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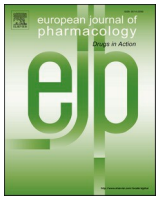

MicroRNA-based therapy for glioblastoma: Opportunities and challenges

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ABSTRACT

Glioblastoma (GBM) is the most common and aggressive primary malignant brain tumor and is characterized by high mortality and morbidity rates and unpredictable clinical behavior. The disappointing prognosis for patients with GBM even after surgery and postoperative radiation and chemotherapy has fueled the search for specific targets to provide new insights into the development of modern therapies. MicroRNAs (miRNAs/miRs) act as oncomirs and tumor suppressors to posttranscriptionally regulate the expression of various genes and silence many target genes involved in cell proliferation, the cell cycle, apoptosis, invasion, stem cell behavior, angiogenesis, the microenvironment and chemo- and radiotherapy resistance, which makes them attractive candidates as prognostic biomarkers and therapeutic targets or agents to advance GBM therapeutics. However, one of the major challenges of successful miRNA-based therapy is the need for an effective and safe system to deliver therapeutic compounds to specific tumor cells or tissues *in vivo*, particularly systems that can cross the blood–brain barrier (BBB). This challenge has shifted gradually as progress has been achieved in identifying novel tumor-related miRNAs and their targets, as well as the development of nanoparticles (NPs) as new carriers to deliver therapeutic compounds. Here, we provide an up-to-date summary (in recent 5 years) of the current knowledge of GBM-related oncomirs, tumor suppressors and microenvironmental miRNAs, with a focus on their potential applications as prognostic biomarkers and therapeutic targets, as well as recent advances in the development of carriers for nontoxic miRNA-based therapy delivery systems and how they can be adapted for therapy.

1. Introduction

Glioblastoma multiforme (GBM) is the most common primary malignant tumor of the central nervous system (CNS), accounting for 81% of malignant brain tumors. It often occurs in children and the elderly and affects 6.6 per million people (Rouse et al., 2016; Ostrom et al., 2015). In contrast to other tumors, GBM morbidity is rare; however, it has a very poor prognosis and significant mortality, with a very low 5-year relative survival rate (approximately 5%) (Morgan, 2015). Due to its invasiveness, high probability of relapse, and substantial inter- and intratumoral molecular heterogeneity, GBM has become a challenge for cancer researchers (Kunadis et al., 2021). The pathogenesis of GBM is unclear and remains to be further elucidated. A small proportion of the GBM pathogenesis is related to Mendelian disorders (Goodenberger and Jenkins, 2012), and mutations in epigenetic regulatory genes play an important role in the pathogenesis of GBM (Dong and Cui, 2019). Based on accumulating evidence, histone 3 is associated with abnormal DNA methylation or demethylation in pediatric high-grade gliomas, such as

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H3K27M-mutant diffuse midline gliomas (DMGs) (Lim et al., 2021; Yoshimoto et al., 2017). Furthermore, changes in the expression levels of methyltransferases, such as euchromatic histone lysine methyltransferase 2 (*EHMT2/G9A*), SUV39H1 histone lysine methyltransferase (*SUV39H1*) and SET domain bifurcated histone lysine methyltransferase 1 (*SETDB1*), and acetyltransferases, such as lysine acetyltransferase 6A (*KAT6A*), sirtuin 2/7 (*SIRT2/7*), and histone deacetylase 4/6/9 (*HDAC4/6/9*), contribute to the pathogenesis and development of GBM (Kunadis et al., 2021). Some research indicates that multiple immune-related signaling pathways, such as tumor necrosis factor alpha (*TNF-α*)/nuclear factor kappa B (*NF-κB*) or p38/mitogen-activated kinase-like protein (*MAPK*), contribute to brain function disorders (Perry et al., 2001; McMahon and Hynynen, 2017; Terrando et al., 2011). Our previous studies showed that tumor necrosis factor α-induced protein 3-interacting protein 1 (*TNIP1*) induces glioma cell proliferation via the *TNF-α/NF-κB* signaling cascade (Lei et al., 2020).

