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Chapter 2

The Roles of MicroRNAs in Glioblastoma Biology and Biomarker

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Abstract

MicroRNAs (miRNAs) are small, noncoding RNAs transcribed from DNA that are 18–24 nucleotides in length. A single miRNA has the capacity to regulate a large number of target messenger RNAs (mRNAs), and the main function of miRNAs is to downregulate gene expression. A large set of miRNAs is overexpressed or downregulated in various human cancers compared with normal tissues, and gene silencing by miRNAs enhances tumor malignancies.

In glioblastomas (GBMs), a number of miRNAs are reported to display aberrant expression patterns, and miRNAs have been shown to strongly influence cell viability, cell proliferation, invasiveness, angiogenesis, metabolism, and microenvironment. Since early findings, the number of studies published on this subject has steadily increased, elucidating numerous interesting miRNA-mediated mechanisms in the tumorigenesis of GBM.

A number of studies have recently suggested that circulating miRNAs could be potential biomarkers for cancer diagnosis. Not only the role of miRNAs in glioma development but also their specificity makes them important candidate biomarkers that could provide important characteristic information about a tumor and improve treatment and prognosis. This review summarizes current progress and future directions in this exciting and steadily expanding field.

Keywords: glioblastoma, microRNA, hallmarks, biomarker, circulating

1. Introduction

Glioblastomas (GBMs) are the most common and intractable primary neoplasms of the central nervous system (CNS). Gross total surgical excision followed by radiotherapy up to a total dose of 60 Gy was the only accepted GBM management for decades because no chemotherapeutic agents significantly improved the survival of patients with GBM until the introduction of temozolomide, an oral alkylating agent (1). Despite aggressive treatments, recurrence is inevitable and fatal in GBMs. In a recent clinical study, the median survival was up to approximately 20 months and the two-year survival rate was approximately 30~40% (2, 3). Therefore, more efforts need to be made to change the poor prognosis of patients with GBM.

MicroRNAs (miRNAs) are small (18–24 nt), noncoding RNAs that are transcribed from the intergenic or intronic regions of DNA. miRNAs regulate the expression levels or translational levels of messenger RNAs (mRNAs) post-transcriptionally and affect to various biological processes, such as cell proliferation, cell survival, differentiation, and metabolism (4). Primary miRNAs (pri-miRNAs), transcribed from DNA and have short stem-loop structures (SLSs), are processed into premature miRNAs (pre-miRNAs) and then finally processed into mature miRNAs (5). A single miRNA has many targets of mRNAs, binding its target sites mostly in the 3′ untranslated region (3′-UTR) of the mRNA. The main function of miRNAs is to down-regulate gene expression, and miRNAs can function either as tumor suppressors or as oncogenes (6).

A number of miRNAs are reported to display aberrant expression patterns in GBMs (7). In 2005, the first miRNA dysregulation was identified in GBMs (8). miR-21 detected by northern blot was overexpressed in GBM tissues when compared with non-neoplastic control tissues. On the other hand, a systemic screen for miRNA aberrations by miRNA microarray in GBM was performed by other researchers (7). Using miRNA microarray analysis, we previously reported that miR-10b, miR-21, miR-183, miR-92b, and miR-106b are highly expressed in GBMs compared with normal brain tissue (9). Several other reports have also identified these miRNAs as being upregulated in GBMs (8). Recently, we reported that the expression of miR-183 involved in HIF-1α expression and its downstream molecules (10). Since then, it has been documented that miRNA dysregulation could play an important role in development and progression.

2. MicroRNA expression in glioblastoma

Similar to other malignant tumors, GBM has the hallmarks of cancer: biological capabilities of sustaining proliferative signaling, inducing angiogenesis, evading growth suppressors, resistance to apoptosis, activating invasion and metastasis, genomic instability, reprogramming energy metabolism, limitless replicative potential, and evading immune destruction. It is generally accepted that hallmark features of GBM are not only a reflection of genetic abnormalities and aberrant signal transition but also the dysregulation of miRNA-mediated translational control. The miRNA-mRNA interactions transform the short “nonsense”

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sequences into endogenous oncogenes or tumor suppressors. miRNA expression patterns could define a tumor type, implying that certain changes in miRNAs might drive the malignant transformation to a particular tumor. Therefore, whether a miRNA acts as an oncomiR or tumor suppressor-miRNA depends on the regulated genes and cellular context.

The first report on altered miRNA expression in GBMs was published in 2005 (8). In this report, miR-21 was shown to be highly upregulated and to have antiapoptotic capabilities in GBM cells. These findings suggest that overexpressed miR-21 may function as an oncogene in GBMs by blocking expression of key apoptosis-enabling genes (8). In the same year, Ciafre et al. analyzed the global expression levels of 245 microRNAs in both GBM cell lines and patient biopsies (7). A systemic screen for miRNA aberrations by microarray identified a set of dysregulated miRNAs in GBM tissues, including the upregulation of miR-10b, miR-21, and miR-25 and downregulation of miR-128 and miR-181a/181b-/181c (7). Since then, miRNA expression in GBMs has been evaluated in several profiling studies. We previously reported that miR-10b, miR-21, miR-183, miR-92b, and miR-106b are highly expressed in GBMs compared with normal brain tissue, and miR-134, miR-302c, miR-324, miR-379, and miR-368-3p are downregulated in GBMs (9). In 2008, Silber et al. reported 13 miRNAs to be downregulated and three miRNAs to be upregulated in anaplastic astrocytomas and GBMs (11). Furthermore, they revealed that expression levels of miRNA-124 and miRNA-137 were significantly decreased compared with non-neoplastic brain tissue, and both of them induce differentiation of adult neural stem cells. Another study carried out by Godlewski et al. found 8 microRNAs to be upregulated and 11 downregulated when analyzing 245 microRNAs in the setting of GBM (12). A microarray-based study by Rao et al. analyzing 756 microRNAs identified another 55 upregulated and 29 downregulated microRNAs in primary and secondary GBMs and anaplastic astrocytoma compared with controls (13). This study not only validated the role of several deregulated microRNAs but also provided data for the development of a 23 microRNA signature pattern for distinguishing between anaplastic astrocytoma and GBM (13).

3. Hallmarks of glioblastoma and microRNAs

3.1. Growth signal activation and microRNAs

Cell proliferation in GBM could be triggered by somatic alterations within the receptor tyrosine kinase (RTK)-signaling pathways. The most important pathways in GBM are the phosphatidylinositide 3-kinase (PI3K)/AKT and RAS/mitogen-activated protein kinase (MAPK) pathways (14). Abnormally augmented signals downstream from RTKs enable tumor cells to sustain proliferation that is under tight control in normal cells. At least 90% of GBM cases harbor genetic alterations in RTK pathways. Epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor alpha polypeptide (PDGFRA), MET proto-oncogene (MET), and fibroblast growth factor receptor (FGFR) are among the most commonly dysregulated RTKs in GBM (15). EGFR and PDGFRA are well-established oncogenes in GBM (16), and thus, identification of their miRNA regulators following the discovery of functional implications of miRNAs in GBMs is warranted.

miR-7 is a common regulator of the PI3K/ATK and MAPK pathways, both of which are launched by EGFR through its two direct targets, the transcription factors PI3K and Raf-1, respectively (17). Human EGFR mRNA 3′-untranslated region contains three miR-7 target sites (18). Transient expression of miR-7 in GBM cells strongly inhibited *in vivo* GBM xenograft growth (17). Decreased expression of miR-128 correlates with aggressive human glioma subtypes, and miR-128 represses growth and mediates differentiation by targeting oncogenic EGFR and PDGFRA (19). The authors demonstrated that miR-128 suppresses glioma formation in a glioma mouse model, suggesting miR-128 as a glioma tumor suppressor that targets RTK signaling to repress gliomagenesis. miR-218 targets multiple components of RTK-signaling pathways, including the EGFR pathway, and miR-218 repression increases the abundance and activity of multiple RTK effectors (20). The expression of miR-218 is significantly decreased in the mesenchymal subtype of GBM, and the miR-218-RTK-HIF2α-signaling axis promotes GBM cell survival and tumor angiogenesis (20). A large-scale, genomewide miRNA expression analysis revealed miR-219 was downregulated in GBM, and exogenous overexpression of miR-219 in glioma cells inhibited proliferation and soft agar colony formation (21). In addition, overexpression of miR-219-5p reduced the activity of PI3 kinase and MAP kinase in glioma cell lines by targeting to EGFR (21). Moreover, overexpression of miR-133 decreased GBM cell growth and increased cell apoptosis, whereas depletion of miR-133 increased cell growth (22). The protein translation inhibition of EGFR by miR-133 was confirmed by a dual luciferase reporter assay (22). miR-340 overexpression suppressed several oncogenes, including EGFR (23). miR-340 induces glioma cells toward terminal differentiation and regulates glioma cell development by downregulating ROCK1 expression (23). miR-491 directly targets EGFR, CDK6, and Bcl-xL (24). Importantly, miR-491 is commonly codeleted with its adjacent CDKN2A on chromosome 9p21.3 in GBM. As a negative regulator of EGFR expression, miR-491 is a tumor suppressor gene. On the other hand, miR-148a expression was elevated in GBM specimens compared with normal human brain and astrocytes (25). miR-148a is a prognostic onco-miRNA that targets MIG6 and BIM to regulate EGFR and apoptosis in GBM (25). By inhibiting MIG6 expression, miR-148a reduced EGFR trafficking to Rab7-expressing compartments, including late endosomes and lysosomes. This process coincided with reduced degradation and elevated expression and activation of EGFR (25). Furthermore, the protein expression of EGFR decreased in cells with forced overexpression of miR-34a (26). Yin et al. reported that both deletion of miR-34a and amplification of EGFR were associated with significantly decreased overall survival of patients with GBM (26).

