MicroRNAs in Glioblastoma Multiforme: Profiling Studies and Therapeutic Impacts

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Abstract

Glioblastoma multiforme (GBM) is the most lethal primary brain tumor and is characterized by a poor prognosis, resistance to standard therapies, and a highly mutated tumor genome. It is therefore critical to identify new molecular targets that contribute to GBM pathogenesis in order to develop novel targeted therapeutic and diagnostic strategies. MicroRNAs (miRNAs) represent an emerging class of molecules that play significant roles in a number of key cellular processes associated with GBM. This review summarizes the results of recent studies that have attempted to profile the miRNA expression signatures of GBM. Additionally, this review highlights the downstream effectors and activities of key oncogenic or tumor suppressive miRNAs in GBM and describes some promising therapeutic, diagnostic, and prognostic strategies involving miRNAs.

Keywords: Glioblastoma multiforme; Oncogenic miRNAs; Tumor suppressive miRNAs

Introduction

Malignant gliomas are the most common and lethal tumors arising in the central nervous system, and are classified by the World Health Organization (WHO) into four different grades based on malignancy (I, II, III, IV) (1). Grade IV glioblastoma multiforme (GBM) is the most cytologically malignant and active form of primary brain tumor (2), and is characterized by aggressive vascular proliferation, invasiveness, chemoresistance to new and traditional therapies, stem cell-like behavior, and normal brain necrosis (3,4). The current standard of care for GBM is maximal safe surgical resection followed by radiation therapy and chemotherapy with temozolomide (5); however, even with this treatment, the prognosis remains very poor. The median life expectancy of newly diagnosed patients is less than one year and the five-year survival rate is less than 3% (6). GBMs are classified as primary or secondary tumors, with primary GBMs commonly arising de novo in older adults while secondary GBMs commonly develop progressively from grade III anaplastic astrocytoma (AA) in younger patients. It is widely acknowledged that primary and secondary GBMs constitute different diseases entirely, and are associated with distinct genetic aberrations. Additionally, GBMs generally arise in the cerebral hemispheres, and less frequently in the brainstem and spinal cord.

The accumulation of numerous underlying genetic defects contributes to the pathological complexity and lethality of GBM. Among the most common defects in GBM are epidermal growth factor receptor (EGFR) amplification, loss of heterozygosity (LOH) on chromosome 10q23, and phosphate and tensin homologue (PTEN) mutation in primary GBMs and TP53 mutations in secondary GBMs (7). Although these mutations are well-documented, further progress needs to be made to elucidate the mechanisms driving these and other genetic lesions in order to develop novel therapeutic and diagnostic strategies targeting specific deregulated pathways and genes in GBM. Therefore, it is critical to better understand the aberrant genetic pathways and mechanisms associated with the hallmark signaling mutations in GBM.

MicroRNA (miRNA) molecules may hold potential as novel therapeutic and diagnostic targets that contribute to the mutant genome of GBM. MiRNAs are an abundant class of small (20-22 nucleotide), non-coding RNA molecules that post-transcriptionally regulate gene activity by degrading
or suppressing the translation of target mRNAs (8,9).
MiRNAs originate from a long primary transcript (pri-mRNA) that is processed within the nucleus into 70-100 nucleotide hairpin precursor RNAs (pre-miRNAs) by the ribonuclease Drosha/DGCR8 (10). The pre-miRNA undergoes translocation to the cytoplasm by Exportin-5 in a RanGTP-dependent manner. In the cytoplasm, it is cleaved by a different ribonuclease, Dicer, into a mature miRNA duplex. This duplex is then incorporated into the RNA-induced silencing complex (RISC), which degrades the duplex into single-stranded, mature miRNA. Within the RISC, the mature miRNA can bind to its target mRNAs by complementary base pairing at its 3’ untranslated region. The miRNA can either inhibit translation or induce degradation of the target mRNA, and this decision is determined by the degree of complementarity between the miRNA and the target mRNA (11,12).

MiRNAs constitute only 1-3% of the human genome, yet they are estimated to control ~30% of all gene expression (11,13). Due to their size and the relatively small number of nucleotides needed for binding complementarity between a single miRNA and a given target mRNA, miRNAs can affect a vast number of target mRNAs. In fact, single miRNAs have been observed to control over 100 target mRNAs (14). Conversely, a single mRNA can be modulated by multiple miRNAs (15). Over 1,000 miRNAs have been identified in humans according to a registry (miRBase) cataloguing all discovered miRNAs (16).