Some studies have revealed that microRNAs (miRNAs/miRs), which regulate many target genes with multipathway targeting abilities, alter and contribute to the pathogenesis and process of GBM (Mondal and Kulshreshtha, 2021). Many miRNAs are dysregulated in GBM and act as pro-oncogenic or tumor-suppressive genes and GBM microenvironment modulators. Specifically, exosomes, one type of extracellular vesicles (EVs), are discoid vesicles ranging between 40 and 100 nm in size (Simpson et al., 2009) and were shown to participate in intercellular communication of the GBM-permissive microenvironment by transporting various biomacromolecules, such as proteins, lipids and miRNAs, long noncoding RNAs, and circular RNAs, which contribute to various malignant behaviors of GBM cells, including tumorigenesis, proliferation, invasion, angiogenesis, apoptosis, and radiation and chemotherapy resistance (Cheng et al., 2020). Here, we discuss the current state of knowledge of miRNAs as oncogenes or tumor suppressor genes in GBM, the roles in the microenvironment modulators, and we decipher the potential of some miRNA candidates as relevant drug targets and discuss miRNA-based therapeutics for GBM. Particularly, we review the challenges of discovering miRNA therapeutic delivery vehicles to bypass the blood–brain barrier (BBB) with nontoxicity and immunogenicity for miRNA-based therapeutics.

2. The biogenesis and function of miRNAs in GBM

MiRNAs are a class of endogenously expressed, small (approximately 19–25 nucleotides in length), evolutionarily conserved, novel post-transcriptional regulators identified as nonprotein-coding single-stranded RNAs (Tafrihi and Hasheminasab, 2019). As shown in Fig. 1, miRNAs are transcribed from individual genes that contain their own promoter or intragenically from spliced regions of protein-coding genes (Bertoli et al., 2015). The biogenesis of mature miRNAs involves several sequential steps. Similar to protein-coding genes, miRNAs with their own promoters are almost exclusively transcribed by the RNA polymerase II enzyme (*pol II*) to form an approximately 500–3000 nucleotide-long primary transcript called a pri-miRNA with a cap at the 5′ end and a poly-A tail at the 3′ end, which forms a specific hairpin-shaped, stem–loop secondary structure (Ding et al., 2018). Then, the pri-miRNA enters into the microprocessor complex (500–650 kDa) that contains the universal RNase III endonuclease Drosha and an essential cofactor, the double-stranded RNA-binding protein DiGeorge syndrome critical region gene 8 (*DGCR8/Pasha*), which contains two double-stranded RNA-binding domains and interacts with the stem–loop structure within the miRNA, in which the complementary sequences are not perfectly aligned. The pri-miRNA is subsequently processed in the microprocessor complex into a 60–70 nucleotide sequence hairpin precursor called pre-miRNA, which possesses a 5′ phosphate group and a 2 nt overhang stretch at the 3′ end. Subsequently, the pre-miRNA is transported from the nucleus to the cytoplasm by exportin-5 (*Exp5*) in a Ran-GTP-dependent manner (Malla et al., 2019). In the cytoplasm, the pre-miRNA is further processed into double-stranded RNA duplexes of approximately 22 nucleotides by the second RNase III endonuclease enzyme Dicer in conjunction with transactivation response RNA-binding protein (*TRBP*) and Argonaute RISC catalytic component 2 (*AGO2*, Dicer complex). Then, the duplex unwinds and is separated into 2 single-stranded molecules by helicases. In most cases, the less paired base strand on the 5’ end is the mature miRNA, which is finally

