Review Article

Genetics and Epigenetics of Glioblastoma: Therapeutic Challenges

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Abstract

Glioblastoma is a brain tumor that develops due to both genetic and epigenetic risk factors. Crosstalk between the genetic and the epigenetic offers new possibilities for therapy. Abnormal methylation of methylguanine-DNA methyltransferase (MGMT) promoter region and isocitrate dehydrogenase 1 (IDH1) mutations are prognostic and therapeutic response markers in glioblastoma. Mutations in genes such as epidermal growth factor receptor, TP53, and P16 have been reported in glioblastoma; therefore, they might associate with survival and worth to be used in estimating survival risks. MKI67 expression associates with posttreatment such as adjuvant radiotherapy results evaluation. On the other hand, monosomies, such as deletions of chromosome 10, especially q23 and q25–26, are good markers for estimating the progression and aggressiveness of glioblastoma. The profile of MGMT methylation is modified in glioblastoma and hence can be a good target for epigenetic drugs. Other useful strategies in the treatment of gliomas include several micro-RNAs (MiRs) which are alerted in glioblastoma and which affect the regulation of mRNAs are associated with gene expression profiles of the disease. Epigenetic drugs, such as azacitidine and decitabine, which belong to the DNA methyltransferases (DNMT) inhibitor 5-aza-2'deoxycytidine (5-aza-dC), can suppress DNMT1 and stimulate tumor suppressor genes expression. MGMT methylation status and IDH mutational status are two valuable prognosis and therapeutic response markers in glioblastoma. Regulation of glioblastoma through epigenetic drugs, such as not only inhibitors of EZH2, histone deacetylase, and DNMT, but also MiRs, are promising approaches in glioblastoma treatment. Improves in understanding cancer genetic and epigenetic disruptions is the key point in solving the puzzle of glioblastoma treatment.

Keywords: *Epigenetic drugs, epigenetic, gene editing, genetic*

Introduction

Glioblastoma is one of the most common brain tumors[1,2] with poor prognosis and limited chemotherapy efficiency as a result of the blood–brain barrier.[3] To improve the prognosis of glioblastoma, the minocycline, telmisartan, and zoledronic acid (MTZ) regimen were recommended, which includes MTZ.[4] Heterogeneity of glioblastoma investigates the variability of genetic and epigenetic of this tumor, changes in methylation pattern. Various mutations in different genes are responsible for glioblastoma.[1] Glioblastoma invades other organs through blood mostly and lymphatic pathways; however, this tumor has low potential of metastasis to out of central nerve system as a result of blood–brain barrier and absence of lymphatic vessels.[5] Mutations in genes such as epidermal growth factor receptor (EGFR), TP53, and P16 have been reported in glioblastoma, therefore, they might associate with survival, and they are worth being used in estimating survival risks.[6] A study on six metastatic glioblastomas investigated CDKN2A/P16 deletion; loss of alleles on chromosomes 1p, 10q and 19q, TP53 mutation, and EGFR amplification and interestingly metastasis occur mostly in young patients with TP53 mutation.[7] Recent studies demonstrated that the metastasis process can be affected by various molecules such as chemokines, pro-angiogenic factors, growth factors, extracellular matrix-remodeling proteins, and several micro-RNA (MiRs).[8] A study on IDH1 gene mutation in glioblastoma, with oligodendroglia appearance and1p19q deletion, showed a better response to chemotherapy in comparison to other mutations.[9] The most invasive mutation in astrocytic gliomas, a subtype of glioblastomas, is 9p21 deletion which can activate MYC signaling pathway.[10] At the molecular level, glioblastoma is characterized by different genetic and epigenetic

How to cite this article: Rasras S, Zibara K, Vosughi T, Zayeri ZD. Genetics and Epigenetics of Glioblastoma: Therapeutic Challenges. Clin Cancer Investig J 2018;7:43-9.

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Access this article online

Website: www.ccij-online.org

DOI: 10.4103/ccij.ccij_82_17

Quick Response Code:

changes that affect different oncogenes and tumor suppressor genes. However, few of these changes are known as prognostic and treatment response markers such as abnormal methylation of methylguanine-DNA methyltransferase (MGMT) promoter region and isocitrate dehydrogenase 1 (IDH1) mutations.[11] Genetic studies can assist in finding a therapeutic target, however; our knowledge is not enough yet.[12] Recent studies suggests that the origin of glioblastoma in the brain can be helpful in choosing the therapeutic method and estimating patients response.[13] Epigenetic modifications of tumor cells have been investigated in glioblastoma whereas epigenetic drugs are considered as good targets for glioblastoma therapeutic studies.[14] Recent therapeutic approaches, such as DNMT and histone deacetylase (HDAC) inhibitors, which overturn epigenetic effects, are intensively considered in neoplastic disorders and malignancies.[15] In this review, we discuss the genetics and epigenetics of glioblastoma and the effect of mutations on its features. We also discuss various treatment strategies such as epigenetic drugs, MiRs, and gene editing. The challenge is to classify glioblastomas according to genetic and epigenetic defects and to manage the treatment strategies according to tumor's genetic and epigenetic origins.