Considering the vast numbers of miRNAs and their potential mRNA targets, it is not surprising that miRNAs play crucial regulatory roles in virtually all cellular processes, including growth, proliferation, metabolism, development, and apoptosis (8,17). Because miRNAs exhibit such widespread regulatory activity within the cell, the aberrant expression of miRNAs has naturally been implicated in several human diseases, such as cerebrovascular illness (18), diabetes (19), arthritis (20), kidney disease (21), and neurodegenerative disorders (22).

MiRNA deregulation is also known to play a pivotal role in cancer etiology (23). Studies of genome-wide miRNA expression have indicated that a majority of human miRNA genes are located at fragile genomic sites associated with cancer (24), and that miRNAs can function in both oncogenic and tumor suppressive roles (25). Furthermore, aberrant miRNA expression has been reported in many human cancers, including pancreatic cancer (26), prostate cancer (27), thyroid cancer (28), melanoma (29), ovarian cancer (30), colon cancer (31), and breast cancer (32). Recently, aberrant miRNA expression patterns have been reported in GBM (33) and multiple specific miRNAs have shown clear tumor suppressive (34) or oncogenic (33) roles in GBM.

There is a clear and urgent need to further understand the mechanisms underlying GBM pathogenesis. MiRNAs represent one set of novel and emerging molecules that play major regulatory roles in numerous cellular processes associated with cancer. A better understanding of the roles of these molecules in GBM may result in the development of new diagnostic and therapeutic strategies targeting aberrant miRNA expression in GBM, thus leading to improved patient outcomes. In this review, we summarize the results of recent miRNA profiling studies in GBM and describe the roles and mechanisms of key oncogenic and tumor suppressive miRNAs that contribute to GBM. We further outline some therapeutic, diagnostic, and prognostic strategies directed at deregulated miRNAs in GBM.

**MiRNA Expression Profiles in GBM**
Global expression profiling of miRNA is frequently used to identify deregulated miRNAs within a tumor genome. Several profiling studies have been performed recently in attempts to broadly characterize the miRNA expression pattern of GBM tumors either by microarray or RT-PCR (Table 1). These studies have identified multiple miRNAs that are differentially expressed in GBM compared to normal tissues. Similar studies have focused on miRNA expression profiles that differentiate GBM (WHO grade IV) from WHO grade III AA or GBM stem cells from non-stem cells. MiRNA-21, 181, 221, 137, 124, 128, and 451 are among the most aberrantly expressed miRNAs in GBM that have been identified by these studies. Overall, these studies have led to the development of a profile of consistently deregulated miRNAs in GBM, which has allowed other researchers to begin to characterize the downstream targets and activities of these specific miRNAs.

In the first global expression analysis of miRNA in GBM, Chan et al (33) identified miRNA-21 as being consistently upregulated in GBM tumors and cell lines compared to normal adult and fetal brain tissues and glial cell lines. Other miRNAs were found to be aberrantly expressed as well, but the expression levels of these miRNAs were not
Table 1. MiRNA profiling studies in GBM