PDGFRA is a direct target of miR-34a, a downregulated miRNA in the proneural subtype compared with the mesenchymal subtype of GBM (27, 28). miR-34a specifically affects the growth of proneural glioma cells *in vitro* and *in vivo* (28). miR-34a repression in proneural gliomas is only modestly dependent on p53. Derepression of PDGFRA by the downregulation of miR-34a in the proneural subtype that results in a more proliferative phenotype may be responsible for the difference in response to clinical treatment for GBM. Gene mutation or amplification in MET is a relatively rare event in GBM (29). However, miR-34a expression inhibited c-Met reporter activities in glioma cells. In addition, miR-34a levels in human gliomas were inversely correlated with c-Met levels. Transient transfection of miR-34a into gliomas strongly inhibited cell proliferation, cell cycle progression, cell survival, and cell invasion (30).

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MET is highly expressed in the mesenchymal subtype of GBM (31). miR-182 sensitized glioma cells to TMZ-induced apoptosis, promoted glioma initiating cell (GIC) differentiation, and reduced tumor cell proliferation via knockdown of c-Met, Bcl2L12, and HIF2A (32). miR-144-3p specifically bound to the MET 3′-untranslated region (3′ UTR) and inhibited its expression. miR-144 strongly repressed GBM cell proliferation and invasion by suppressing MET *in vitro* and *in vivo* (33).

RAS signaling, upstream of MAP kinase, is reported to be regulated by miRNAs. Although only 1% of GBMs have a RAS mutation or amplification, 10% of GBMs contain neurofibromin 1 (NF1) inactivating genetic alterations that lead to hyperactive RAS activity by enhancing the intrinsic GTPase activity (29). Two recent reports focused on miRNAs targeting RAS in GBMs and showed that miR-143 directly targets N-RAS (34) and that let-7a directly targets K-RAS (35). Low expression of let-7a was correlated with a poor prognosis of primary GBM patients (35). In addition, miR-124 governs glioma growth and angiogenesis and enhances chemosensitivity by targeting R-RAS and N-RAS (36). In addition, miR-124 inhibits the MAP kinase pathway by repressing SOS1 mRNA (37). Moreover, these miRNAs are all downregulated in GBM specimens, underlying the malignant transformation.

Phosphatase and tensin homolog (PTEN) and NF1 are the most important negative regulators of cell proliferative pathways. The inactivated genetic mutations of NF1 and PTEN are found in 10 and 41% of GBM cases, respectively (29). NF1 is targeted by miR-9, which is an miRNA upregulated in GBM and promotes the proliferation and migration of glioma cells (38, 39). In addition, NF1 is a direct target of miR-514a, and over-expression of miR-514a inhibited NF1 expression, which correlated with increased survival cells (40). The protein phosphatase activity of PTEN is involved in cell proliferation, preventing cells from growing. miR-26a, frequently amplified at the DNA level in human gliomas, is identified as a direct regulator of PTEN expression (41). miR-26a-mediated PTEN repression in a murine glioma model enhances *de novo* glioma formation (41). Overexpression of miR-26a in PTEN-competent and PTEN-deficient GBM cells promoted tumor growth *in vivo* and further increased growth in cells overexpressing CDK4 or CENTG1 (42). Guo et al. reported that c-Myc modulates genes associated with oncogenesis in GBM through deregulation of miRNAs via the c-Myc-miR-26a-PTEN-signaling pathway (43). CREB, a proto-oncogenic transcription factor that is overexpressed in gliomas, can promote gliomagenesis by modulating the expression of oncogenic miR-23a, which represses PTEN directly (44). miR-23a-mediated suppression of PTEN led to the activation of AKT/ERK pathways and epithelial-mesenchymal transition (EMT) (45). miR-17-5p (46), and miRNA-1908 (47) directly target PTEN in GBMs. Overexpression of miR-17 prolongs GBM cell survival and increases cell motility, and induces HIF-1α activation in response to stress by targeting PTEN. (46). miR-1908 promotes proliferation, invasion, and sphere formation in GBM cells by targeting PTEN, and PTEN levels are inversely correlated with miR-1908 levels in GBM tissues (47). The expression of PTEN-targeting miR-17-5p, miR-19a, miR-19b, miR-21, miR-130b, miR-221, and miR-222 was significantly higher in irradiated glioma cells than in nonirradiated cells, and the PTEN expression levels were lower in the irradiated glioma cells than in the nonirradiated cells (48).

In recent years, molecular target therapy-targeting RTK pathways has been one of the most exciting developments in cancer therapy, and certain molecular targeted drugs have been clinically validated. However, for GBMs, no effective molecular targeted drug has yet been developed. By understanding the roles of miRNAs in growth signal activation, miRNA-based treatments should also be taken into consideration either alone or in combination.

3.2. Sustained angiogenesis and microRNAs

New growth in the vascular network is important because the proliferation, as well as metastatic spread, of tumor cells depends on an adequate supply of oxygen and nutrients and the removal of waste products. GBMs have a strong angiogenetic property because GBMs possess glomeruloid microvascular proliferation, a hallmark of GBMs (49). GBMs stimulate new blood vessel formation through processes driven primarily by vascular endothelial growth factor A (VEGF-A), the most established proangiogenic protein in the VEGF family. The overexpression of VEGF-A, and subsequent activation of its receptors, is an important event during glioma progression (50).

miRNA-205, an miRNA significantly downregulated in GBMs, can specifically suppress expression of VEGF-A directly (51). Moreover, miRNA-205 induces apoptosis and depresses the invasion of glioma cells *in vitro* (51). VEGF-A upregulation can be induced by hypoxia inducible factor 1 alpha subunit (HIF-1α), which is negatively regulated by the von Hippel-Lindau (VHL) tumor suppressor (52). HIF-1α levels were upregulated in glioma cells following transfection with miR-183 mimic RNA, and VEGF-A and glucose transporter 1 (GLUT1), which are downstream molecules of HIF-1α, were upregulated in cells transfected with miR-183 (10). miR-21, miR-23b, and miR-566 are reported to target VHL and decrease the production of the VHL protein, upregulating VEGF-A expression. miR-21 directly targets VHL and peroxisome-proliferator-activated receptor a (PPARa), and miR-21 regulates EGFR/AKT signaling through VHL/β-catenin and the PPARa/AP-1 axis (53). miR-21 significantly colocalized with the hypoxia- and angiogenesis-associated markers HIF-1α and VEGF (54). Downregulation of miR-23b triggered growth inhibition, induced apoptosis, and suppressed invasion of glioma *in vitro* (55). miR-23b deletion decreased HIF-1α/VEGF expression and suppressed β-catenin/Tcf-4 transcription activity by targeting VHL (55). Inhibition of miR-566 expression increases the expression levels of VHL, decreases the expression levels of VEGF, and inhibits the invasive and migratory abilities of GBM cells (56). Moreover, miR-7 downregulates the expression of O-linked *N*-acetylglucosamine transferase (OGT) involved in the VEGF-signaling pathway, leading to a profound reduction in vascularization, similar to the antiangiogenic drug sunitinib (57).

miR-101 is downregulated in GBMs and targets EZH2, a histone methyltransferase affecting gene expression profiles in an epigenetic manner. Inhibition of EZH2 by miR-101 attenuated GBM cell growth, migration, and GBM-induced endothelial tubule formation (58). Ectopic expression of miR-137 inhibited angiogenesis in a SCID mouse xenograft model. EZH2 was identified as a direct target of miR-137, and EZH2 overexpression can rescue the inhibitory effect of miR-137 on cell proliferation and angiogenesis (59). A key step in angiogenesis is the upregulation of growth factor receptors on endothelial cells. miR-296 level is increased in

primary tumor endothelial cells isolated from GBM tissues (60). Overexpressed miR-296 enhances angiogenesis by directly targeting the hepatocyte growth factor-regulated tyrosine kinase substrate (HGS), results in upregulation of the VEGF receptoe-2 and PDGF receptor-β (60). miR-93 promotes angiogenesis by suppressing integrin-β8 (61). *In vivo* studies revealed that miR-93-expressing cells induced blood vessel formation, allowing blood vessels to extend to tumor tissues in high densities (61). Angiogenesis promoted by miR-93 in return facilitated cell survival, resulting in enhanced tumor growth. miR-17∼92 promotes angiogenesis and tumor growth by downregulation of clusterin (62). Specifically, miR-17-5p and miR-20 reduce the expression of the type-II TGFβ receptor and miR-18 limits the expression of Smad4, namely miR-17∼92 attenuates the TGFβ-signaling pathway to shut down clusterin expression, thereby stimulating angiogenesis and tumor cell growth.

