Origin of Gliomas

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Abstract
Malignant glioma is a common type of brain tumor that remains largely incurable. Although a definitive cell of origin of gliomas remains elusive, numerous population studies, sequencing efforts, and genetically engineered mouse models have contributed to our understanding of the early events that may lead to gliomagenesis. Herein we summarize our current knowledge on the population epidemiology of gliomas, heritable genetic risk factors, the somatic events that contribute to tumor evolution, and mouse models that have shed light on the glioma cell of origin. Future studies will increase our understanding of the sequence of early events within susceptible cells and their niche that trigger the path to malignant transformation. Such knowledge will allow us to design more effective treatment options to control tumor growth or screening methods for early detection.

Keywords
► epidemiology
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► hereditary syndromes
► heritable genetic risk factors

Multi-institutional collaborations and molecular profiling of gliomas in the last decade have greatly advanced our understanding of these clinically and molecularly heterogeneous neoplasms. Approximately 80% of low-grade gliomas (LGGs) and secondary glioblastomas (GBMs) share recurrent mutations in the isocitrate dehydrogenase genes 1 and 2 (IDH1 and IDH2).1,2 IDH mutations in LGGs most frequently occur at IDH1 R132, in the enzyme’s active site, and confer a new gain of enzymatic function, the ability to convert α-ketoglutarate to D-2-HG. IDH mutations cause global glioma DNA hypermethylation leading to the glioma CpG island hypermethylator phenotype (G-CIMP) in primary tumors.3–5 Genome-wide analyses of LGGs have revealed three molecular subclasses: (1) IDH mutated and 1p/19 codeleted, (2) IDH mutated and 1p/19q intact, and (3) IDH wild-type.6 Most LGGs without IDH mutations are molecularly and clinically similar to GBM, suggesting that they may be precursor lesions to IDH wild-type GBM or represent incomplete surgical sampling.5

In contrast to IDH-mutated LGGs, primary, IDH wild-type GBMs have complex alterations in their genomes and a higher somatic mutation frequency compared with LGGs.7,8 GBM patients have a median overall survival of 14 months, and LGGs, although some can follow an indolent path if IDH mutated, ultimately undergo malignant transformation with a few treatment options.5,7

In addition, numerous efforts have focused on the epidemiology of gliomas to identify environmental and germline risk factors that confer glioma susceptibility. The cell of origin that gives rise to gliomas still remains elusive, although several studies have shed light on the cell types within the brain that are most susceptible to malignant transformation. In this review, we will discuss the current state of the field and recent advances that have significantly contributed to our understanding of the origin of gliomas (as summarized in ►Fig. 1).

Population Epidemiology
The overall annual incidence rate for primary brain tumors in the United States between 2009 and 2013 was ~21.5 per 100,000 population.9 Overall, men have a higher risk of developing gliomas compared with women. Hormonal factors and genetic features have been suggested as possible explanations for these gender differences.10 Epidemiologic studies have also reported that Asian countries have lower rates of brain tumors compared with European countries and the United States.11,12 It is postulated that regional
differences may be partially attributed to polymorphisms, although a definitive causal factor remains elusive. In the United States, the incidence rates for gliomas are at least twice as great in the white population than the black population. Genetic factors may play a role in this discrepancy, although a biological basis is undetermined. There is an age-related increase in the overall incidence of gliomas, with the highest incidence in the eighth decade of life. An underlying basis for the increased glioma risk with age is not yet determined; however, age-related changes in the cell of origin, the brain microenvironment, accumulation of mutations, and reduced immune surveillance may be possible risk factors.