<table>
<thead>
<tr>
<th>Study Specifications</th>
<th>Samples</th>
<th>Upregulated</th>
<th>Downregulated</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Microarray with 180 target miRNAs</td>
<td>3 GBM tissues, 8 nonneoplastic brain tissues, 6 GBM cell lines</td>
<td>miRNA-21, 138, 347, 135, 291-5'</td>
<td>miRNA-198, 188, 202</td>
<td>Chan 2005</td>
</tr>
<tr>
<td>Microarray with 245 target miRNAs</td>
<td>9 GBM tissues + 9 ABTs</td>
<td>miRNA-10b, 130a, 221, 125b-1, 125b-2, 9-2, 21, 25, 123</td>
<td>miRNA-128a, 181c, 181a, 181b</td>
<td>Ciafre 2005</td>
</tr>
<tr>
<td>Microarray with 756 target miRNAs</td>
<td>26 GBM, 13 AA, 7 normal brain tissues</td>
<td>miRNA-21, 146b-5p, 155, 16, 193-3p, 199a/b-3p, 335, 142-5p, 34a, 513a-5p, 451</td>
<td>miRNA-126, 22, 143, 381, 24, 552, 886-5p, 128, 509-3-5p, 376c, 886-3p, 219-2-3p</td>
<td>Rao 2010</td>
</tr>
<tr>
<td>Microarray with 245 target miRNAs</td>
<td>Unspecified # GBM tumor samples + ABTs</td>
<td>miRNA-383, 519d, 21, 516-35p, 26a, 10b, 486, 451</td>
<td>miRNA-124a, 137, 323, 139, 218, 128-2, 483, 128-1, 299, 511-1, 190</td>
<td>Godlewski 2008</td>
</tr>
<tr>
<td>Microarray with 435 target miRNAs</td>
<td>5 GBM cell lines, 1 AA cell line, and 1 normal brain tissue</td>
<td>miRNA-137, 23b, 23a, 222, 221, 106, 15b, 21</td>
<td>miRNA-451, 124, 495, 223, 329, 126, 219, 1, 330, 342, 323, 127, 128, 132, 95</td>
<td>Zhou 2010</td>
</tr>
<tr>
<td>RT-PCR assay with 8 target miRNAs</td>
<td>10 GBM tissues, 10 AA tissues, 8 LGA tissues</td>
<td>miRNA-21-221</td>
<td>miRNA-181b</td>
<td>Conti 2009</td>
</tr>
<tr>
<td>RT-PCR assay with 192 target miRNAs</td>
<td>GBM v. AA tissues</td>
<td>miRNA-21, 155, 210</td>
<td>miRNA-101, 128a, 132, 133a, 133b, 149, 153, 154*, 185, 29b, 323, 328, 330</td>
<td>Silber 2009</td>
</tr>
<tr>
<td>Microarray with unspecified # of target miRNAs</td>
<td>CD133+ (stem) vs. CD133- (non-stem) cells from 6 GBM tissues</td>
<td>miRNA-451, 486, 425, 16, 107, 185</td>
<td>None found</td>
<td>Gal 2008</td>
</tr>
</tbody>
</table>

Results of studies attempting to determine the miRNA expression signature of glioblastoma multiforme. GBM: glioblastoma multiforme; AA: anaplastic astrocytoma; LGA: low-grade astrocytoma

validated by Northern Blot. A larger study (35) following the study by Chan et al found nine upregulated miRNAs including miRNA-221 and four downregulated miRNAs including miRNA-128a, 181a, 181b, and 181c in GBM human tissues. In GBM cell lines, the same study found nine upregulated miRNAs which included miRNA-21 and 221 and seven downregulated miRNAs including miRNA-128b, 181a, 181b, and 181c, confirming many of the results from the human tissue samples.
Slaby et al (36) further confirmed many of these results, finding miRNA-21, 221, 222, 181b, 181c, and 128a to be significantly deregulated and miRNA-21 to be the most upregulated miRNA in GBM tissues. Interestingly, the authors also found that miRNA-181b and 181c are the most downregulated miRNAs in patients who responded to radiation therapy (RT) and temozolomide (TMZ). This not only suggests that miRNA-21, 221, 222, 181, and 128a could serve as potential biomarkers in GBM in general, but also that miRNA-181b and 181c may serve as predictors for RT/TMZ response, which could potentially identify a subpopulation of GBM patients who will benefit from the RT/TMZ treatment.

A large-scale profiling study assessed miRNA expression levels in GBMs, Grade III AAs, and normal tissues (37). A 23 miRNA profile that differentiates GBM from AA and normal tissues was identified, which included miRNA-21. A similar study (38) found several miRNAs that were aberrantly expressed in GBM tissues compared to normal adjacent brain tissues. Among the deregulated miRNAs were miRNA-124a, 137, and 128, which were downregulated, while miRNA-451 and 21 were overexpressed. These data largely confirmed the results of previous profiling studies.

Recently, Zhou et al (39) found eight miRNAs to be upregulated in GBM cell lines and tissue specimens, including miRNA-137, 221, 222, 221, and 21. MiRNA-21 again showed the most significant increase relative to normal brain tissue. The study also identified 18 miRNAs that were downregulated, which included miRNA-451, 124, 128a, and 128b.

A study by Conti et al (40) attempted to confirm and expand upon results seen in the previous studies by assessing expression levels of the most commonly deregulated miRNAs in GBM in astrocytic tumors at various stages of malignancy. The miRNAs examined were miRNA-21, 221, 128a, 128c, 181a, 181b, and 181c. MiRNA-21 was overexpressed in both low- and high-grade tumors, while miRNA-221 was only overexpressed in GBMs but not in AAs or low-grade astrocytomas. Meanwhile, miRNA-181b was consistently downregulated in tumors of all grades. In order to build on these results, Silber et al (41) assessed the global expression levels of 192 miRNAs in high-grade (grade III) AAs and GBMs. Significant upregulation of miRNA-21 was again observed. In addition, a set of 16 differentially expressed miRNAs was found to sufficiently distinguish GBMs from high-grade AAs, with 13 of these being downregulated, including miRNA-128a, and three being upregulated, including miRNA-21. This study also found a set of 29 total miRNAs that are differentially expressed in both AAs and GBMs. The most significant of these were miRNA-7, 124, and 137.