Because angiogenesis is a hallmark of GBM, targeting therapies against angiogenesis using the VEGF antibody was previously considered to be promising in GBM. However, recently, two phase-III studies revealed that the addition of the VEGF antibody bevacizumab to radiotherapy-temozolomide did not improve survival in patients with GBM, although improved progression-free survival and maintenance of baseline quality of life and performance status were observed with bevacizumab (2, 3). Therefore, new therapeutic targets or strategies need to be developed. To make progress, a better understanding of the miRNAs contributing to angiogenesis will lead to more effective antiangiogenic therapy for patients with GBM.

3.3. Insensitivity to antigrowth signals and microRNAs

To proliferate without limit, GBM cells must circumvent biologically programmed pathways that negatively regulate cell proliferation. There are two major tumor suppressor genes, retinoblastoma 1 (RB1) and tumor protein p53. Similarly, a better understanding of the dysfunction of the RB1 and p53 pathways should also implicate the roles of dysregulated miRNAs. The mutation of p53 results in the inability to stop further cell-cycle progression triggered by oncogenic signals (63). miR-10b, one of the most studied miRNAs in GBMs, is highly upregulated in human GBM and pleiotropically regulates invasion, angiogenicity, and apoptosis of GBM cells. The pleiotropic effect of miR-10b is caused by its suppression of multiple tumor-suppressive genes, including p53 (7, 64, 65). miR-10b directly targets p53 in GBM, giving the tumors a way to evade growth control and enable persistent cell proliferation by perturbing the miRNAs expression.

Moreover, p53 signaling is under the precise control of the negative regulator mouse double minute 2 (MDM2). MDM2 regulates the ubiquitin-dependent degradation and transcriptional activity of p53 (66). MDM2 mRNA is upregulated in both GBM cell lines and samples (67), and the upregulation could be a consequence of the downregulation of miR-17, miR-181b, miR-25, or miR-32, which directly target MDM2 gene expression (46, 67, 68). It is worth noting that miR-25 and miR-32 are two miRNAs repressed by p53, suggesting a feedback circuit between p53 and MDM2 mediated by miRNAs (67). The feedback circuit can explain the overexpression of miR-25 in GBM reported by several separate studies, in which the miRNA is meant to be downregulated to increase MDM2 expression and thus inactivate p53 (7, 39, 69). In addition,

miR-181b is downregulated in GBM samples, further indicating that miRNA contributes to the complexity of the pathological progression of gliomas (68).

Another key tumor suppressive is p16INK4a, an important inhibitor of RB pathway (70), namely p16INK4a can bind specifically to CDK4/6 and inhibit the catalytic activity of CDK4/6-cyclin D complexes (71). Approximately, 80% of GBMs have one or more alterations affecting the RB1 function. In addition to targeting p53 in GBMs, miR-10b also targets p16INK4a, and the inhibition of miR-10b leads to cell cycle arrest (65). miR-26a also targets RB1 in GBMs. Additionally, CDK-cyclin complex-mediated phosphorylation is one of the main mechanisms by which RB1 protein is inactivated (72). The frequent gain-of-function mutations on CDK4/6-cyclin D complexes underscore their importance and potential in the development and progression of GBM. miR-124 is reported to radiosensitize human glioma cells by downregulating CDK4 (73), whereas CDK6 is a direct target of miR-138 (74) and miR-491-3p/5p (24). miR-195 inhibited glioma cell proliferation by downregulating expression of cyclin D1 and cyclin E1, via directly targeting cyclin D1 and cyclin E1 mRNA (75). Notably, those miRNAs that target cyclin-CDK complexes are all downregulated in GBM samples (69).

3.4. Invasion in the brain and microRNAs

The invasive propensity of glioma cells remains the major obstacle to improving the poor outcomes of patients with GBM. GBM cells prefer to migrate through the tortuous extracellular spaces of the brain. Therefore, it is supposed that the interaction of invading glioma cells with the extracellular matrix (ECM) is crucial in the initiation of invasion and migration. Generally, cell attachment is mediated by interactions between cell-cell and cell-ECM receptors, including integrins and cadherins, and degradation of ECM components by metalloproteinases is essential for cell detachment (76). The matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinases (ADAMs) are two distinct types of metalloproteinases secreted by glioma cells to overcome the dense matrix, and the proteolytic activity can be blocked by endogenous metalloproteinase inhibitors, such as tissue inhibitors of metalloproteinase (TIMPs) and reversion-inducing-cysteine-rich protein with kazal motifs (RECK) (76).

miR-218 expression has been found to be significantly downregulated in GBM tissue samples (77). Several studies have shown that miR-218 is a negative regulator of invasion in GBM through various pathways (77, 78). miR-218 has been reported to target the mRNA of Lef1, the transcription factor that is upregulated by β-catenin (77). Suppression of Lef1 leads to the reduction of MMP-2, MMP-7, and MMP-9 activity and inhibition of invasion *in vitro* (79). Another mechanism through which miR-218 negatively regulates GBM invasion is targeting IKKβ mRNA along with reducing the transcription of NF-kB. NF-kB is a transcription factor that is important in many cellular processes and has been strongly linked to the migration and invasion of various cancer cells (78). NF-kB's target genes include MMP-9; therefore, its inhibition by miR-218 causes a decrease in invasion.

MMP-9 is a direct target of miR-491-5p, which is upregulated in GBM (24). miR-491-5p expression has been reported to reduce cell proliferation and invasion by targeting MMP9 (80). miR-491-3p also reduces the invasiveness of GBM cells by targeting the mRNA of insulin-like growth factor-binding protein 2 (IGFBP2). MMP-9 is also a direct target of miR-211 (81). MMP-3

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is targeted by miR-152 (82). Additionally, ADAM17, a non-MMP, is under the direct regulation of miR-145 (83). It is clear that if glioma cells lose the control of MMPs and ADAMs by miRNAs, ECM homeostasis is compromised and the combined activity of these proteases remodels the ECM to favor tumor invasion. miR-101 is a tumor suppressor that is downregulated in GBM, as well as other cancers. (58). It has been shown to downregulate the invasion of glioma cells, as well as proliferation and migration, by targeting the transcription factor Kruppel-like factor 6 (KLF6). This suppression of KLF6 reduced the expression of chitinase-3-like protein 1 (CHI3L1) and inactivated MEK1/2 and PI3K signaling (72). miR-101 downregulation has been shown to result in EZH2-induced proliferation, migration, and angiogenesis in GBM (74).

Similarly, miR-152 is a tumor suppressor that is often downregulated in cancers, including in GBM. It was shown to suppress the invasion of glioma stem cells, as well as cell proliferation, migration, invasion, and apoptosis. It has been reported that miR-152 exerts its tumor-suppressing effects by targeting the transcription factor Kruppel-like factor 4 (KLF4). This suppression of KLF4 causes the transcriptional downregulation of galectin-3 (LGALS3) and the inactivation of MEK1/2 and PI3K signaling (75).

Because the MMPs and ADAMs each comprises more than 20 members, targeting a single target appears to be nonessential in cancerous diseases, which provides miR-21 with an opportunity to broadly inhibit metalloproteinase function. miR-21 regulates multiple genes associated with glioma cell migration and invasion, including the RECK and TIMP3 genes, which are inhibitors of matrix metalloproteinases (84).

In addition, interactions with the ECM are mostly mediated by integrins, which enable cells to sense the extracellular environment and adjust their behavior to environmental cues. miR-124, frequently downregulated in GBM, targets β1 integrin and is shown to affect glioma cell migration and invasion *in vitro* (85). PPP1R13L (protein phosphatase 1, regulatory subunit 13 like), an inhibitory member of the apoptosis-stimulating protein of p53 family (IASPP), was found to be a direct target of miR-124 in GBM cells. miR-124-mediated PPP1R13L regulates invasion of GBM cells (86). Cai et al. found that miR-542-3p expression was decreased in GBM cells, and miR-542-3p suppressed GBM cell invasion by not only targeting AKT1 but also directly downregulating its two important upstream regulators, ILK (integrin-linked kinase) and PIK3R1 (87). miR-181 family is downregulated in GBM and is inversely correlated with activities of NF-kB-targeting genes. Furthermore, miR-181b was shown to suppress epithelial-mesenchymal transition (EMT) by targeting KPNA4 (88). miR-181c overexpression inhibits TGF-β signaling by downregulating TGFBR1 (transforming growth factor, beta receptor 1), TGFBR2 and TGFBRAP1 (transforming growth factor, beta receptor-associated protein 1) expression. miR-181c expression levels are correlated with poor prognosis of patients with GBM.

