REVIEW ARTICLE

Molecular biology of glioblastoma: Classification and mutational locations

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Abstract

Glioblastomas are regarded as the most common malignant brain tumours with great morphological and genetical heterogeneity. They comprise 12% to 15% of all intracranial tumours, with its peak observed in the 8th decade of life. The five-year survival is only 5%. Primary glioblastomas are more common in elders while secondary glioblastomas mostly involve younger people. Based upon gene expression profile, researchers have classified glioblastomas into several subtypes. Genetic mutations provide an advanced standard platform essential for diagnosis, therapeutic remedies and prognosis of glioblastomas. Common mutations observed in glioblastomas are loss of heterozygosity at 10q followed by epidermal growth factor receptor amplification (34%) and others. Vascular occlusion model (Figure) and tumour stem cell model can explain the possible mechanism in glioblastomas pathogenesis. This review highlights glioblastomas' classifications, genetic mutations, pathogenesis and prognosis of different sub-types.

Keywords: Glioblastoma, Molecular classification, Genetic mutations, Pathogenesis, Prognosis.

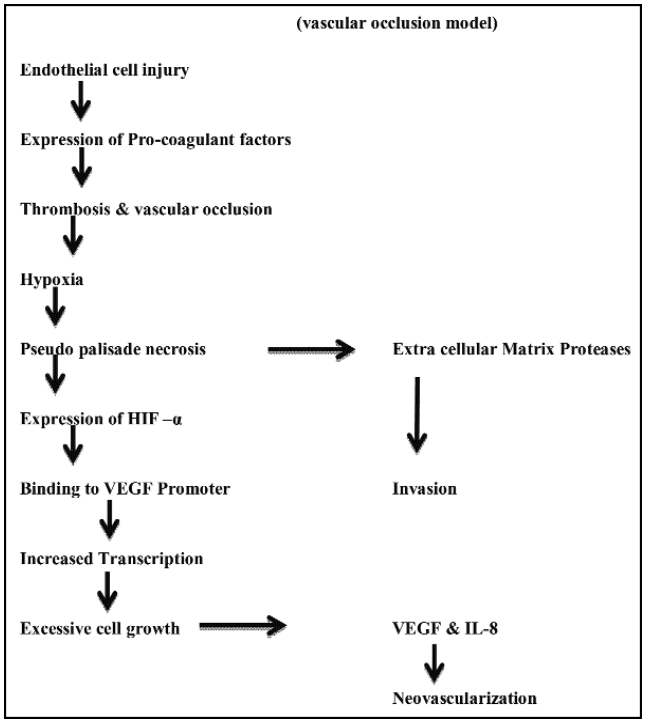

Figure: Mechanism of glioblastoma genesis.

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Introduction

The term 'glioma' includes all the tumours arises from glial cells, including grades I, pilocytic astrocytomas, pleomorphic xanthoastrocytomas, and subependymal giant cell astrocytomas, grade II oligodendrogliomas and astrocytomas, grade III anaplastic oligodendrogliomas, anaplastic astrocytomas, anaplastic oligoastrocytomas, anaplastic ependymomas and grade IV glioblastoma multiform (GBM).[1]

GBMs are the most common malignant tumours of central nervous system,[2] accounting for 12% to 15% of all intracranial tumours and 50% to 60% of astrocytic tumours. GBMs have annual incidence of 5.26 per 100,000 people. Around 17,000 new cases are diagnosed per year.[3] The incidence of GBMs increases with age and reaches the maximum between 75 to 84 years of age. They are more common in white males. GBMs have poor prognosis and the 5 year survival is less than 10%. Patients usually survive for 12 to 15 months after the final diagnosis.[4,5]

Histopathological Classification

Gliomas develop from glia cells, especially astrocytes and oligodendrocytes lineage cells. There are many different ways to classify gliomas. According to tumour growth pattern, Gliomas can be classified as circumscribed and diffuse variety. Circumscribed gliomas include juvenile fibrillary astrocytomas, pleomorphic xanthoastrocytomas and subependymal giant cell astrocytomas. Due to location and low aggressiveness, circumscribed gliomas can be surgically resected.

Diffuse gliomas are highly invasive. There is no clear boundary between tumour and surrounding tissues. Due to this reason curative surgical resection can never be achieved and residual neoplastic cells lead to recurrence.