A unique profiling study (42) attempted to assess differential expression levels of miRNAs between GBM stem (CD133+) cells and non-stem (CD133-) cells, as GBM is largely characterized by stem cell-like behavior and the regeneration of brain tumor-initiating stem cells (3). Interestingly, several miRNAs were found to be overexpressed in the non-stem cell population, including miRNA-451, miR-486, miR-16, miR-107, and miR-185, but none were found to be downregulated in this cell population. The authors hypothesize that overexpression of these miRNAs in the non-stem cell population drives stem cells to differentiate and lose their stem cell character, and increasing the levels of these miRNAs in the stem cell population may inhibit cell growth and tumor proliferation by reducing self-renewal potential.

**Specific deregulated miRNAs in GBM and their proposed mechanisms**

Like other cancers, GBM is characterized by the accumulation of genetic aberrations associated with multiple cellular processes vital to carcinogenesis, including growth, survival, and proliferation (43,44). Although the GBM miRNome is emerging out of the previously mentioned profiling studies, key deregulated miRNAs need to be functionally characterized in order to better understand how specific miRNAs contribute to the genetic aberrations that modulate tumor development. This will be crucial to produce effective targeted therapies and identify reliable biomarkers based on miRNA functions and effectors.

Depending on their downstream targets, miRNAs can either be oncogenic or tumor suppressive. Oncogenic miRNAs are commonly overexpressed in cancer while tumor suppressive miRNAs are downregulated. Several studies have attempted to identify and characterize the targets and mechanisms of action of commonly deregulated oncogenic and tumor suppressive miRNAs in GBM (Table 2).

**Oncogenic miRNAs**

MiRNA-21 is the most frequently upregulated miRNA in GBM tumors (33,35,36,37,38, 39,40,41).
Table 2. Deregulated miRNAs, their functions, and known molecular targets in GBM

<table>
<thead>
<tr>
<th>MiRNA</th>
<th>Role</th>
<th>Molecular Target(s)</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>miRNA-7</td>
<td>Tumor suppressive</td>
<td>EGFR, IRS-1, IRS-2</td>
<td>Kefas 2008</td>
</tr>
<tr>
<td>miRNA-124</td>
<td>Tumor suppressive</td>
<td>CDK6, PTBP1</td>
<td>Silber 2008, Makeyev 2007</td>
</tr>
<tr>
<td>miRNA-128</td>
<td>Tumor suppressive</td>
<td>Bmi-1, E2F3a</td>
<td>Godlewski 2008, Zhang 2009</td>
</tr>
<tr>
<td>miRNA-137</td>
<td>Tumor suppressive</td>
<td>CDK6</td>
<td>Silber 2008</td>
</tr>
<tr>
<td>miRNA-181a,b,c</td>
<td>Tumor suppressive</td>
<td>Bcl-2</td>
<td>Chen 2010</td>
</tr>
<tr>
<td>miRNA-221/222</td>
<td>Oncogenic</td>
<td>p27kip1, BIRC1, BIRC5 (survivin-1), caspase-3, PUMA</td>
<td>Gillies 2007, Lukiw 2009, Zhang 2010</td>
</tr>
<tr>
<td>miRNA-451</td>
<td>Oncogenic/Tumor Suppressive</td>
<td>CAB39,PI3K/Akt</td>
<td>Nan 2010, Godlewski 2010</td>
</tr>
</tbody>
</table>

Furthermore, it has been identified as a key oncogene in numerous tumor types, including prostate cancer (45), breast cancer (46), pancreatic cancer (47), and lung cancer (48), among many other cancers (49). Chan et al (33) was the first to identify miRNA-21 as being significantly overexpressed in GBM tumors and cell lines. The authors further found that inhibition of miRNA-21 by antisense oligonucleotides in GBM cell lines activates caspases and leads to increased apoptosis, suggesting that miRNA-21 acts as an oncogene in GBM by blocking apoptosis-enabling genes.