miR-125a are downregulated in GBMs and directly target the 3′-UTR of podoplanin (PDPN) and inhibit invasion, apoptosis, and proliferation of GBMs. In addition, miR-125a inhibits Nrg1, one of the most active members of the EGF-like family, and suppresses the proliferation and migration of GBM cells *in vitro* and *in vivo* (89). miR-203 expression is decreased in anaplastic astrocytoma and GBM tissues. Forced expression of miR-203 was shown to suppress glioma cell proliferation, migration, and invasion, by disrupting the Robo1/ERK/MMP-9-

signaling axis (90). In addition, miR-203 inhibits the proliferation and invasion by directly targeting phospholipase D2 (PLD2) (91). miR-29b expression, downregulated in GBMs, was inversely proportional to that of BCL2-like 2 (BCL2L2) mRNA or protein (92). Interestingly, BCL2L2 mRNA is highly expressed in the mesenchymal type of GBM. BCL2L2 repression is of central importance to miR-29b antitumor activity in migration, invasion, and angiogenesis. Moreover, miR-29b regulates PDPN, which promotes glioma invasion.

miR-10b is overexpressed in GBM and induces glioma cell invasion by modulating expression of RhoC, MMP-14, and uPAR (urokinase-type plasminogen activator receptor) via HOXD10 (93). Multifocal lesions of malignant gliomas were associated with higher expression levels of miR-10b (9). Dong et al. reported that not only miR-10b inhibition but also miR-21 inhibition could exert synergistic inhibition of the invasion of glioma cells. miR-10b pleiotropically regulates invasion, angiogenicity, and apoptosis of glioma cells by the suppression of multiple tumor suppressors, including p53, FOXO3, HOXD10, and NOTCH1 (64). miR-155 has been identified to be an oncomiR and is highly expressed in several solid cancers, including GBM. Knockdown of miR-155 sensitizes glioma cells to the chemotherapy of temozolomide by targeting p38 isoforms of mitogen-activated protein kinase 13 (MAPK13) and MAPK14 (94).

In sum, the roles of miRNAs in glioma cell invasion or migration further our understanding of the genesis of the aggressive glioma phenotype. Some of these miRNAs have been discovered, whereas more still remain to be found. The blockade of excessive pro-invasive miRNAs or the restoration of weakened anti-invasive miRNAs will provide extra treatment options for advanced-stage gliomas that are marked by poor prognoses for decades (**Table 1**).

Hallmarks of glioblastoma	miRNAs	Targets	Expression	Function	Ref
Growth signal activation	miR-7	EGFR, PI3K, Raf-1	down	downregulate PI3K/ATK and MAPK pathways	Liu (17), Webster (18)
	miR-128	EGFR, PDGFRA	down	downregulate PI3K/ATK and MAPK pathways	Papagiannakopoulos (19)
	miR-218	EGFR	down	downregulate PI3K/ATK and MAPK pathways	Mathew (20)
	miR-219	EGFR	down	downregulate PI3K/ATK and MAPK pathways	Rao (21)
	miR-133	EGFR	down	downregulate PI3K/ATK and MAPK pathways	Xu (22)
	miR-340	EGFR	down	induce terminal differentiation	Huang (23)

Hallmarks of glioblastoma	miRNAs	Targets	Expression	Function	Ref
	miR-491	EGFR, CDK6, Bcl-xL	down	inhibit cell proliferation, negative regulator of EGFR	Li (24)
	miR-148a	MIG6, BIM	up	reduce EGFR trafficking	Kim (25)
	miR-34a	PDGFRA	down	inhibit cell proliferation and cell cycle progression inhibit cell survival invasion	Genovese (27), Silber (28)
	miR-182	MET, Bcl2L12, HIF2A		promote glioma initiating cell differentiation	Kouri (32)
	miR-144-3p	MET	down	repress GBM cell proliferation and invasion	Lan (33)
	let-7a	K-RAS	down	downregulate PI3K/ATK and MAPK pathways	Wang (35)
	miR-143	N-RAS	down	inhibit cell proliferation	Wang (34)
	miR-124	SOS1, R-RAS	down	inhibit cell proliferation	Shi (36), Lv (37)
	miR-9	NF1	up	inhibit cell proliferation	Tan (38)
	miR-26a	PTEN	up	promote tumor growth	Huse (41)
	miR-23a	PTEN	up	activate of AKT/ERK pathways and EMT	Tan (44), Tian (45)
	miR-17-5p	PTEN	up	formation of colonies and neurospheres	Li (46)
	miRNA-1908	PTEN	up	promote the tumor forming potential and anchorage-independent growth	Xia (47)
	miR-19a	PTEN	up	promote tumor growth	Tokudome (48)
	miR-19b	PTEN	up	promote tumor growth	Tokudome (48)
	miR-21	PTEN	up	promote tumor growth	Tokudome (48)
	miR-130b	PTEN	up	enhance stem cell-like phenotype	Tokudome (48)
	miR-221	PTEN	up	promote tumor growth	Tokudome (48)
	miR-222	PTEN	up	promote tumor growth	Tokudome (48)
Sustained angiogenesis	miR-205	VEGF-A	down	inhibit expression of VEGF-A induce apoptosis and depress the invasion	Yue (51)
	miR-183	IDH2	up	increase HIF1 expression	Tanaka (10)
	miR-21	VHL, PPARa	up	regulate EGFR/AKT signaling	Harmansen (54)

Hallmarks of glioblastoma	miRNAs	Targets	Expression	Function	Ref
	miR-23b	VHL	up	inhibit tumor growth and invasion, induce apoptosis	Chen (55)
	miR-566	VHL	up	inhibit invasion and migration	Xiao (56)
	miR-7	OGT	down	reduce vascularization	Babae (57)
	miR-101	EZH2	down	attenuate GBM growth and migration/invasion	Smits (58)
	miR-137	EZH2	down	inhibit cell proliferation and angiogenesis	Sun (59)
	miR-93	Integrin-β8	up	promote tumor growth and angiogenesis	Fang (61)
Insensitivity to antigrowth signals	miR-10b	p53, p16INK4a	up	promote cell cycle	Gabriely (65)
	miR-17-3p	MDM2		inhibit tumor progression	Li (46)
	miR-181b	MDM2	down	inhibit tumor progression	Suh (67)
	miR-25-3p	MDM2, p53	up	inhibit cellular proliferation	Suh (67)
	miR-32-5p	MDM2, p53	up	inhibit cellular proliferation	Suh (67)
	miR-26a	RB1	up	promote cell proliferation	Lundberg (72)
	miR-124	CDK4	down	cell cycle arrest	Deng (73)
	miR-138	CDK6	down	cell cycle arrest	Qiu (74)
	miR-491-3p/5p	CDK6	down	cell cycle arrest	Li (24)
	miR-195	cyclin D1, cyclin E1	down	cell cycle arrest	Hui (75)
Invasion and metastasis	miR-218	IKKβ, Lef1	down	inhibit cell invasion	Liu (77), Song (78)
	miR-491	IGFBP2, MMP9	down	reduce cell proliferation and invasion	Yan (80)
	miR-211	MMP9	down	reduce cell invasion	Asuthkar (81)
	miR-101	KLF6, EZH2	down	reduce invasion of glioma stem cells	Smits (58), Qiu (74)
	miR-152	MMP3, KLF4	down	reduce cell invasion and angiogenesis suppress invasion of glioma stem cells	Zheng (82), Hui (75)

Hallmarks of glioblastoma	miRNAs	Targets	Expression	Function	Ref
	miR-145	ADAM17	down	reduce glioma cell invasion and angiogenesis	Lu (83)
	miR-21	TIMP3, RECK	up	promote cell invasion	Gabriely (84), Zhao (86)
	miR-124	β1 integrin, IASPP	down	suppress cell migration and invasion	Fowler (85)
	miR-542-3p	AKT1	down	suppress cell invasion	Cai (87)
	miR-181b	KPNA4	down	inhibit cell invasion and proliferation	Wang (88)
	miR-181c	TGFBR1, TGFBR2, TGFBRAP1	down	inhibit cell invasion and proliferation	Wang (88)
	miR-125a	PDPN, Nrg1	down	inhibit invasion and proliferation	Yin (89)
	miR-203	PLD2	down	suppress cell proliferation and invasion,disrupt the Robo1/ERK/MMP-9 signaling axis	Dontula (90), Chen (91)
	miR-29b	BCL2L2, PDPN	down	inhibit glioma invasion	Chung (92)
	miR-10b	HOXD10, FOXO3, NOTCH1	up	induce glioma cell invasion	Sun (93)
	miR-155	MAPK13, MAPK14	up	regulates glioma cells invasion and chemosensitivity	Liu (94)
Antiapoptosis	miR-21	PDCD4, HNRPK, TP53BP2, p63	up	antiapoptosis	Corsten (95), Gaur (96)
	miR-92a	BCL2L11	up	antiapoptosis	Papagiannakopoulos (97)
	miR-92b	NLK	up	antiapoptosis	Niu (98)
	miR-93	ITGB8	up	antiapoptosis	Fang (61)
	miR-221	p27, p57	up	antiapoptosis	Medina (100)
	miR-222	p27, p57	up	antiapoptosis	Medina (100)
Genome instability and mutation	miR-106a		up	DNA replication and mitosis	Liu (101)
	miR-106b	RBL1, RBL2	up	DNA replication and mitosis	Liu (101)