Diffuse gliomas have the strongest ability of infiltration and neovascularisation which can be used to distinguish them from necrotic tissues. Diffuse gliomas can be further classified into astrocytic, oligodendroglial and mixed (oligodendroglial-astrocytic) subtypes of World Health Organisation (WHO) grade II (low grade), III (anaplastic) or IV (glioblastoma).[6]

GBMs, the most common intrinsic and foetal tumours of brain in adults, arise from glial cells but can also develop from astrocytic or neural stem/progenitor cells.[7] These tumours can be classified into primary and secondary GBMs, based on pre-existing lesion. The primary subtype accounts for 90%~95% of GBMs. They are more common among elders, especially after the age of 50 years. Secondary GBMs develop from evolution of low-grade astrocytic tumours and anaplastic astrocytomas over the course of 4-5 years. They account for 5% to 10% of GBMs, and are more common among young people.[8]

Molecular Classification of Glioblastoma

Studies over last two decades have mentioned the role genetic of mutations in GBMs but still it is not clear that which mutation is more responsible for specific characteristics of GBMs. Classification only based on histo-pathology could not describe all the malignant features of GBMs, specially their responses to treatment. For example, some GBMs are particularly sensitive to radio-chemotherapy while others are resistant. Some GBMs are very much aggressive with early recurrence, whereas others progress slowly for prolonged period.[7,9] Due to these pitfalls of traditional classifications, molecular parameters have been added for GBMs classification recently. Molecular markers that carry both diagnostic and prognostic information add useful tools to traditional classification by redefining tumour subtypes within each WHO category. Nowadays, molecular markers have become an integral part of tumours assessment in modern neurooncology. Biomarkers status now helps for clinical decisions in GBMs, one of the most aggressive subtype.[10]

In The Cancer Genome Atlas (TCGA) Verhaak[11] divided GBMs into four subtypes based on gene expression profile. These are proneural, neural, classical and mesenchymal subtypes. Proneural GBMs have lower incidence rate and characteristics of oligodendroglial cells. They develop mainly in younger patients with secondary glioblastoma. Neural GBMs arise from astrocyte and oligodendrocyte lineage cells and manifest the expression of neuron-related genes. Their main characteristics are high level of epidermal growth factor receptors (EGFRs) and expression mode most similar to normal brain tissues. Classical GBMs express the markers of neuron precursor cells and stem cells. They show the characteristics of astrocytes while mesenchymal GBMs share the characteristics of cultured astrocytic gliomas.

After examining 183 samples of GBMs, Phillips et al. classified them into three subtypes: proneural (31%), proliferative (20%) and mesenchymal (49%). Proneural GBMs express genes which correlate with neuron development. They always occur around the age of 40 and have good prognosis. Proliferative and mesenchymal subtypes express genes which correlate with cells proliferation and angiogenesis or mesenchyma, respectively. Both (proliferative and mesenchymal) varieties always occur over the age of 50 years and have poor prognosis.[7]

Chinese glioma genome atlas (CGGA), with the study of 89 samples of GBMs, has been divided into three subgroups (G1, G2 and G3). Co-relating with the TCGA classification system, it was observed that the G1, G2 and G3 subgroups were enriched with proneural, neural and mesenchymal GMBs, respectively. G1 type has been observed mainly in young patients having good prognosis while G3 type in old age patients with poor prognosis. Clinical characteristics of G2 type are between G1 and G3 type.[12]

Genetic Mutations in Glioblastomas

At the molecular level in primary GBMs, loss of heterozygosity (LOH) at 10q is the most frequent genetic alteration in primary GBMs observed in 69% patients, followed by EGFR amplification (34%), tumour protein 53 (TP53) mutations (31%), p16INK4a deletion (31%), and phosphatase and tensin homolog (PTEN) mutation (24%). In secondary GBMs, LOH 10q and TP53 mutations are the most frequent as they are present in 63% and 65% patients, respectively, while other genetic alterations are

Table: Some Common mutations in Glioblastomas.