In GBM, miRNA-21 is known to target multiple components of the p53, transforming growth factor-β (TGF-β), and mitochondrial apoptosis tumor-suppressive pathways (50). Within the p53 pathway, miRNA-21 targets a p53 homologue (p63) and the p53 activators JMY, TOPORS, TP53BP2, DAXX, and HNRPK. These targets are required for p53 tumor suppressor activity, so their downregulation by miRNA-21 prevents p53-mediated apoptosis and promotes growth (50). MiRNA-21 also targets TGF-β1/2, the receptors TGF-βR2/3, and the apoptotic mediator DAXX (death associated protein 6) within the TGF-β pathway. DAXX thus plays a role in both the p53 and TGF-β pathways, making it particularly appealing as a therapeutic target that works at the intersection of these two key pathways. Overall, these alterations lead to growth repression, increased apoptosis, and cell cycle arrest, which all correspond to the observed anti-apoptotic and proliferative effects of miRNA-21.

Additionally, miRNA-21 promotes GBM invasion by directly targeting matrix metalloproteinase (MMP) inhibitors like RECK and TIMP3 (51). MMPs impart the ability to invade and metastasize in cancerous tissues, so their inhibitors play key roles as tumor suppressors (52). Importantly, MMPs are overexpressed in human gliomas and their expression correlates with tumor cell invasiveness (53). The ability of miRNA-21 to suppress MMP inhibitors thus explains the upregulation of MMPs and increased invasiveness in GBM. Kwak et al (54) also found that miRNA-21 potentiates invasion by inhibiting Spry2, a negative regulator of growth factor signaling. This action increases the strength and duration of Ras/MAPK signaling, which is associated with growth and increases levels of oncogenic MMPs.
A recent study (39) revealed that knockdown of miRNA-21 resulted in the inhibition of EGFR and Akt activity and suppression of STAT3 expression. Furthermore, miRNA-21 knockdown upregulated the tumor suppressor genes BID, FAS, PRS6, and SOCS4 in both PTEN wild-type and PTEN-mutant GBM cells, suggesting that the oncogenic effect of miR-21 is independent of PTEN status.

Although miRNA-21 has also been implicated to control a number of other key survival and apoptotic pathways such as the tumor suppressors PTEN (55), tropomyosin 1 (TPM1) (56), and programmed cell death 4 (PDCD4) (57) in other cancer types, these targets have not been validated in GBM. However, it is worth noting that some of these targets, such as PDCD4, have been found to be downregulated in glioma samples (58), suggesting a yet unproven connection between consistent upregulation of miR-21 and downregulated of PDCD4 in GBM. Further work needs to be done in order to accurately elucidate the role of miRNA-21 overexpression in the deregulation of these key tumor suppressor molecules in GBM; nonetheless, it is evident that miRNA-21 plays a major oncogenic role in GBM development, invasiveness, and growth.

miRNA-221 is another consistently upregulated miRNA in GBM tissues and cell lines which may carry out an oncogenic function in GBM (35,39,40,59). It is worth noting that miRNA-221 and miRNA-222 are co-regulated, have the same target specificity, and are often both upregulated in GBM. Gillies et al (60) found that antagonism of either miRNA-221 or miRNA-222 in GBM cells increased expression levels of p27kip1, a known cell cycle regulatory protein that is inactivated in many human cancers. Furthermore, the authors analyzed the 3’ UTR (untranslated region) of p27kip1 and found that miRNA-221 and miRNA-222 directly bind to sites in the 3’ UTR of p27kip1, p27kip1 is a member of the Cip/Kip family of cyclin-dependent kinase (CDK) inhibitors and acts as a tumor suppressor to prevent cell cycle progression through the G1 phase by binding to CDK2 and cyclin E complexes. By directly targeting p27kip1, miRNA-221/222 can inhibit the tumor suppressive activity of p27kip1, leading to aggressive cellular growth. This mechanism of action of miRNA-221/222 was confirmed contemporaneously by a separate study (61) that also showed that miRNA-221/222 promotes cancer cell proliferation by inhibiting levels of p27kip1 in GBM cells.

Lukiw et al (59) found that upregulation of miRNA-221 in GBM also alters apoptotic signaling and promotes neural cell proliferation by downregulating the survivin homolog BIRC1, a neuronal inhibitor of apoptosis protein (NAIP), and increasing expression of BIRC5 (survivin-1) and caspase-3. The BIRC (baculoviral IAP repeat-containing) proteins are a family of apoptosis regulatory proteins that inhibit apoptosis and suppress differentiation (62), and BIRC5 is abundantly expressed in GBM cell lines (63). The authors propose that miR-221 may alter apoptotic and cell cycling pathways in GBM by modulating alternative expression levels of the BIRC family of apoptotic regulators.