Hallmarks of glioblastoma	miRNAs	Targets	Expression	Function	Ref
	miR-17-92		up	DNA replication and mitosis	Liu (101)
	miR-20			DNA replication and mitosis	Liu (101)
	miR-221	p27	up	Cell cycle chechpoint regulation	Gillies (103)
	miR-222	p27	up	Cell cycle chechpoint regulation	Gillies (103)
	miR-155	MLH1, MSH2, MSH6	up	DNA mismatch repair	Liu (94)
	miR-125b	p53, MXD1	down	cell cycle arrest to repair damaged DNA spindle assembly checkpoint	Wan (104), Le (105)
	miR-29	PIK3R1, CDC42	down	upregulate p53	Park (106)
	miR-34	CCND1, CCNE2, CDK4, MET, MYC, SNAI1, and SIRT1	down	DNA damage response upregulate p53 downstream molecules	He (107)
	miR-101	ATM, PRKDC	down	DNA repair regulate non-homologous end joining of DNA double strand breaks	Yan (109) Li (111)
Tumor-promoting inflammation	miR-21	LRRFIP1	up	regulate NF-kB signaling pathway	
	miR-146a	TRAF6, IRAK1		regulate NF kB activation	Taganov (112), Park (113)
	miR-124		down	inhibit STAT3 pathway	Wei (117)
Reprogramming energy metabolism	miR-451	CAB39	up	regulate LKB1/AMPK signaling activity	Godlewski (124)
	mir-145	c-Myc, Sox9, ADD3	down	reduce Lin28/Lin28b transcription	Rani (130), Gan (132)
	miR-34c	c-Myc		regulate c-Myc mRNA stability and translation	Masui (134)

Hallmarks of glioblastoma	miRNAs	Targets	Expression	Function	Ref
	let-7	RAS, Myc, CCND1, LIN28, HMGA2	down	regulate glucose metabolism	Boyerinas (135)
	let-7a	K-ras	down	regulate glucose metabolism	Boyerinas (135)
	miR-326	PKM2, NOB1	down	regulate glucose metabolism RNA metabolism	Kefas (136), Zhou (137)
Evading immune destruction	miRs-29b		down	differentiation of tumor-associated macrophage	Graff (140)
	miR-125a		down	differentiation of tumor-associated macrophage	Graff (140)
	miR-146a		down	differentiation of tumor-associated macrophage	Graff (140)
	miR-155	C/EBPβ	up	lead to and inversion of M2 into M1 macrophages	Graff (140)
	miR-221	STAT1,2	up	phosphorylation of STAT1 and STAT2	Zhang (143)
	miR-222	STAT1,2	up	phosphorylation of STAT1 and STAT2	Zhang (143)
	miR-20a	NKG2DL	up	immune evasion of glioma cells at the level of the NKG2D recognition pathway	Codo (146)
	miR-93	NKG2DL	up	immune evasion of glioma cells at the level of the NKG2D recognition pathway	Codo (146)
	miR-106b	NKG2DL	up	immune evasion of glioma cells at the level of the NKG2D recognition pathway	Codo (146)
	miR-138	CTLA-4, PD1	down	tumor regression	Wei (147)

Table 1. Hallmarks of glioblastoma and microRNAs.

3.5. Antiapoptosis and microRNAs

Among the tumor-associated miRNAs, miR-21 is frequently overexpressed in various types of tumors, including GBMs (7, 9), and plays a critical role in cell death and apoptosis. Knockdown of miR-21 in cultured GBM cells triggered activation of caspases and led to increased apoptotic cell death (8). miR-21 knockdown disrupts glioma growth *in vivo* and displays synergistic cytotoxicity when combined with the agent tumor necrosis factor-related apoptosis (s-TRAIL), leading to an increase in caspase activity (95). In particular, it was reported that the downregulation of miR-21 in GBM-derived cell lines resulted in an increased expression of a specific target, PDCD4 (programmed cell death 4), a known tumor suppressor gene, with a consequent decrease in proliferation and increase in apoptosis (96). Furthermore, miR-21 regulated a network of p53, TGF-b, and mitochondrial apoptosis tumor suppressor genes in GBM cells, targeting HNRPK (heterogeneous nuclear ribonucleoprotein K), TP53BP2 (tumor protein p53-binding protein 2), and p63, a member of the p53 family of genes (97).

miR-92a and miR-92b, upregulated in glioma, target BCL2L11 (98) and NLK (99), respectively. Their inhibition promotes tumor-suppressive phenotypes through the induction of apoptosis. In addition, miR-93 was found to be upregulated in glioma specimens and resulted in an enhancement in cell survival, promoting sphere formation, and augmenting tumor growth by suppressing the expression of ITGB8 target (61).

miR-221 and 222 were extensively investigated in glioma. These miRNAs were found to be upregulated in this cancer, and both were reported to target the cell growth-suppressive cyclin-dependent kinase inhibitors p27 and p57 (100).

3.6. Genome instability and microRNAs

Genomic instability is defined as a high frequency of mutations within the genome, including changes in nucleic acid sequences, chromosomal rearrangements, or aneuploidy. The stability of the human genome is maintained by multiple mechanisms such as the cell cycle checkpoint, DNA damage response, and mitotic separation machinery. Defects in the DNA damage response could cause genomic DNA mutations, deletions, insertions, or gross chromosomal gains and losses upon cell division and subsequently lead to cancer. Genomic instability is present in GBM and affects the prognosis of patients.

Several members of the miR-106b-25 cluster and its paralog miR-17-92 cluster were associated with DNA replication and mitosis. Overexpression of miR-106b-5p in glioma tumor cells significantly promoted cell proliferation, suggesting a role of this miRNA in cell cycle regulation (101). A mechanistic study revealed that two target genes, retinoblastoma-like 1 (RBL1) and RBL2, were involved in miR-106b-5p's regulation of cell proliferation (101). RBL proteins were involved in genomic instability, coinciding with decreased DNA methylation and increased acetylation of histone H3.

p27, one of the cyclin-dependent kinase inhibitor, inhibits cell cycle progression at the G1 phase by blocking the activation of cyclin-CDK complexes (102). The downregulation of p27 expression by miRNAs could abrogate the cell cycle checkpoints and could increase DNA damages in GBM cells. The regulatory effect of miR-221/222 on p27 and the subsequent effect

on cell proliferation were also demonstrated in GBM cells (103). Moreover, p27 inhibition abrogated the growth advantage of cells with miR221/miR-222 downregulation (103).

DNA mismatch repair (MMR) is a system for recognizing and correcting insertions, deletions, and misincorporated bases at DNA replication and recombination. Deficiency of MMR system can be a cause for the development and progression of GBMs. There are two essential members of the DNA MMR genes, MutS homologs and MutL homologs. Computational algorithms predicted, and subsequently *in vitro* studies confirmed, several essential MMR genes, including MLH1, MSH2, and MSH6, as potential binding sites for miR-155. miR-155 is known to elevate its expression levels in primary and secondary GBMs (94).

As an important sensor in the DNA damage response, p53 functions to block the cell cycle to repair damaged DNA. Several miRNAs affect p53 or p53-regulated genes via different mechanisms. For instance, miR-125b, which has been shown to play a potential role in the development of glioma stem cells (104), directly targets p53 by binding to 3′-UTR of p53 and negatively regulates p53 expression (105). In addition, miR-125b negatively regulates MXD1 expression, an adaptor protein for MAD2L2 in the spindle assembly checkpoint. Moreover, miR-29, commonly downregulated in GBMs, indirectly upregulates p53 by targeting PIK3R1 and CDC42 (106). Previous studies have shown that miR-34 is directly regulated by p53 and represses various oncogenes, such as CCND1, CCNE2, CDK4, MET, MYC, SNAI1, and SIRT1. However, these miR-34-targeting oncogenes are upregulated in GBM, because miR-34 is usually downregulated (107). Several studies have shown that miR-34 is indispensable for the DNA damage response, and miR-34a-regulated genes are strongly related with DNA repair and apoptosis (108).

Ataxia telangiectasia mutated (ATM) is an important mediator in connecting DNA damage signals to downstream events, including damage repair. miR-101, a miRNA downregulated in GBM, could directly target ATM via the canonical action mechanism (109). In addition, miR-101 also targets PRKDC (protein kinase, DNA activated, catalytic polypeptide) to regulate nonhomologous end joining of DNA double-strand breaks (109).