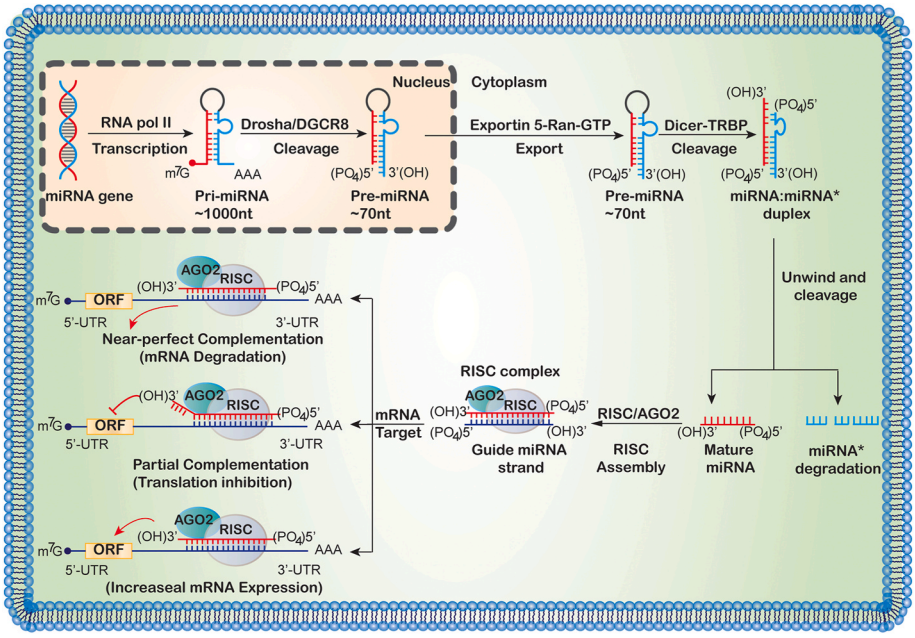

Fig. 1. MiRNA biogenesis and the mode of miRNA silencing mechanisms. Each miRNA is transcribed by *pol II* from genomic DNA in the nucleus to form approximately a 500–3000 nucleotide-long pri-miRNA capped with 7-methylguanosine at the 5′-end and a polyadenylated tail at the 3′-end harboring one or more stem–loop structures. Following loop formation, the pri-miRNA is recognized and processed in the nucleus by a microprocessor composed of the *Drosha/DGCR8* complex to release the stem loop, giving rise to precursor miRNA (pre-miRNA, approximately 70 nt). Next, the pre-miRNA is exported from the nucleus by *Exp5* and Ran GTPase into the cytoplasm. Then, the pre-miRNA is loaded into a pre-RISC that contains the RNase III enzyme Dicer, *TRBP*, one of the Argonaute proteins (*AGO1–AGO4*), and chaperones, such as heat shock proteins70/90 (*HSP70/HSP90*). Dicer removes the loop from pre-miRNA to generate an approximately 22 nucleotide dsRNA with a two-nucleotide 3′-overhang miRNA:miRNA* (miR-5p:miR-3p) duplex. Then, the cytoplasmic helicase unwinds the duplex, and one of the strands forms the mature miRNA guide strand and assembles into the RISC (miRNA:RISC complex) loaded with AGO protein. The miRNA* is degraded. The mature miRNA leads the miRISC complex to imperfect base pairing via the homologous miRNA “seed” to the 3′-UTR target sequence of the mRNA, which blocks protein expression by mRNA destabilization and represses translation by blocking access to the translational machinery. In addition, some miRNAs bind the 5′-UTR or coding regions to upregulate the translation of target mRNAs.

incorporated into the RNA-induced silencing complex (RISC) to form a miRNA-induced silencing complex (miRISC). The passage strand can also be loaded in the RISC or usually undergoes degradation; alternatively, it may be involved in the maintenance of the optimal level of miRNAs in cells (Jafri et al., 2015). Some studies have revealed that either one or both miRNA strands yields individual miRNA:RISC complexes to regulate the expression of the targets (Anthiya et al., 2018).

The mature miRNA and several other proteins, such as AGOs, helicases, deadenylases, mRNA decapping proteins, and methyltransferases, are located in the RISC complex, which is able to silence the expression of target genes through sequence-specific binding between the 3′-untranslated region (3′-UTR) of the mRNA via the 'seed sequence' of the 5′ end of the miRNA (2–8 positions) (Mathe et al., 2015). This binding interaction usually inhibits the ribosome from translating the gene and/or mRNA cleavage, leading to reductions in gene expression and the final protein output. Additionally, some miRNAs bind the 5′-UTR or coding regions to upregulate the translation of target mRNAs. Notably, miRNAs play crucial roles in GBM tumorigenesis, angiogenesis, invasion, and apoptosis, and the expression of miRNAs is substantially altered in GBM (Buruiana et al., 2020).