An additional mechanism by which miRNA-221/222 modulates cell survival was recently identified. MiRNA-221 and miRNA-222 were found to inhibit apoptosis by targeting the pro-apoptotic gene PUMA in GBM cells and a mouse xenograft model (64). Knockdown of miRNA-221/222 induced apoptosis, reduced tumor growth, and upregulated PUMA expression, while ectopic expression of miR-221/222 led to the opposite effects. Furthermore, miRNA-221 and miRNA-222 were found to directly interact with the 3’UTR of PUMA. The authors suggest that miRNA-221/222 promotes survival by negatively regulating PUMA, which leads to an increase in Bcl-2 expression and a decrease in BAX.

**Tumor suppressive miRNAs**

Among the most commonly downregulated miRNAs in GBM are miRNA-181a, 181b, and 181c (34,35,36,40), and these miRNAs likely serve as tumor suppressors in GBM. Shi and colleagues (34) showed that transfection of miRNA-181a and 181b triggered growth inhibition and apoptosis and suppressed invasion in glioma cells. The authors also found that while miRNA-181a was inversely correlated with tumor grade, miRNA-181b was differentially expressed only between low grade and high grade (III and IV) gliomas. Furthermore, upregulation of both miRNA-181a and miRNA-181b induced apoptosis in glioma cell lines, but miRNA-181b overexpression led to significantly more apoptotic cells than miRNA-181a, indicating that these two miRNAs might function via two different signaling pathways in GBM.

Slaby et al (36) further found that miRNA-181b and 181c were the most downregulated miRNAs in patients who responded to RT/TMZ, suggesting that these two miRNAs may serve as predictive markers for RT/TMZ reponse. A separate study (65) revealed that miRNA-181a sensitizes GBM U87MG cells to
radiation by targeting Bcl-2 (B cell lymphoma 2). Bcl-2 is a key pro-survival protein that inhibits the release of pro-apoptotic factors and activation of the caspase cascade (66). Additionally, Bcl-2 expression can help cells adapt to a harmful environment, and has been implicated in cancer cell resistance to radiation (67). These results suggest that miRNA-181a, 181b, and 181c sensitize GBM cells to radiation and thus may serve as therapeutic targets in GBM radiation treatment.

MiRNA-128 is one of the most commonly downregulated miRNAs in GBM (35,36,38,39, 40,41). Increasing miRNA-128 levels in GBM inhibits proliferation and self-renewal, and may suppress tumor growth in GBM by reducing levels of Bmi-1 (38). Bmi-1 is a well-known oncogene that normally promotes stem cell renewal by silencing Ink4a and Arf (68). These data suggest that miRNA-128 may suppress cancer pathogenesis by inducing differentiation out of a stem cell-like state. Therefore, decreased expression of miRNA-128 in GBM leads to the maintenance of stem cell self-renewal, which is known to play a significant role in GBM progression (3). Additionally, Zhang et al (69) found that miRNA-128 directly targets the transcription factor E2F3a, a regulator of cell cycle progression, and that the targeting of E2F3a by miRNA-128 inhibited glioma cell proliferation.

MiRNA-7 is also downregulated in GBM (41,70) and is another potential tumor suppressor. Kefas et al (70) found that miRNA-7 acts in a tumor suppressive role by reducing epidermal growth factor receptor (EGFR) expression. EGFR overexpression is one of the most frequent genetic mutations contributing to GBM (71); thus, miRNA-7 downregulation may play a paramount role in GBM pathogenesis by contributing to overexpression of EGFR. Kefas et al (70) also found that miRNA-7 also suppresses the Akt survival pathway, which is downstream of EGFR, by targeting two of its upstream regulators, IRS-1 (insulin receptor substrate 1) and IRS-2 (insulin receptor substrate 2). Moreover, this effect was observed independently of EGFR amplification status. Taken together, these data suggest that miRNA-7 acts as a tumor suppressor by targeting two key pathways related to growth and survival in GBM.