3.7. Tumor-promoting inflammation and microRNAs

NF-kB is a transcription factor with pleiotropic activity owing to its central roles in inflammatory processes. A critical regulator of NF-kB activation is the IkB kinase (IKK-β) complex (110). Ectopic expression of miR-218, which is downregulated in GBM, reduced NF-kB activity, whereas inhibition of miR-218 enhanced the transcriptional activity of NF-kB (78). miR-218 could inactivate NF-kB signaling by directly targeting the 3′-UTR of the IKK-β (78). miR-21 was revealed as another regulator involved in the NF-kB-signaling pathway in GBM. Li et al. confirmed LRRFIP1 (leucine-rich repeat interacting protein 1) as a direct target of miR-21 (111). LRRFIP1 is a transcriptional repressor that preferentially binds to the GC-rich consensus sequence and regulates expression of TNF, EGFR, and PDGFA signaling. miR-21 contributes to drug resistance through the depression of LRRFIP1 expression, leading to the reduction of cytotoxicity of chemotherapeutic drugs through the activation of the NF-kB pathway (111).

miR-146a is known as miRNA associated with the innate immune response to microbial infection (112). Promotor analysis revealed that miR-146a is a NF-kB-dependent miRNAs (112). miR-146a has complementary sequences in the mRNA of the TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK1), key adaptor molecules downstream of toll-like and cytokine receptors (113). miR-146a overexpression enhanced apoptosis and suppressed NF-kB activation in TMZ-treated GBM cells (114). Increased expression of miR-146a was observed in glioneuronal lesions (115), and miR-146a expression in human glial cells was strongly induced by IL-1β (115). miR-146a expression by transfection in astrocytes regulated many mRNA expression levels, including those of IRAK-1, IRAK-2, and TRAF-6. In addition, the expression of IL-6 and COX-2 was suppressed by miR-146a (115).

Recent evidence suggests that the signal transducer and activator of transcription (STAT) family proteins play a crucial role in selectively inducing and maintaining a procarcinogenic inflammatory microenvironment, both upon the initiation of malignant transformation and during cancer progression (116). Upon upregulating miR-124, a miRNA downregulated in GBM, specifically in glioma cancer stem cells (gCSC), the STAT3 pathway was inhibited, and miR-124 reversed gCSC-mediated immunosuppression of T cell proliferation and induction of regulatory T cells (Treg) (117). Systemic administration of miR-124-transfected T cell transfers exerted potent antiglioma therapeutic effects in murine models of GBM (117).

Human glioma immune activation is potently elicited by a cytokine combination. Cytokines such as IL-1β and TNFα induce the expression of miR-155 and miR-155*, the miRNAs crucial in immunity and inflammation-induced oncogenesis, and this expression is dose dependently suppressed by IRF3. Importantly, IRF3 also inhibits glioma proliferation, migration, and invasion (118).

3.8. Reprogramming energy metabolism and microRNAs

Cellular metabolism of malignant tumor is considerably different from that of normal cell, because of limitless proliferation and motility. (119). GBM cells uptake a large amount of glucose, and aerobic glycolysis generates various substrates, such as fatty acids and nucleotides that are required for rapidly proliferating cells, and is associated with a survival advantage in the tumor cells (Warburg effect). MR spectroscopy analyses have shown that as much as 90% of the glucose converts into lactate by aerobic glycolysis in GBM cells. Clinically, it is known that the levels of lactate are elevated in GBM tissue compared with those in contralateral normal brain tissue. In addition, lactate was shown to be high-grade gliomas than for low-grade gliomas, and glutamate levels were significantly elevated for GBMs. Pretreatment F^{18}-2-fluoro-2-deoxy-D-glucose (FDG)-PET provides significant additional prognostic information in high-grade gliomas (120).

In normal cells, the 5'-adenosine monophosphate-activated protein kinase (AMPK) pathway is the major cellular sensor of energy availability (121), but its function in cancer is not clear. AMPK is activated by metabolic stress to promote energy conservation and glucose uptake, allowing cells to survive periods of low-energy availability. Allosteric interaction with elevated intracellular AMP, which acts to inhibit dephosphorylation of AMPK (122), and phosphory-

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lation at Thr172 by the protein kinase LKB1 are necessary for AMPK activation under conditions of bioenergetic stress (123).

Godlewski et al. identified miR-451 as a glioma-expressed miRNA that regulates the balance of proliferation and migration in glioma cells in response to changes in glucose levels (124). miR-451 regulates LKB1 activity through direct targeting of CAB39, a component of the active LKB1 complex. Glucose deprivation was shown to reduce miR-451 levels (124). Thus, miR-451 plays an essential role in LKB1/AMPK signaling in glioma cells. When glucose is sufficient, elevated miR-451 levels lead to reduced LKB1/AMPK pathway activation, which facilitates cell proliferation by allowing unrestrained mTOR activity and reducing apoptosis. In contrast, when glucose is limiting, miR-451 levels decline, allowing for increased CAB39 expression and activation of AMPK by LKB1-mediated phosphorylation. This effect promotes cell survival in response to metabolic stress and activates pathways involved in glioma motility.

Previous reports indicate that Myc is frequently upregulated or amplified in gliomas. As a transcriptional factor, Myc interacts with many tumor-related oncogenes in glioma cells, such as SPARC (125), GAS1 (126), and VEGF (127). c-Myc has been shown to stimulate glutamine metabolism by increasing the expression of amino acid transporters and glutaminase (128). Mechanistically, c-Myc expression has been shown to be suppressed by miR-145, which results in reduced Lin28/Lin28b transcription (129). miR-145 is one of the miRNAs significantly downregulated during malignant transformation in GBMs (130). miR-145 overexpression suppresses the activity of oncogenic proteins Sox9, leading to reduction of cell proliferation and invasion of GBM cells (130). Reduced levels of miR-145 may lead to metabolic remodeling in glioma cells via Sox9, because Sox9 is known as a regulator of c-myc.

mTORC2 regulates c-Myc and glycolysis through FoxO acetylation, and mTORC2 controls FoxO acetylation through class IIa HDACs, independently of Akt. FoxOs antagonize c-Myc (131) by increasing the expression of miR-145 (132) and miR-34c (133), limiting c-Myc mRNA stability and translation. In GBM cells, miR-34c levels were suppressed by FoxO1/FoxO3 knockdown, and miR-34c regulated c-Myc levels in GBM cells (134). The let-7 family has nine members, and previous studies have identified the let-7 miRNA as a tumor suppressor that regulates many important target genes during tumor development (135), such as RAS, Myc, CCND1, LIN28, and HMGA2, which are involved in cell cycle progression and cell stemness. The majority of let-7a miRNA functions in glioma malignancy are believed to involve K-Ras, suggesting that let-7a-mediated manipulation of K-Ras may also be involved in regulating glucose metabolism and GBM cell growth. The M2 isoform of pyruvate kinase (PK) is upregulated in most cancers, including GBM. The regulation of PKM2 was shown to occur via miR-326 (136). There are four isoforms of PK in mammals, and PKM2 is highly expressed in undifferentiated tissues and tumors. Because PKM2 is regulated by tyrosine phosphorylation and is overexpressed in malignant tumors, PKM2 is considered as key molecule of aerobic glycolysis. In addition, Zhou et al. reported that the human Nin one binding protein (NOB1), which is required for the biogenesis and function of the 26S proteasome and plays a role in RNA metabolism, is a direct target of miR-326 (137).

3.9. Evading immune destruction and microRNAs

In the past, the infiltration of innate and adaptive immune cells into the tumor microenvironment was considered an immune attack against tumors. However, now, it is widely accepted that immune cells also promote tumor initiation, progression, and metastasis (138). Tumor-associated macrophages (TAMs) control the majority of immunological processes within tumors exerting both regressive (M1) and progressive (M2) effects on tumor development (139). However, the majority of TAMs exhibit an M2-like phenotype. Remarkably, miRs-29b, miR-125a, miR-146a, and miR-155 are involved in the differentiation of TAMs (140). Overexpression of miRNA-155 was shown to attenuate the production of cytokines (IL-6 and TNF-α) by suppressing C/EBPβ expression, which led to an inversion of M2 into M1 macrophages (141). M2 TAMs are capable of releasing anti-inflammatory cytokines such as the immunosuppressive cytokine IL-10, which promotes tumor growth (142).

Natural killer T (NKT) cells are a subfraction of T cells. A large number of published studies have demonstrated that NKT cells have miscellaneous functions in immune regulation, one of which is that NKT cells are tumor cell killers, based on the production of antitumor cytokines. In addition to contributing to immune protection, NKT cells are involved in immune tolerance in the body. Tang et al. showed that glioma cells can induce immune-tolerant IL-6+ and IL-10+ NKT cells via miR-92a.

Another study revealed miR-221 and miR-222 as possible regulators of IFN pathways. Type-I IFN receptor activation triggers the JAK-mediated tyrosine phosphorylation of STAT family proteins. Zhang et al. found that STAT1 and STAT2 expression and phosphorylation were upregulated after repression of miR-221/222 in U251 cells (143). Upregulation of STAT pathway is controlled by the IFN-α activation after knockdown of miR-221/222 cluster in U251 glioma cells (143).