Genetic alterations	Primary Glioblastoma (%)	Secondary Glioblastoma (%)
LOH 10q Mutation[8]	70	63
EGFR Amplification[8]	35	8
TP53 Mutation[8]	30	65
PTEN Mutation[8]	25	4
IDH Mutation[18]	5	80
MGMT promoter methylation[22]	42	79

LOH: Loss of heterozygosity.
EGFR: Epidermal growth factor receptor.
TP53: Tumor protein 53
PTH: Phosphatase and tensin homolog.
IDH: Isocitrate dehydrogenase.
MGMT: 0^6-methyl guanine-DNA (deoxyribonucleic acid] methyltransferase.

infrequent (4%-19%). LOH 10q alteration has been observed in both primary and secondary GBMs while TP53 mutation mostly in secondary GBMs[13] (Table).

a) EGFR amplification and EGFR variant - rearrangement

The EGFR gene, located on chromosome 7p12, encodes a transmembrane tyrosine kinase receptor. The EGFR protein has three functional domains: the extracellular binding domain, transmembrane domain and intracellular tyrosine kinase domain. After ligand binds with EGFR, it undergoes phosphorylation and activates downstream targets of signalling pathways, thus promotes cells proliferation and migration. The amplification of the EGFR gene has high incidence in gliomas, found in 40% to 50% of all GBMs.[14] Among them, 63-75% cases also carry rearrangements of the EGFR gene, resulting in tumours expressing both wild-type (wt) and mutated EGFR.[15] In anaplastic astrocytomas, the incidence of EGFR amplification is 17%. EGFR amplification in TCGA's classical subtypes and Phillip's proliferative/ mesenchymal subtypes has been observed in 94% cases. Cells with EGFR amplification usually have other forms of EGFR mutations, the most common being EGFRvIII rearrangement, which is resulted from the deletion of exons 2-7 in EGFR messenger ribonucleic acid (mRNA). EGFRvIII rearrangement reduces kinase degradation and over-activates the downstream targets of signalling pathways. In GBM patients, EGFRvIII mutation indicates poor prognosis. EGFR-targeted therapies have showed no obvious curative effect, but several clinical trials have found that anti-EGFRvIII vaccines can improve the prognosis in these patients.[16]

b) Isocitrate dehydrogenase (IDH) mutation

IDH is a rate-limiting enzyme in tricarboxylic acid (TCA) cycle which catalyzes Isocitric acid into α-ketoglutaric acid and reduces co-enzyme II nicotinamide adenine dinucleotide phosphate (NADPH) by oxidative decarboxylation.[17] The IDH gene family has three isomerases, IDH1, IDH2 and IDH3. IDH gene mutation leads to the abnormal formation of 2 - hydroxyglutaric acid from α-ketoglutaric acid. Thus it inhibits the enzymes which depend upon α-ketoglutaric acid for their functions. This causes epigenetic changes (hypermethylation at large number of genes) which results in different gene expression profile and inactivation of tumour suppressors, ultimately occurrence of GMBs.[17-19]

In diffuse gliomas, IDH1 is the most frequently observed mutation in more than 90 per cent cases, IDH2 in only 10% cases while mutation in IDH3 hasn't been found yet. The IDH1 mutation usually occurs in young people. Mutation incidence in the secondary GBMs is 80%, much higher than primary GMBs in which it is only 5%. IDH mutations are associated with an better prognosis throughout every grade of glioma.[8,18,19]

c) MGMT (O6-methyl guanine-DNA methyltransferase) promoter methylation

O6-methylguanine-DNA methyltransferase (MGMT) is an excision repair enzyme located in the nucleus responsible for deoxyribonucleic acid (DNA) repair. This enzyme is encoded by MGMT gene, located at 10q26.[20] When DNA is damaged it is transferred into the nucleus where it binds to O6-methyl-guanine with the help of alkylating agent, demethylates it and effectively repairs DNA damage. At the same time, MGMT itself is irreversibly deactivated. Therefore, MGMT is called a suicidal enzyme.

Transcription of the MGMT gene appears to initiate at a single site within a guanosine-cytosine-rich, non-TATA box-containing promoter. Methylation of a promoter acts to inhibit (silence) transcription of the associated gene. Methylation results inactivation of tumour suppressor genes, DNA repair genes, and proapoptotic genes. Thus induction of carcinogensis.[21,22] In GBMs, about 50% of MGMT promoter methylation occurs in the secondary GBMs, more common in elder patients.[23] GBMs having MGMT promoter methylation are sensitive to chemo-radiotherapy. Simple radiotherapy combined with adjuvant chemotherapy can prolong survival in elderly patients with MGMT promoter methylation.[24] In patients with MGMT promoter methylated tumours, monotherapy with temozolomide is superior to radiotherapy alone.[25]