MiRNA-124 has been commonly identified as a downregulated miRNA in GBM (38,41). Supplementation of miR-124 induced cell cycle arrest in GBM cells by directly inhibiting CDK6 (41). The same study found that miRNA-137, which is also downregulated in GBM (33,38,41), exhibits the same effect by the same mechanism. These results would seem to indicate that miRNA-124 and miRNA-137 act as tumor suppressors in GBM by promoting cell cycle arrest via CDK6 inhibition.

Additionally, treatment of GBM stem cells with miRNA-124 or miRNA-137 led to morphological changes and marker expressions consistent with neuronal differentiation (41), suggesting that the decreased expression of these two miRNAs in GBM decreases tumor cell differentiation and leads to proliferation of undifferentiated cells. These data are supported by a separate study (72) that found that miRNA-124 promotes neuronal differentiation by depressing levels of stem cell maintenance proteins. In particular, miRNA-124 was found to regulate PTPB1, a repressor of alternative pre-mRNA splicing in non-neuronal cells. PTPB1 levels are reduced by miRNA-124 during neuronal differentiation, which results in alternative splicing patterns that promote neuronal-specific development from non-neuronal cells.

Interestingly, miRNA-451 has been found to possess both tumor suppressive and oncogenic roles in GBM. Two studies (39,42) both found miRNA-451 to be downregulated in GBM. Increased expression of miRNA-451 via miRNA-451 mimic oligonucleotides inhibited cell growth, inducing cell cycle arrest, and increased apoptosis in GBM cells (73). Furthermore, expression levels of several downstream targets of the PI3K/Akt pathway, including Akt1, cyclin D1, p27, MMP-2, MMP-9, and Bcl-2 were all decreased upon supplementation of miRNA-451. The PI3K/Akt pathway is commonly mutated in GBM and is a key contributor to survival and growth in GBM cells (74,75). These findings suggest that miRNA-451 may act as a tumor suppressor in GBM by regulating the PI3K/AKT survival pathway.

Contrary to the previous findings, miRNA-451 has also been found to be overexpressed in GBM cells (38,76) and may function in an oncogenic role. Godlewski and colleagues (76) proposed that miRNA-451 allows GBM cells and tissues to adapt to altered energy availability and survive dynamic metabolic stress that is commonly found in rapidly growing tumors like GBM (77). The authors found that miRNA-451 is regulated by glucose levels and modulates the AMPK pathway (76), which plays a key role as the major cellular sensor of energy availability (78) and promotes survival in periods of low energy availability. MiRNA-451 modulates the AMPK pathway by controlling expression of its upstream activator, LKB1, via direct regulation of
Figure 1. MiRNA-targeted therapies in GBM. Several delivery techniques may be able to penetrate the blood-brain barrier and provide miRNA-targeted therapies directly to GBM tissues. These therapies include miRNA mimics and viral gene restoration therapy in order to upregulate certain tumor suppressor miRNAs or anti-miRNA oligonucleotides, antagoniRs, DNAzymes, antisense molecules, miRNA masking, and miRNA sponges to downregulate specific oncogenic miRNAs.
CAB39 expression, which can reduce migration by promote proliferation (76). Therefore, miR-451 acts to maintain the balance between migration and proliferation in response to metabolic stress in the tumor microenvironment. Because of the apparent dual-nature of miRNA-451, additional work needs to be done to elucidate the complete mechanism by which miRNA-451 promotes or inhibits GBM pathogenesis.

**MiRNA-based therapeutic strategies**

Due to the tumor suppressive or oncogenic involvement of miRNAs in GBM, miRNAs represent a viable set of potential therapeutic and diagnostic targets. Some studies have demonstrated effective *in vitro* and *in vivo* suppression of tumor-like characteristics such as cell survival and proliferation by delivering tumor suppressive miRNAs or downregulating oncogenic miRNAs. Zhou et al (39) demonstrated that the downregulation of miRNA-21 with an antisense oligonucleotide induced apoptosis, inhibited proliferation, and halted cell-cycle progression *in vitro*. Furthermore, the authors found that the downregulation of miRNA-21 with a lipofectamine-mediated anti-sense-miRNA-21 gene therapy vector in a xenograft mouse model effectively suppressed tumor growth and increased apoptosis. A similar study (79) found that the combined effect of miR-21 antagonism by locked nucleic acid (LNA)-antimiRNA-21 oligonucleotides and treatment with the cytotoxic agent tumor necrosis factor–related apoptosis inducing ligand led to a synergistic increase in caspase activity and significantly decreased cell viability in human glioma cells *in vivo*. Zhang et al (64) targeted the oncogenes miRNA-221 and miRNA-222, and found that ectopic expression of miRNA-221/222 induced cell survival while knockdown increased apoptosis *in vitro*. *In vivo*, they found that miRNA-221/222 knockdown increased apoptosis and decreased tumor growth in a xenograft model.