Glioblastoma Genetic and Possible Classification

Genome-wide profiling studies have investigated genomic heterogeneity among glioblastoma tumors, and different molecular signatures defined subclasses that can be useful in stratification of treatment.[16] However, mutation occurrence in glioblastoma is lower than other solid tumors.[17] On the other hand, loss of heterozygosity (LOH) among markers of the long arm of chromosome 10 (10q), which contains cancer genes such as PTEN, FGFR2, and MKI67, is detectable in up to 80% of glioblastoma cases.[11] In fact, monosomies such as deletions of chromosome 10, especially q23 and q25–26, are good markers for estimating the progression and aggressiveness of glioblastoma.[18] In astrocytomas and oligodendroglial tumors, which are subtypes of glioblastoma tumor, IDH mutations usually happen earlier than 1p deletion (del),9q del and tumor protein p53 (TP53) mutations.[19] Amplification of CDK and MDM2 oncogenes in glioblastoma disrupts P53 and RB pathways, and their mutations are associated with tumor progression.[17] Indeed, TP53, PTEN, and EGFR genes are the most frequently mutated genes in glioblastoma [Table 1].[20]

Epigenetics in Glioblastoma

Epigenetic risks such as allergies, atopic diseases, and systemic infections seem to be important in triggering glioblastoma, however; neither cigarette smoking nor alcohol consumption have been reported as risk factors.[46,47] Sturm *et al*. dentified six epigenetic glioblastoma subgroups displaying characteristic global DNA methylation patterns harboring distinct hotspot mutations, DNA copy-number alterations, and transcriptomic patterns.[1] The most common epigenetic change in glioblastoma is the LOH of chromosome 10q.[44] Several cancer mutations cause changes in DNA methylation profile, histone modifications, and nucleosome positioning which disrupt vital signaling pathways.[48] Studies showed that several epigenetic changes such as methylation of LINE-1 to be associated with poor prognosis in primary glioblastoma patients.[49] Changes in promoter DNA methylation pattern are important in glioblastoma, especially if the methylation occurs in a promoter involved with crucial biologic pathways.[50] Abnormal methylation of the MGMT promoter region and mutations in IDH1 are two valuable prognosis and therapeutic response markers in glioblastoma.[51,52] For instance, epigenetic changes such as changing MGMT methylation profile might result in a decrease in MSH2, MSH6, and PMS2 proteins in glioblastoma.[53] In fact, hypermethylation of several tumor suppressors, DNA repair genes, and cell-cycle regulators is associated with increased mutation rate and poor outcome in glioblastoma.[54] In addition, several studies showed that MGMT promoter methylation status can be a predictor of temozolomide response in glioblastoma.[55] Moreover, CHK2 that inhibits cell-cycle progression through decreasing cyclin-dependent kinases (CDK) activity has been found to be hypermethylated in gliomas.[56]

Developing Therapeutic Approaches According to Genetic and Epigenetic Changes

Mesenchymal stem cells (MSCs) have inhibitory effects on growth, invasion, and metastasis of solid tumors. Therefore, they can be considered as a therapeutic approach in tumor treatment although their exact role in tumor progression is still unknown.[57] Since glioblastoma tumors do not respond efficiently to chemotherapy, radiation, and they are not surgically curable, novel treatment methods are needed.[58] MiRs affect gene expression and are candidates for glioblastoma therapy. For instance, MiR-873 downregulate IGF2BP1 expression affecting negatively the carcinogenesis and metastasis of glioblastoma.[59] On the other hand, MiR610 decrease the proliferation and cell growth of glioblastoma through inhibiting CCND2 and AKT3 expression at the transcriptional and translational levels.[60] Long noncoding RNAs (lncRNAs) such as ASLNC22381 and ASLNC20819, which target IGF-1, play important roles in glioblastoma development and progression. Therefore, targeting lncRNAs might be an effective therapeutic approach.[61] Epigenetic modifications are altered in tumor cells, in comparison to normal tissues, which can be reverted by inhibitors interfering in epigenetic enzymatic activities. For example, 5-aza-2'-deoxycytidine (5-AZA-CdR) is an epigenetic drug which increases apoptosis in glioblastoma cells through caspase-8 pathway.[62]