3. The multiple roles of miRNAs in GBM

By dissecting the functional mechanisms and relevant signaling pathways in GBM tissues and cells, studies have found that the upregulation of some pro-oncogenic miRNAs induces proliferation, cell cycle progression, aggressiveness, migration and tumor cell differentiation; nevertheless, inhibiting the expression of some tumor-suppressive miRNAs accelerates the deterioration of GBM, inhibits apoptosis, and contributes to a poor prognosis. In addition, many studies have focused on the roles of miRNAs as modulators in the EVs/miRNAs communication, immnue, the hypoxic and reverse pH microenvironment of GBM.

3.1. The dual role of miRNAs in GBM

Many studies have indicated that the involvement of miRNAs in GBM appears to be context specific, with a dual role as an oncogene or tumor suppressor based on results from GBM tissues or cell lines.

3.1.1. Oncomirs in GBM cells and tumor tissue

In GBM tissues and cells, the expression of many miRNAs is upregulated, which enhances tumor cell proliferation, aggressiveness, and migration or inhibits the translation of tumor suppressor genes that are linked to a significant increase in survival of patients with GBM. These miRNAs are termed proto-oncogenes or 'oncomirs'. Many miRNAs with pro-oncogenic roles in GBM and their mechanisms of action have been described in multiple studies.

Chan et al. reported that the expression of miR-21 was markedly upregulated in human GBM tumor tissues, early-passage GBM cultures, and six established GBM cell lines (A172, U87, U373, LN229, LN428, and LN308), which effectively inhibited the expression of the apoptosis-related genes caspase 3/9 (*CASP3/9*) (Chan et al., 2005). Chuang and his coworkers showed that miR-21-5p was enriched in exosomes and enhanced the tolerance of GBM to temozolomide (TMZ) in M2 GBM-associated macrophages in clinical high-grade GBM samples. The suppressor of signal transducer and activator of transcription 3 (*STAT3*), pacritinib, enhanced the therapeutic effect of TMZ by inhibiting the secretion of miR-21-enriched exosomes (Chuang et al., 2019). Yachi et al. found that miR-23a regulated the glial-mesenchymal transition to promote the invasion of GBM cells by targeting and inhibiting the translation of homeobox D10 (*HOXD10*) in U373 and LN443 GBM cell lines. In addition, miR-23a induces the expression of invasion-related molecules, such as urokinase-type plasminogen activator receptor (*uPAR*) and Ras homolog family member A/C (*RhoA/C*), and changes the expression of glial-mesenchymal transition markers, such as snail family transcriptional repressor 1 (*Snail*), snail family transcriptional repressor 2 (*Slug*), matrix metallopeptidase 2/9/14 (*MMP2/9/14*), and E-cadherin (Yachi et al., 2018). The latest research showed that the miR-1290 mimic significantly increased miR-1290 expression in the GBM cell line U87 and enhanced resistance to chemoradiation by targeting the 3′-UTR of suppressor of cytokine signaling 4 (*SOCS4*) to inhibit its translation (Khalighfard et al., 2020). In normal cells, BCL2-like 11 (*BCL2L11/BIM*) is a pro-apoptotic factor. According to Gabriely et al., miR-10b directly targets *BCL2L11/BIM* to regulate proliferation and the cell cycle in glioma cells from human GBM and a mouse model of human glioma (Gabriely et al., 2011a, b). Sasayama et al. showed that miR-10b plays a key role in high-grade gliomas because miR-10b expression is negatively correlated with the expression of the invasive factors *RhoC* and *uPAR* in 43 glioma samples (Sasayama et al., 2009).