NKG2D is one of the major activating receptors of natural killer (NK) cells and binds to several ligands (NKG2DL). NKG2D recognizes different MHC class I-homologous ligands (NKG2DL), including the MHC class I-chain-related molecules A (MICA) and B (MICB) and the UL16- binding proteins (ULBP)1-6 (144), which are also present on the surface of glioma cells (145). Codo et al. reported that miR-20a, miR-93 or miR-106b regulates NKG2DL expression in glioma cells (146), suggesting that the expression of miRNA-targeting NKG2DL may contribute to the immune evasion of glioma cells at the level of the NKG2D recognition pathway.

In addition, miRNA regulates immunocheckpoint molecules. miR-138 could bind the 3′-UTR of CTLA-4 and PD-1, and transfection of human CD4+ T cells with miR-138 suppressed expression of CTLA-4 and PD-1 (147). *In vivo* treatment in immunocompetent mice using miR-138 revealed marked tumor regression and prolonged survival time. Moreover, inoculated tumors showed decrease in intratumoral regulatory T cell, CTLA-4, and PD-1 expression (147). miR-138 exerts antiglioma efficacy by targeting immune checkpoints that may have rapid translational potential as novel immunotherapeutic agents.

On the other hand, miRNA expression is transcriptionally regulated by various cytokines. Ohno et al. analyzed the effect of IFN-β treatment on miR-21 expression in glioma cells and

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intracranial glioma xenografts (148). Systematic delivery of IFN-β markedly reduced the level of miR-21 in glioma cells 6 hours after the addition of IFN-β.

4. Circulating microRNA in glioma patients

In 2008, Chim et al. firstly demonstrated the existence of placental miRNAs in maternal plasma (149). In the same year, several miRNAs were detected in the serum of the tumor patients. Quantitative real-time polymerase chain reaction (RT-PCR) analyses revealed sera levels of miR-155, miR-210, and miR-21 are higher in diffuse large B-cell lymphoma patient sera than healthy controls (150). To prevent degradation in the circulation, miRNAs are released by cells in both exosomes and miRNA/protein complexes. Exosomes are lipid vesicles ranging between 50 and 100 nm in size and contain a range of molecules, including mRNA, miRNA, DNA, and proteins. The detection of biomarkers within serum is attractive because of the relatively non-invasive process of collection (**Figure 1**).

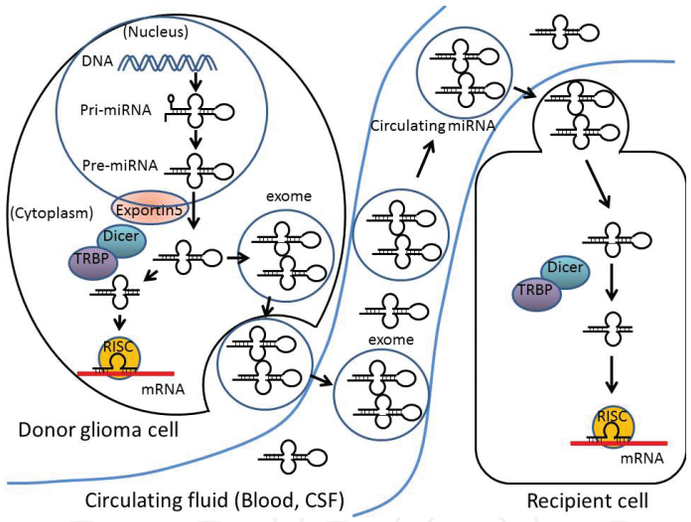

Figure 1. Cell-cell communication through circulating microRNAs. MicroRNAs contained in exosomes are released from glioma cells where they can enter the blood-stream or CSF-stream and circulate through the body to distant sites. These exosomal miRNAs are taken up by recipient cells, where the miRNAs can then suppress target genes in the recipient cells. Circulating miRNAs released by glioma cells may involve in growth signal, angiogenesis, anti-apoptosis, tumor metabolism, and immunoregulation.

Over the past few years, researchers have investigated the capability of using miRNAs as blood biomarkers for the diagnosis of tumors. Circulating miRNAs can be isolated from serum or plasma, but recent studies have indicated plasma to be a more adequate source for miRNA extraction (150). However, depending on plasma preparation, the level of circulating miRNA can be altered. Cheng et al. showed that processing differences resulted in a variation in residual platelet contamination in plasma and significant differences in miRNA abundance (151). Consequently, supplementary centrifugation, the refusal of samples with platelet counts

above a certain limit and the quantification of hemolysis are mandatory in accurately determining miRNA levels in plasma.

Cerebrospinal fluid (CSF) is another useful biofluid and is in direct contact with the extracellular fluid of the brain. CSF has various functions, such as protecting the brain, transporting biological substances, and excreting toxic and waste substances. Although the composition of CSF reflects that of the blood plasma, active transport and secretions from the brain tissues contribute to the composition of CSF. Therefore, the analyses of CSF can suggest biological brain processes and is indispensable for understanding disorders of brain (152).

Clinically, histopathological examinations have been widely used to diagnose glioma, but they are invasive because microsurgical resection or stereotactic biopsy is needed to acquire specimens. In this context, it is of great interest to develop novel biomarkers for glioma. Currently, several miRNAs have been identified as noninvasive biomarkers for the diagnosis of cancers, including breast, lung, and gastric cancer. Previous studies have reported that miRNAs can be detected in circulating exosomes in the serum or CSF of glioma, suggesting that miRNAs might be useful biomarkers for glioma diagnosis. However, to effectively apply these findings to clinical detection, further studies must be conducted (**Table 2**).

No.	Authors	Year	Source	Number of glioma	Number of control	microRNA
Blood						
1	Skog	2008	serum	2 (GBM)	none	let-7a, miR-15b, miR-16, miR-19b, miR-21, miR-26a, miR-27a, miR-92, miR-93, miR-320, miR-20
2	Roth	2011	blood	20 (GBM)	20 (Healthy controls)	miR-128(↑), miR-342-3p(↓)
3	Wang	2012	plasma	50 (GBM)	10 (Healthy controls)	miR-21(↑), miR-128(↓), miR-342-3p(↓)
4	Yang	2013	serum	122 (Astro cytoma)	123 (Healthy controls)	miR-15b*(↓), miR-23a(↓), miR-133a(↓), miR-150*(↓),miR-197(↓), miR-497(↓), miR-548b-5p(↓)
5	Dong	2014	serum	3 (GBM)	3 (Healthy controls)	miR-576-5p(↑), miR-340(↑), miR-626(↑), miR-320(↓), let-7g-5p(↓), miR-7-5p(↓)
6	Manterola	2014	serum	25 (GBM)	25 (Healthy controls)	miR-320(↑), miR-574-3p(↑)
7	Shao	2015	plasma	70 (Glioma)	70 (Healthy	miR-454-3p(↑)

No.	Authors	Year	Source	Number of glioma	Number of control	microRNA
					controls)	
8	Wu	2015	serum	83 (Glioma)	69 (Healthy controls)	miR-29b(↓)
9	Liu	2015	serum	120 (Glioma)	120 (Healthy controls)	miR-29b(↓)
10	Lai	2015	blood	136 (GBM)	50 (Healthy controls)	miR-210(↑)
11	Sun	2015	serum	151 (Glioma)	53 (Healthy controls),52 (Meningioma)	miR-128(↓)
12	Yue	2016	serum	20 (Glioma)	5 (Healthy controls)	miR-205(↓)
13	Wei	2016	serum	33 (Glioma)	33 (Healthy controls)	miR-125b(↓)
Cerebrospinal fluid (CSF)						
1	Baraniskin	2012	CSF	10 (Glioma)	10 (Neurologic disorders), 23 (CNS lymphoma), 7 (Metastatic tumor)"	miR-15b(↑), miR-21(↑)
2	Teplyuk	2012	CSF	19 (GBM)	15 (Non-neoplastic control),44 (Metastatic tumor), "	miR-10b(↑), miR-21(↑) , miR-200 family(↑)
3	Akers	2013	CSF	13 (GBM)	13 (Non-neoplastic control)	miR-21(↑)
4	Shi	2015	CSF	8 (Astro cytoma), 25 (Epen dymoma), 45 (GBM)	none	miR-21(↑)

Table 2. Circulating microrna in glioma patients.