Table 1: Genetic changes of glioblastoma

Mutation	Result	Prognosis	References
PTEN (10q23.31) inactivation	generation of cells, angiogenesis, invasion of cells, immune response, differentiation of cells, and cell survival	Poor	[21]
Activation of the FGFR2 oncogene (10q26.13)	-	Poor	[22]
Deletion of the FGFR2 oncogene (10q26.13)	-	Good	[11,23]
MKI67 (10q26.2)	High expression is related with shorter survival, expression can be associated with posttreatment such as adjuvant radiotherapy	Poor	[24]
TERT promoter mutations	Poor survival, require aggressive treatment	-	[25]
Deletion of 10q26	Favorable response to TMZ	Good	[11,24]
IDH1 mutant sGBIV	Longer survival	Good	[26]
PDGFRAgain or amplification	Poor outcome	Poor	[26]
1p/19q codeletion	Predicts response to radiation or chemotherapy	Good	[19,27,28]
BRAF (V600E) mutation	Good response to BRAF/MEK inhibitors	-	[29]
amplification of MDM2 (1q32)	Small fraction of human malignant gliomas escapes p53-dependent growth control	-	[30,31]
Amplification of AKT3	Mutated cells survive, tumor recurrence	Poor	[32]
Amplification of 12q13-15	Decreased survival	-	[33]
Deletion of NFKBIA gene	Associated with poor survival	Poor	[17]
Amplification of EGFR	Multimodal therapeutic resistance, generation of cells, angiogenesis, invasion, immune response, differentiation, and cell survival	Poor	[34]
Deletion of CDKN2A	Pathogenesis of glioblastomas	-	[35,36]
Mutation of TP53	Generation of cells, angiogenesis, invasion, immune response, differentiation, and cell survival	Poor	[20]
Aberrant activation of ras signaling	Tumor development	-	
RTK amplifications	Mechanism of resistance	Poor	[37]
Loss ofRB1 expression	Tumor development	Poor	[38]
Mutation of NF1	Associate with CNS pathogenesis	Poor	[39]
Mutation of PIK3CA	Therapeutics targeting	-	[40]
Mutation of PIK3R1	Increase tumorigenicity	Poor	[41,42]
KUB3, CDK4, and/or CYP27B1 amplification	Decreased survival, resistant to TMZ	-	[33]
MGMT (10q26.3) methylation	Favorable response to temozolomide, predictive marker for prolonged survival	Good	[17,19,43]
LOH at the 10q23.3-26.3(deletion or an aUPD)	Contain PTEN, FGFR2, MKI67, and MGMT, allow to find molecular markers of disease prognosis and response to treatment	-	[44]
H3F3A mutations	Associated with a uniformly	Poor	[45]

IDH: Isocitrate dehydrogenase, TERT: Telomerase reverse transcriptase gene, TMZ: Temozolomide, LOH: Loss of heterozygosity, UPD: Uniparental disomy, RTKs: Receptor tyrosine kinases, CNS: Central nervous system

Epigenetic drugs

Epigenetic drugs are undergoing clinical trials and some of them have been already approved for cancer treatment by the Food and Drug Administration and the European Medicines Agency.[63] Targeting epigenetic regulators such as EZH2 and BMI1proved to be effective *in vitro* and *in vivo*.[64] EZH2 interact with several lncRNAs, therefore, EZH2 inhibitors are used to potentially control glioblastoma progression.[61] Drugs which suppress DNMT1 hypomethylated the DNA across cell divisions and can stimulate tumor suppressor genes to be expressed. For instance, azacitidine and decitabine belong to the DNMT inhibitor 5-aza-2'deoxycytidine (5-aza-dC) which is a category of epigenetic drugs that have been approved by the FDA for the treatment of myelodysplastic syndromes, acute myeloid leukemia, and medulloblastoma.[55,65] Combination treatment of epigenetic drugs such as HDACi and DNMT represent a new hope in glioblastoma treatment.[65]

Micro-RNAs

Micro-RNAs (MiRs) are types of noncoding RNAs that control gene expression at the posttranscriptional level.[66] MiRs play important regulatory roles in biological processes such as apoptosis, migration, and invasion.[67] Recent studies have investigated several MiRs that are alerted in glioblastoma and which affected the regulation of mRNAs associated with gene expression profiles.[68] A summary of several studies based on MiRs therapeutic potential as an epigenetic drug in glioblastoma is presented in Table 2.