A study by Gulluoglu et al. showed aberrant expression of 1332 genes and 319 miRNAs in GBM from sixteen fresh-frozen glioblastoma samples and seven healthy brain tissue samples by performing a whole transcriptome microarray chip analysis. Among these 319 miRNAs, miR-21-5p, miR-92b-3p, miR-182-5p and miR-339-5p were deemed oncomirs, and these miRNAs were substantially upregulated in GBM tissues and *in vitro* experiments. These oncomirs induced the migration and invasion of tumor cells and inhibited cell apoptosis by regulating the *Ras*, hypoxia-inducible factor 1 (*HIF-1*), *MAPK* and other signaling pathways (Gulluoglu et al., 2018). Of the abovementioned oncomirs, miR-92b-3p belongs to the miR-17–92 cluster family. Numerous studies have shown that this cluster is highly expressed in GBM tissues and cells. These miRNA cluster families increase the proliferation of glioma cells by inhibiting the transcription of antiproliferative factors, including transforming growth factor-beta receptor type 2 (*TGFBRII*), SMAD family member 4 (*SMAD4*) and calmodulin binding transcription activator 1 (*CAMTA1*) (Dews et al., 2010; Ernst et al., 2010; Malzkorn et al., 2010). Moreover, the expression of miR-183 was increased in human astrocytoma clinical specimens and U251 cells and was involved in promoting cell proliferation by targeting the neuronal cell differentiation marker neurofilament light polypeptide neurofilament light chain (*NEFL*) (Wang et al., 2016). MiR-296-5p promoted GBM cell self-renewal and stemness and propagated glioma xenografts *in vivo* by targeting chromatin remodeling protein high mobility group AT-hook 1 (*HMGA1*) and regulating SRY-box transcription factor 2 (*Sox2*) expression (Lopez-Bertoni et al., 2016). Lee et al. showed that miR-296-5p increases invasiveness in various GBM cells (LN229, T98G, and U87MG) by targeting caspase-8 (*CASP8*) (Lee et al., 2017). Moreover, miR-595 was upregulated and involved in regulating proliferation, aggressiveness, and migration by suppressing the expression of SRY-box transcription factor 7 (*Sox7*) in GBM tissues and cells (Hao et al., 2016; Ahir et al., 2017). Table 1 summarizes the abovementioned upregulated miRNAs that exert functions as oncogenes in the development of GBM.

3.1.2. MiRNAs functioning as tumor suppressors in GBM cells and tumor tissue

Many studies have documented that several miRNAs are expressed at higher levels in normal gliocytes or brain tissue. These miRNAs are presumed to function as tumor suppressors by inhibiting the proliferation, aggressiveness, and migration of tumor cells or the transcription of proto-oncogenes. A broad number of miRNAs with antitumorigenic roles and their mechanisms of action have been identified in various GBM studies.

In GBM specimens and cell lines, downregulated miR-519a expression significantly correlated with poor outcomes of GBM. The upregulated expression of miRNA-519a suppresses tumor proliferation, migration, and invasion by activating the *STAT3* gene (Hong et al., 2016). Li et al. found that increasing the expression of miR-519a enhanced the sensitivity to TMZ chemotherapy by targeting *STAT3*/B-cell lymphoma 2 (*Bcl-2*) signaling pathways to induce autophagy in human GBM tissues, U87-MG cells and xenografts *in vivo* (Li et al., 2018). In 55 paired GBM tissues and adjacent normal tissues and 6

Table 1
Upregulated oncomirs and their functions in GBM.

miRNA	Targets	Functional Assay	Tumor Grade	Sources	References
miR-21	*CASP3, CASP9, STAT3*	Cell apoptosis	Grade III-IV	Cell line, tissue	(Chan et al., 2005; Chuang et al., 2019)
miR-23a	*HOXD10, uPAR, RhoA, RhoC*	Cell invasion	GBM	Cell line	Yachi et al. (2018)
miR-1290	*SOCS4*	Cell proliferation, migration, invasion, chemoradiotherapy resistance	GBM	Cell line	Khalighfard et al. (2020)
miR-10b	*BCL2L11/BIM, RhoC, uPAR*	Cell proliferation, invasion, cell cycle	Grade III-IV	Tissue	(Gabriely et al., 2011a,b; Sasayama et al., 2009)
miR-92b-3p	*TGFBRII, SMAD4, CAMTA1*	Cell migration, invasion, apoptosis	GBM	Tissue	(Gulluoglu et al., 2018; Ernst et al., 2010; Malzkorn et al., 2010)
miR-17-92 cluster	*TGFβRII, SMAD4, CTGF, CAMTA1, POLD2*	Cell viability, proliferation, apoptosis, angiogenesis	Grade III-IV	Cell line, tissue	Dews et al. (2010)
miR-182-5p/21-5p/339-5p	*Ras, HIF-1, MAPK*	Cell migration, invasion, apoptosis	GBM	Cell line, tissue	Gulluoglu et al. (2018)
miR-183	*NEFL*	Cell proliferation	GBM	Cell line, tissue	Wang et al. (2016)
miR-296-5p	*HMGA1, CASP8*	Cell invasion	GBM	Xenografts	(Lopez-Bertoni et al., 2016; Lee et al., 2017)
miR-595	*Sox7*	Cell proliferation, aggressiveness, migration	GBM	Cell line, tissue	(Hao et al., 2016; Ahir et al., 2017)