**Preferential Brain Locations**

GBMs can occur close to subventricular zone (SVZ) or in regions further away from the SVZ. Tumor location may correlate with distinct subtypes, clinical outcomes, and growth patterns. In particular, proneural or neural GBM subtypes, based on The Cancer Genome Atlas (TCGA) system, are proximal and mesenchymal or classical TCGA subtypes are distal to the SVZ, suggesting differential cells of origin. The frontal lobe is a preferential location for LGGs with IDH mutations. Furthermore, LGGs in the frontal lobe have higher TERT promoter mutation rates compared with midline tumors that are predominantly IDH wild-type and harbor fewer TERT promoter mutations. It is plausible that clinical translation of these noninvasive radiographic analyses of gliomas may guide personalized therapy based on tumor location prior to surgery. An intriguing question is why do certain gliomas have preferential locations? These differences could be attributed to distinct properties of local progenitor cell populations or neurogenic niche signals. Indeed, Chen et al have raised an intriguing possibility that evolutionary changes contributing to the specialized need of the human prefrontal cortex for higher glutamate transmitter flux may have created a favorable microenvironmental niche that inadvertently supports the growth of IDH mutant gliomas.

**Environmental and Heritable Genetic Risk Factors**

There have been significant efforts to identify potential glioma predisposition risk factors, but a definitive link is yet to emerge. The only well-established exception is the association between high-dose ionizing radiation and brain tumors. An inverse association between glioma incidence and allergies, atopic diseases, and systemic infections has been reported by multiple groups. A positive history of chickenpox is associated with a protective effect against glioma. However, a possible role for the immune system in the development of glioma needs to be further elucidated. Additionally, several inherited Mendelian disorders, including neurofibromatosis, tuberous sclerosis, Lynch syndrome, Li–Fraumeni syndrome, melanoma–oligodendroglioma susceptibility syndrome, melanoma–neural–system tumor syndrome, and Ollier disease/Maffucci syndrome, confer increased subtype-specific glioma risks. However, these monogenic disorders account for a small proportion of glioma cases. In addition, an increased incidence of brain tumors was noted among patients with L-2-hydroxyglutaric aciduria, a rare autosomal recessive neurometabolic disorder caused by mutations in the L2HGDH gene. Of note, L-2-HG is the stereoisomer of D-2-HG, the oncometabolite generated by IDH mutations in cancers. Several studies have also indicated familial clustering of gliomas in nonsyndromic families. Efforts to identify recurrent genetic events in familial glioma pedigrees have pointed to a novel low-penetrance susceptibility locus at the 15q23 region. Further, patients with LGGs with wild-type IDH are older compared with LGGs with mutated IDH and are more likely to have a family history of cancer.

Genome-wide association studies (GWAS) have greatly contributed to our understanding of glioma heritability by

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*Fig. 1* Over the last decade, various germline glioma risk factors and highly recurrent somatic genetic alterations have been uncovered. Our next challenge is to model these early events in appropriate cell lines or mouse models to faithfully recapitulate human malignant gliomas. Therefore, the identification of the cell or cells of origin for gliomas remains pivotal to our understanding of glioma biology.
identifying single-nucleotide polymorphisms (SNPs) that influence glioma risk.\textsuperscript{37–42} Interestingly, rs55705857, a glioma risk SNP located at 8q24.21 is strongly associated with risk of non-GBM tumors, suggesting its causal link to the development of IDH\textsuperscript{-}mutated gliomas by potentially deregulating MYC activity.\textsuperscript{43,44} Glioma risk loci near TERC and TERT associate with telomere length and indicate that telomere dynamics may contribute to glioma susceptibility.\textsuperscript{41} A recent large-scale meta-analysis of GWAS studies identified new SNPs that confer increased risk of GBM and non-GBM tumors, increasing the total to 26 risk SNPs.\textsuperscript{45} The study also found that these SNPs significantly overlap with enhancer regions, raising the possibility that they may regulate transcription through cis-regulatory elements to influence glioma risk. Of note, this study identified rs7572263 within intron 3 of C2orf80 as a risk loci of non-GBM tumors that is 50kb telomeric to IDH1.\textsuperscript{45} Given that IDH1 is primarily mutated in non-GBM tumors, it is plausible that this SNP may act as a basis for susceptibility to develop IDH1 mutant gliomas. Future research will shine light on how these SNPs may create a favorable environment for gliomagenesis to occur.