Genome editing technologies

Discovery of the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas system, offered a path to genome engineering. CRISPR/Cas was first generated and applied in 2013; however, limitations such as vector delivering systems into cells are still the challenging point of this technology.[87] CRISPR/Cas-based genome editing technologies are supposed to increase our ability to engineer genetic changes in glioblastoma-derived neural stem cells.[88] Zinc finger-mediated gene editing for the treatment of glioblastoma has been taken to the clinic.[89] Hematopoietic stem cell transplantation and immunotherapy are suggested therapeutic approaches because they can induce tumor-specific T cells production to fight malignant gliomas.[90]

Conclusion and Future Perspectives

Glioblastoma is a brain tumor with high frequency of mutations and poor prognosis. Glioblastoma involves various genetic and epigenetic changes that render diagnosis and treatment very difficult. Various mutations in EGFR, TP53, and P16 have been reported in these tumors. Important genetic markers implicated in estimating prognosis include 1p19q deletion, MGMT methylation, and IDH mutational status. Hypermethylation of several tumor suppressors, DNA repair genes, and cell-cycle regulators is associated with increased mutation rate and poor outcome in glioblastoma. Since most of the genetic changes lead to epigenetic modifications, we hypothesize that glioblastoma develops as a result of epigenetic

Table 2: Studies on micro-RNAs are with therapeutic potential in glioblastoma

MiRs	Study on	Result	References
MiR-124 and MiR-137	Adult mouse neural stem cells	Inhibit proliferation of glioblastoma cells/ induce differentiation of brain tumor stem cells	[69]
MiR-101	HBMVECs; cell systems ACBRI-376	EZH2-induced proliferation, migration, and angiogenesis	[70]
MiR-21 downregulation	The human U251 and LN229 glioblastoma cell lines	Inhibits EGFR pathway/suppresses the growth of human glioblastoma cells independent of PTEN status	[68]
MiR-221 and MiR-222	U251 and LN229 cells	Induce cell survival/inhibited cell apoptosis by targeting pro-apoptotic gene PUMA in human glioma cells	[71]
MIR-451 and Imatinib mesylate	A172 cells	Inhibit tumor growth in glioblastoma stem cells	[72]
MiR-17-92 downregulation	Glioblastoma spheroid cultures enriched in tumor-initiating cells	Increased apoptosis and decreased cell proliferation	[73]
MiR-9/9 inhibition*	R28 cells	Decrease glioblastoma cell survival	[74]
MiR-29b and miR-125a	LN229, U87, LN319, and U251 cell lines	Suppress invasion in glioblastoma	[75]
MiR-181d	A1207, T98G, LN340, and LN18 glioblastoma cells	Downregulates MGMT expression	[76]
MiR-182	GICs	Integrates apoptosis, growth, and differentiation programs in glioblastoma	[77]
MiR-99a	U87MG and U118MG cells	Inhibit FGFR3 and PI3K/Akt signaling mechanisms/control growth of human glioblastoma	[78]
MiR-124	Glioma cell lines and GCSCs	Inhibits STAT3 signaling/enhance T cell-mediated immune clearance in glioma	[79]
MiR-15b and MiR-152	9L rat glioma cell line	Reduce glioma cell invasion and angiogenesis	[80]
MiR-218	Mice	Promote the development of targeted therapies in mesenchymal glioblastoma	[81]
MiR-125b	Glioma cell line	Inhibits connexin43 expression/increase cell growth and anti-apoptosis	[82]
MiR-7	CHG5, TJ899, and TJ905 human glioblastoma cell lines	Inhibits glioblastoma growth by interfering with PI3K/ATK and Raf/MEK/ERK	[83]
MiR-124a	A172, T98G, U87MG, MO59J, MO59K, and CCF-STG1 cell lines	Inhibit migration and invasion of glioblastoma	[84]
MiR-34a1	Immunodeficient mice compared with wild-type U251 glioblastoma cells	Act as tumor suppressor modulating EGFR in glioblastoma	[85]
MiR-483–5p	U87, U251, and SHG44 human glioma cell lines and HEK293T	Suppresses the proliferation of glioma cells through targeting ERK1	[86]

MiR-9, MiR-9 (referred to as MiR-9/9*). HBMVECs: Human brain microvascular endothelial cells, GICs: Glioma-initiating cells, gCSCs: Glioma cell line stem cells, HEK293T: Human embryonic kidney cells, MiRs: Micro-RNAs, EGFR: Epidermal growth factor receptor

defects and that we could overcome glioblastoma by controlling the epigenetic changes. Hence, genetic and epigenetic changes can be benefit approaches in detecting the prognosis and treatment responses in glioblastoma. Regulation of glioblastoma through epigenetic drug such as not only inhibitors of EZH2, HDAC, and DNMT, but also MiRs, can be promising approaches in glioblastoma treatment because recent studies in these fields developed based on animal studies. Understanding cancer genetic and epigenetic disruptions are crucial for solving the puzzle of glioblastoma treatment. We recommend several studies based on combination regimens involving epigenetics and immunotherapy might be useful in increasing the hope of the treatment of glioblastoma.

Acknowledgment

We wish to thank all our colleagues in Golestan Hospital clinical research development unit, Ahvaz Jundishapur University of Medical Sciences. A special thanks to Professor Najmaldin Saki Hematology Phd in Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran who helped us in enriching this review.

Financial support and sponsorship

Nil.

Conflicts of interest

This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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