4.1. Serum and plasma

The first analysis of exosomes in serum identified the presence of 11 miRNAs in the samples from two different patients with primary GBM in 2008 (153). However, the levels were generally lower in exosomes but correlated well with the tumor profile (153). In 2011, Roth et al. analyzed miRNA profiles from the blood of 20 patients with GBM and 20 matched healthy controls (154). Among 1158 tested miRNAs, 52 were significantly deregulated, and of these, two candidates, miR-128 (upregulated) and miR-342-3p (downregulated), remained the most significant miRNAs. The altered expression of these two miRNAs was confirmed in a validation cohort by RT-PCR. In this model, the discrimination between blood samples of patients with GBM and healthy controls reached an accuracy of 81%, specificity of 79%, and sensitivity of 83%. In 2012, Wang et al. determined the plasma miRNA levels of 50 patients with glioma and 10 healthy donors using RT-PCR (155). The plasma level of miR-21 was increased and the levels of miR-128 and miR-342-3p were significantly decreased in the patients with glioma compared with those in normal controls. These miRNAs were able to discriminate patients with glioma from healthy controls with high specificity and sensitivity. However, there were not significant differences between patients with glioma and other brain tumors such as meningioma or pituitary adenoma. Yang et al. performed genomewide serum miRNA analysis using serum samples of 122 untreated astrocytoma patients and 123 normal controls (156). The seven-miRNA panel (miR-15b*, miR-23a, miR-133a, miR-150*, miR-197, miR-497, and miR-548b-5p) demonstrated a high sensitivity (88.00%) and specificity (97.87%) for malignant astrocytoma prediction (156). These identified miRNAs also exhibited a global decrease in tumor tissues relative to normal tissues. Interestingly, these miRNAs in serum were markedly elevated after tumor removal.

In miRNA microarray analysis of the serum of patients with GBM and normal controls, 115 miRNAs were upregulated in the GBM group and 24 miRNAs were downregulated (157). In these microRNAs, a six-membered serum miRNA expression profile (upregulated miRs; miR-576-5p, miR-340, and miR-626, downregulated miRs; miR-320, let-7g-5p, and miR-7-5p) could serve as a noninvasive biomarker for GBM diagnosis (157). Manterola et al. found that the serum expression levels of miR-320 and miR-574-3p were significantly altered in the patients with GBM (158). In addition, small noncoding RNA (RNU6-1) was an independent predictor of a diagnosis of GBMs. Shao et al. compared the expression levels of miR-454-3p between preoperative plasmas from 70 patients with glioma and 70 healthy controls and between these preoperative and postoperative plasmas (159). The expression levels of miR-454-3p in plasma in patients with glioma were significantly higher, and the area under receiver operating characteristic (ROC) curve (AUC) of the expression of miR-454-3p for glioma diagnosis was 0.9063. In addition, the expression levels of miR-454-3p in the postoperative plasmas were significantly downregulated relative to the preoperative plasmas. Wu et al. analyzed serum from 83 patients with glioma and 69 healthy controls and evaluated the availability of the serum miR-29 family in the screening of glioma (160). The predictive value of the serum miR-29 family for glioma was moderate (AUC = 0.74), but that in high-grade glioma detection was sufficient (AUC = 0.81). Another study also showed that the expressions of miR-29b in blood were significantly different compared with those of a healthy control (161).

miR-125b is widely considered a tumor suppressor-miRNA and an ideal biomarker for clinical diagnosis in various human cancers. The study of serum miR-125b from 33 gliomas and 33 healthy controls revealed that the serum miR-125b level was significantly lower in patients with glioma (162). The ROC curve analysis yielded an AUC value of 0.839 (162). Furthermore, a meta-analysis was conducted to assess the diagnostic accuracy of miR-125b in cancer diagnosis, and the results revealed that employing miR-125b as a biomarker for cancer detection achieved a sensitivity of 82% and a specificity of 77% (162).

miR-210 is reported to be another potentially useful biomarker in the serum of patients with glioma. Lai et al. analyzed blood samples collected from patients with glioma (n=136) and healthy controls (n=50) and revealed that an approximately sevenfold increase in miR-210 expression was detected in serum samples from patients with GBM relative to healthy controls (163). miR-210 has been found to be upregulated in a variety of other solid tumor types and potentially influences cellular function through diverse pathways; the miRNA has also been correlated with hypoxia (164). A number of targets of miR-210 have been reported, including VEGF (165), BCL2 (166), and E2F transcription factor 3 (167). Sun et al. analyzed the expression levels of miR-128 in serum samples from 151 gliomas, 52 meningiomas, and 53 normal donors and showed that miR-128 expression was significantly decreased in glioma preoperative serum compared with others. ROC analyses showed that serum miR-128 levels were reliable in distinguishing patients with glioma from normal controls and patients with meningioma, with AUC values of 0.9095 and 0.8283, respectively (168). Although the mechanism has not yet been clarified, the study demonstrated that serum miR-128 expression was significantly elevated after surgery. miR-128 functions as a vital suppressor of tumorigenesis in glioma cells and is reported to downregulate p70S6K1 and its downstream signaling molecules, including VEGF and HIF-1. Another study showed that serum miR-205 expression was significantly lower in patients with glioma than in healthy controls ($p < 0.001$) (169). Interestingly, serum miR-205 expression levels were inversely correlated with pathological grades (169).

4.2. Cerebrospinal fluid

Baraniskin et al. (170) reported the first investigation of microRNA expression in CSF from patients with glioma in 2012. The results demonstrated that miR-15b and miR-21 were differentially expressed in CSF samples from patients with glioma compared with control subjects with various neurologic disorders, including CNS lymphoma and carcinomatous brain metastases. The combination of miR-15b and miR-21 resulted in increased diagnostic accuracy, with 90% sensitivity and 100% specificity in distinguishing patients with glioma from control subjects. Interestingly, miR-15 levels were significantly higher in glioma than in CNS lymphoma or metastatic tumor; however, miR-21 levels were significantly lower in glioma than in CNS lymphoma or metastatic tumor.

Teplyuk et al. (171) determined the CSF levels of several cancer-associated miRNAs for 118 patients diagnosed with different types of brain cancers. The levels of miR-10b and miR-21 were significantly increased in the CSF of patients with GBM and brain metastasis of breast and lung cancer compared with non-neoplastic conditions. Members of the miR-200 family were useful markers for discriminating between GBM and metastatic brain tumors. In

addition, quantification of as few as seven microRNAs in CSF (miR-10b, miR-21, miR-125b, miR-141, miR-200a, miR-200b, and miR-200c) enabled differential recognition of GBM and metastatic brain tumor with high accuracy (91%–99%) (171).

Akers et al. analyzed extracellular vesicles (EVs) containing miRNAs in the CSF from patients with GBMs. The EV fraction was isolated by differential centrifugation. Although the analytic algorithm for quantitatively assessing EV miRNA remains underdeveloped, the authors showed that the CSF miR-21 levels of patients with GBM were 10-fold higher than those in the CSF of nononcologic patients (172).

Recently, another study reported results to those of Akers and coworkers. Exosomal miR-21 levels in the CSF of patients with glioma were found to be significantly higher than in controls, although no difference was detected in serum-derived exosomal miR-21 expression. Interestingly, the CSF-derived exosomal miR-21 levels correlated with a level of dissemination and a location of recurrence. Therefore, exosomal miR-21 in CSF may be a predictive marker for the early stages of tumor recurrence or metastasis (173).

5. Future prospects

Over the past decade, our understanding of genetic alterations and the tumor-specific signal pathways in glioma has been made, although this knowledge to date has not been translated into the prognosis of the patients with glioma. However, a better understanding of key pathways associated with gliomagenesis and malignant transformation could promise further improvements in glioma therapy. Maximum safe resection followed by radiotherapy up to a total dose of 60 Gy and TMZ-based chemotherapy have been established as a standard therapy, but new molecular targeting drugs, including small-molecule cell cycle inhibitors and biological and immune-based therapies have begun to yield promising results in clinical studies.

Over the past two decades, miRNAs have emerged as molecules central to glioma biology. miRNAs represent an additional layer of complexity in tumor biology and have been further validated as regulators of processes fundamental to glioma. This field is rapidly changing. Herein, we have reviewed the emerging roles of miRNAs as drivers of glioma formation and progression with a focus on glioma hallmarks. miRNAs may be essential in glioma growth, angiogenesis, invasion, genomic instability, tumor-promoting inflammation, and glioma-specific metabolism. In recent years, functions of miRNAs in gliomagenesis and microenvironment have been considerably elucidated, and we have validated the tumor suppressive or oncogenic functions of miRNAs in glioma. Now, we should move toward a next phase involving clinical trials with miRNAs as glioma therapies. For example, miR-34, a tumor-suppressive miRNA that simultaneously downregulates the expressions of MET, PDGFRA, and CDK6 is currently in clinical trials as a directed therapy for advanced solid tumors. In glioma, the restoration of miR-34a can possibly outperform any single targeted agents tailored to those targets. There remain several problems to use miRNAs in glioma therapy. These include not only accumulation of our knowledge of miRNAs' biological functions in glioma,

but also the development of new and safe methods for delivery to glioma cells. Although the clinical application of miRNAs in GBM has yet to be realized, we believe the future of glioma therapy could be bright.

In this review, we have also focused on circulating miRNAs of serum, plasma, and CSF in patients with glioma. The use of miRNAs has several limitations, such as the diversity of methodologies that exist for miRNA detection and the small cohort size for the validation steps reported in current studies. Nonetheless, circulating miRNAs may exhibit diagnostic values, the combination of biomarkers may improve the diagnostic accuracy, and the combination of circulating miRNAs and other screening methods may be particularly useful in glioma detection. However, a standard protocol of sample treatment and suitable internal controls should be further established to make the level of detection comparable to that of other methods before miRNA biomarkers are fully utilized in clinical practice.

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