment approaches still have not been clear with contradictory findings in previous clinical trials.[88,89]

It was demonstrated that a point mutation in IDH1R132H, expressed in gliomas and other tumors, is presented on human major histocompatibility complex (MHC) class II and induces a mutation-specific $CD4^+$ antitumor T-cell response in patients and a syngeneic tumor model in MHC-humanized mice.[90] Conceptually, patients with low-grade and anaplastic gliomas, secondary GBM with a high prevalence of the IDH1 (R132H) mutation represent a patient population that may particularly benefit from an IDH1R132H specific tumor vaccine.[91-93]

Conclusions

These recurrent genetic aberrations occur in a specific context of cellular origin, co-oncogenic hits and are present in distinct patient populations. Primary and secondary GBMs are distinct disease entities that affect different age groups of patients and develop through distinct genetic aberrations. These differences are important, especially because they may affect sensitivity to radio- and chemo-therapy and should thus be considered in the identification of targets for novel therapeutic approaches. The biological distinction of GBM subgroups should therefore guide the design of future clinical trials.

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Conflicts of interest

There are no conflicts of interest.

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