### Somatic Genetic Alterations

Genome-wide profiling has exposed the genomic landscape of malignant gliomas and revealed glioma subtypes with well-defined molecular and epigenetic signatures.\textsuperscript{5,6,7} Furthermore, these studies have shed light on the timescale of genomic alterations that have taken place during the early evolution of malignant gliomas. GBM, the most aggressive form of malignant brain tumor in adults, presents with a high degree of intratumoral genomic and spatial heterogeneity.\textsuperscript{46–49} Ozawa et al applied a mathematical modeling method to infer a temporal sequence of genetic alterations in non-G-CIMP GBM and identified chromosome (chr) 7 gain and chr10 loss, observed in 80\% to 90\% of these tumors, as early genetic events during GBM pathogenesis.\textsuperscript{50} Interestingly, increased PDGFA and decreased PTEN expression were significantly associated with chr7 gain and chr10 loss, respectively, and these events were identified as potential early drivers.\textsuperscript{50} Collectively, these data suggest that focal EGFR amplification, which accompanies chr7 gain, may be a later event involved in GBM pathogenesis that influences tumor progression or maintenance.\textsuperscript{7,50} In addition to copy number alterations, sequencing of primary GBMs has identified PIK3CA mutations as early clonal events and FGFR3-TACC3 fusions as shared truncal events.\textsuperscript{51–53} Commonly mutated genes in GBMs converge on a core set of pathways including inactivation of p53 and Rb pathways, dysregulation of growth factor signaling pathways, and activation of phosphoinositide 3-kinase signaling, leading to GBM pathogenesis.\textsuperscript{5,54}

In contrast to chromosomal abnormalities and the extensive heterogeneity observed in non-G-CIMP GBM tumors, virtually all G-CIMP gliomas harbor IDH\textsuperscript{mutation}.\textsuperscript{6} Earlier studies pointed to IDH1 mutations as the earliest event in gliomas.\textsuperscript{55} Exceptions include Li-Fraumeni associated astrocytomas with TP53 germline mutations that acquire the rare IDH1 R132C mutation as a somatic event.\textsuperscript{56} Deep sequencing analysis identified IDH1, TERT promoter mutations, and 1p/19q codeletion or IDH1, TP53, and ATRX mutations as truncal events, suggesting that these combinations are necessary to promote gliomagenesis, as mutant IDH alone is not sufficient for malignant transformation.\textsuperscript{57,58} Further work is warranted to understand the causal link between the sequence of events and their functional role in malignant transformation.

### Cell of Origin of Gliomas

In the adult mammalian brain, neural stem cells (NSC) reside within at least two neurogenic niches, the SVZ lining the lateral ventricles and the subgranular zone of the dentate gyrus of the hippocampus.\textsuperscript{59} NSCs are self-renewing, multipotent, and express Gfap. NSCs give rise to progenitor cells that produce progeny along either neuronal or glial lineages. Both NSCs and progenitor cells express nestin along with various other markers.\textsuperscript{60,61} The developmental potential and plasticity of NSCs make them ideal candidates as cells of origin for glia. NSCs exhibit similar features of gliomas, such as the transcriptional circuitry governing NSC identity, activation of developmental signaling pathways, motility, association with blood vessel basement membranes and white-matter tracts, and expression of cell surface antigens.\textsuperscript{62–68}

Several genetically engineered mouse models (GEMMs) have been developed to test combinations of glioma-specific genetic alterations (tumor suppressor genes such as Pten, Ink4a/Arf, Nf1, p53 and Rb1, and oncogenes such as Egfr, kRas, Akt, PDGF ligands and IDH1 R132H) in particular cell types. Several studies indicate NSCs as the likely cell of origin for gliomas in mouse models, since mature astrocytes failed to form gliomas in mice with combined activation of Ras and Akt or combinatorial deletions in Pten, Tp53 Nf1, and Rb1.\textsuperscript{69–71} Further, inactivation of Nf1, Pten, and Tp53 in neural progenitor cells and oligodendrocyte precursor cells (OPC) gives rise to different GBM subtypes that differ with regard to their molecular profile, their location in the brain, and overall survival.\textsuperscript{72} However, others have shown that oncogenic signaling is dominant to cell of origin in determining glioma phenotype.\textsuperscript{73} Conditional knock-in mice with central nervous system (CNS)-specific Idh1 R132H expression in nestin-positive mouse NSC as early as embryonic day 10.5 led to perinatal lethality due to extensive brain hemorrhage.\textsuperscript{74} However, conditional, inducible expression of the Idh1 R132H in the adult mouse SVZ stem cell niche led to cellular and molecular features associated with gliomagenesis, such as increased global methylation, overexpression of Wnt, and activation of pathways involved in telomere maintenance, stemness, and the cell cycle.\textsuperscript{58} These results highlight the importance of the developmental period in which glioma-specific IDH mutations arise.