d) PTEN Genetic Mutation

PTEN, located on chromosome 10q23, is a member of the protein tyrosine phosphatase gene family. It is an important tumour suppressor gene which expresses function by protein phosphorylation. PTEN can inhibit cell invasion, cell adherence to the surrounding matrix and blood vessel formation. It is also involved in signalling transduction pathways. In case of excessive cell differentiation, it can regulate cell cycle and induce apoptosis, thus inhibits tumour growth. Around 86% of patients with GBMs have loss of PTEN gene and changes of receptor tyrosine kinase / phosphoinositide 3-kinase (RTK/PI3K) signalling pathway. PTEN point mutation rate in the primary GBMs is 26%~34% while in anaplastic astrocytomas, the mutation rate is 18%. GBM patients with PTEN mutation tend to have poor prognosis.[26]

e) Neurofibromatosis type 1 (NF1) gene mutation and deletion

NF1 gene, located on chromosome 17q11.2, encodes the

neurofibromatosis protein. It is a potential tumour suppressor and negative regulator of Ras and mechanistic target of rapamycin (mTOR) signalling pathways in astrocytes. Inactivation of neurofibromatosis type 1 (NF1) gene in GBMs, which occurs via two mechanisms: excessive proteasomal degradation and genetic loss results in excessive growth and tumorigenesis.[27] NF1 mutations are more common in mesenchymal subtype of GBMs.[28] By experiments on mouse astrocytes, it was found that NF1 deletion results in a significant rise in cell proliferation and migration, through Ras-mediated trans-activation of mTOR.[29] In the case of NF1 gene mutations or deletions, Stat3 (potential downstream target genes) is regulated by the mechanistic target of rapamycin complex 1 (mTORC1) and Ras-related C3 botulinum toxin substrate 1 (Rac1) and the expression levels of cyclin D1 increases which results in increased cells proliferation.[29] Genetically engineered mouse models have demonstrated that in astrocytes, double knockout of NF1 gene can promote cells growth both in vivo and in vitro experiments, but it is not enough to induce GBMs.[30] Interestingly, NF1-/- astrocytes need heterogeneity to induce GBMs in brain environment.[31] These results highlight the importance of genetic changes, heterogeneity and cell specificity in the process of tumorigenesis.

Mechanisms of glioblastoma genesis

a. Vascular occlusion model of glioblastoma

Tumour genetic alterations lead to change in biological behaviours like rapid cell proliferation, invasion and angiogenesis. Excessive cells proliferation results in the formation of thrombosis and vascular occlusion. In models of GBM, the endothelial injury and the expression of procoagulant factors by the neoplastic cells result in intravascular thrombosis and increasing hypoxia, which trigger adaptive changes of psuedopalisade necrosis in tumour cells. Pseudopalisade cells over-express hypoxia-inducible factor-1α (HIF-1α), which binds the vascular endothelial growth factor (VEGF) promoter and increased its transcription resulting in excessive cells proliferation. In addition, hypoxic psuedopalisade cells express increased levels of extracellular matrix proteases associated with invasion. During the migration, tumour cells secrete proangiogenic VEGF and interleukin 8 (IL-8), which promote tumour neovascularisation. Thus microvascular hyperplasia in GBM that provides a new vasculatures. An altered vascular network determines irregular blood flow and promotes rapid peripheral tumour invasion[32] (flow chart).

b. Tumour stem cell model

The cancer stem cell theory postulates that tumours are sustained by a selected cell population with specific features such as self-renewal ability and the capacity to give rise to a heterogeneous mass of tumour cells. The existence of such cells has been verified for GBMs, known as glioma stem cells (GSCs).[33]

Pallini et al.[34] have found that these cells exert influence on the survival of patients: if the number of tumour stem cells increases, overall survival will be shortened. The precursor cells of tumour stem cells are still unclear. They may come from normal neural stem cells or mature neurons which are genetically changed and thus obtain a more primitive phenotype. In addition to genetic changes, tumour microenvironment also plays an important role in signal transduction and the regulation of tumour stem cell phenotype. Tumour stem cells have high tumourigenic capacity and chemoresistance which usually results in tumour recurrence.[35]

Conclusion

Research in the past 20 years has helped us have a deeper understanding about GBM, while more challenging and controversial issues have been raised. The interpretation of GBMs' complex inter-tumoural and intra-tumoural heterogeneity provides us important information about tumour behaviour, thus enhancing the development of treatment strategies for future.

Future research should be focused on the role of tumour stem cells and tumour microenvironment in GBMs' occurrence, progression and response to treatments. Targeted therapies against different mechanisms for tumorigenesis may be beneficial in future.

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