MiRNAs may also function in chemoresistance, which is also common characteristic of GBM (4). Ujifuku and colleagues (80) found that a TMZ-resistant GBM cell line expressed several upregulated miRNAs (miRNA-195, miRNA-455-3p, miRNA-10a) compared to its parental line, and that these miRNAs played a role in acquired TMZ-resistance. Similarly, miRNA-181b and 181c may contribute to sensitivity to radiation therapy (RT) and act as predictors for RT response (36), while miRNA-181a has been shown to sensitize human malignant glioma to radiation by targeting the pro-survival protein Bcl-2 (65).

Targetable miRNAs can play either tumor suppressive or oncogenic roles. If a miRNA of interest is tumor suppressive, miRNA mimics or viral vector-based gene restoration therapy may be used to upregulate the miRNA in order to enhance its tumor suppressive functions. On the other hand, an oncogenic miRNA can be downregulated through the use of several anti-miRNA therapies, including anti-miRNA oligonucleotides, antagonirs, DNAzymes, ribozymes, antisense molecules, and miRNA masking (81). A miRNA sponge may also provide a novel method of silencing specific miRNAs (82).

Effective delivery of miRNAs is the most significant roadblock in translating discoveries at the bench to treatments at the bedside (83). This is especially true for GBM, as brain tumors present unique challenges to effective delivery, such as the blood-brain barrier (BBB), which makes systemically-administered vectors unlikely candidates for delivery mechanisms. Local delivery techniques will likely be necessary if miRNA-based therapies are to reach their full therapeutic potential. One possible local delivery modality that has emerged recently is convection-enhanced delivery (CED), which involves prolonged injection of a therapeutic agent into the brain tissue under positive pressure (84). Additional therapeutic techniques such as focused ultrasound enhancement (85), permeability modulation (86), and injection of targeted exosomes (87) have also been developed to improve drug delivery to the brain. These newly developed treatment strategies may enhance the effectiveness and viability of miRNA-based therapies in GBM in the future.

Viruses encoding miRNAs or their inhibitors are currently the most suitable delivery vectors for miRNA-based therapies. These include adenovirus, lentivirus, and adeno-associated virus. Additionally, liposomal nanoparticles are another viable delivery vector (83). Other nonviral miRNA delivery methods that are non-specific to the brain have been developed, including lipid encapsulation, complex formation via a variety of liposomes or cationic polymers, and chemical conjugation of siRNAs to peptides, aptamers or antibodies (88).

MiRNA-based therapies have been shown to effectively inhibit tumor growth *in vitro* and in mouse xenograft models, yet many challenges still remain if these advances are going to be translated to the clinic. In order to optimize the efficacy of
miRNA-based therapies, effective delivery strategies and vectors must first be developed to overcome the unique challenges posed by GBM tumors. Several different delivery modalities are emerging that may be able to overcome these challenges in order to provide effective delivery to the tumor and ultimately improve patient outcomes (Fig. 1).

Aside from possessing clear therapeutic potential, miRNAs may additionally become valuable diagnostic and prognostic targets in GBM. Tumor classification based on miRNA profiling is more accurate than mRNA-based profiling in characterizing human cancers, and is an accurate predictor of a patient’s overall prognosis (89). Recently, Srinivasan et al (90) identified a ten-miRNA expression signature that can accurately predict survival in GBM patients based on miRNA expression and survival data of 222 GBM patients. These data suggest that miRNAs may hold additional clinical potential outside of the therapeutic realm by serving in a diagnostic or prognostic role.

Conclusion
Glioblastoma multiforme (GBM) is the most lethal and aggressive type of primary brain tumor, and is characterized by a dismal prognosis and numerous aberrations in important signaling pathways. MiRNAs represent an emerging set of molecules that play key roles in GBM pathogenesis. Differential expression levels of specific tumor suppressive or oncogenic miRNAs can lead to signal transduction abnormalities that are associated with increased survival, growth, and proliferation. As the roles of miRNAs in GBM are better understood and novel delivery methods are developed and optimized, miRNA-based targeted therapies may emerge to possess significant therapeutic potential for GBM treatment.

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Conflicts of Interest
The authors have no conflicts to disclose.

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