In contrast, the role of postmitotic, differentiated astrocytes in glioma formation is still controversial. Several studies have shown that mature astrocytes are less susceptible to transformation in vivo unless converted to an undifferentiated state through retroviral transduction of PDGF or through the loss of the Ink4a-Arf locus to block cellular senescence.\textsuperscript{75–77} In contrast to these studies, a recent study by the Verma group demonstrated that differentiated neurons and glial cells can
dedifferentiate and initiate gliomas in mice upon loss of tumor suppressors Nf1 and Tp53. Together, these results from GEMMs suggest that various CNS cell types are susceptible to glioma-specific mutations (either alone or in combination); however, these different cell types may differ in their likelihood of transformation when presented with genetic alterations. Also, there exists the possibility that progenitor cells may be different in humans.

Recent large-scale single cell transcriptomic sequencing of primary IDH mutant gliomas identified a shared common progenitor for IDH mutant gliomas regardless of their molecular subclassification (either TP53 and ATRX mutations or TERT promoter mutations and 1p/19q codeletions). Further, most of the transcriptional differences observed between these two IDH mutant subtypes were explained by their genetic differences and the composition of the tumor microenvironment, but not by distinct cell of origin in the malignant cells. It is plausible that subsequent genetic alterations (e.g., 1p/19q codeletion or ATRX mutation) acquired by the IDH mutant cell may create different microenvironments leading to tumors with distinct clinical courses. Of note, single cell transcriptomic sequencing has not revealed a shared developmental architecture in IDH wild-type GBMs; these results are supported by the intratumoral heterogeneity of GBMs and the variable results from the GEMM models.

**Clinical Implications and Concluding Remarks**

The cell of origin for glioma remains elusive. Given the heterogeneity of the disease, it is likely that different molecular subtypes may arise from different cells of origin or harbor distinct molecular states influenced by developmental age. Identifying the roles of initiating events in candidate cell types at defined developmental time windows may resolve some of the features associated with gliomagenesis. Through GWAS studies and genome-wide analyses of primary gliomas, we have identified germline risk factors and greatly advanced our knowledge of the early genetic events that occur during glioma evolution. Our current challenge is to decipher how early events or germline risk loci cooperate with the cell of origin, specific signaling pathways, and the local niche to create an environment susceptible to malignancy. A thorough understanding of origin of gliomas can inform the design of effective and minimally toxic therapies and may identify biomarkers to stratify patients for personalized treatments. It is tantalizing to envision that expression of oncogene-driven early effector genes in brain tumors can be quantitatively measured using noninvasive technology. Such advances would allow the early detection of gliomas, or screening for patients at high risk of developing brain tumors. In addition, experimental mouse models that are developed to study cell of origin of gliomas can be used to study the multistep process toward malignant transformation and can be utilized as valuable preclinical mouse models for drug screening. For example, recently available CNS-specific mouse models of Idh1 R132H will facilitate testing of cooperating alterations that are recurrently found in IDH mutant lower grade gliomas. In addition to mouse models, human cerebral organoids may be uniquely appropriate models to investigate the early stages of gliomagenesis, since they resemble the complex spatial organization of the human brain. Advances in single cell sequencing approaches and sequencing-based methods that preserve spatial information along with spatially resolved proteomics will advance our understanding of glioma evolution in the context of its microenvironment. We are at an exciting time in glioma research and believe that bringing together multiple perspectives will shine light on potential therapeutic targets or biomarkers that will be a major step forward in our fight against this disease.

**References**