Abbreviations: CASP3 (caspase 3), CASP9 (caspase 9), STAT3 (signal transducer and activator of transcription 3), HOXD10 (homeobox D10), uPAR (urokinase-type plasminogen activator receptor), RhoA (Ras homolog family member A), RhoC (Ras homolog family member C), SOCS4 (suppressor of cytokine signaling 4), BCL2L11/BIM (BCL2 like 11), TGFBRII (transforming growth factor-beta receptor type 2), SMAD4 (SMAD family member 4), CAMTA1 (calmodulin binding transcription activator 1), CTGF (connective tissue growth factor), POLD2 (DNA polymerase delta 2, accessory subunit), HIF-1 (hypoxia-inducible factor 1), MAPK (mitogen activated kinase-like protein), NEFL (neurofilament light chain), HMGA1 (high mobility group AT-hook 1), CASP8 (caspase 8), Sox7 (SRY-box transcription factor 7).

GBM cell lines (U118, LN-299, H4, A172, U87-MG, and U251), upregulating the expression of miR-758-5p with mimics suppressed cell proliferation, migration and invasion by targeting zinc finger and BTB domain containing 20 (*ZBTB20*). Xenograft experiments also showed that miR-758-5p suppressed tumor growth and metastasis (Liu et al., 2018). As shown in the study by Shi et al., upregulating the expression of miR-125b inhibits growth by inducing cell cycle arrest at the G1/S transition through the inhibition of the overexpression of cyclin-dependent kinase 6 (*CDK6*) and cell division cycle 25A (*CDC25A*) in U251 glioma stem cells (GSCs) (Shi et al., 2010). Shi et al. also found that high expression of miR-125b suppressed the expression of *MMP2/9* to inhibit the invasion of $CD133^{+}$ glioma cells (Shi et al., 2012a). In addition, the authors showed that miR-125b-2 expression was upregulated in GBM tissues and the corresponding stem cells (GBMSCs), which conferred resistance to TMZ (Shi et al., 2012b). In addition, miR-124 and miR-137, two tumor suppressors, were significantly downregulated in anaplastic astrocytomas (World Health Organization grade III) and glioblastoma multiforme (World Health Organization grade IV) relative to nonneoplastic brain tissue. Transfection of miR-124 and miR-137 regulated cell cycle arrest at G1 or S phase and thereby suppressed the proliferation of GBM cells by binding *CDK6* and induced the differentiation of GSCs in $CD133^{+}$ human glioblastoma multiforme-derived stem cells (SF6969) and U251 cell lines (Silber et al., 2008). Multiple 'TTAGGG' repeating tandem sequences called telomeres are present at the ends of linear eukaryotic chromosomes. With aging, the length of telomeres will shorten gradually (Whittemore et al., 2019). In tumor cells, telomerase is reactivated to prevent shortening and contribute to tumorigenesis (Chiba et al., 2017). Vinchure et al. showed downregulated expression of miR-490. High levels of miR-490 directly target telomeric repeat-binding factor 2 (*TERF2*) of the shelterin complex, tankyrase 2 (*TNKS2*) and serine/threonine-protein kinase SMG1 nonsense-mediated mRNA decay-associated PI3K-related kinase (*SMG1*) to shorten telomeres and inhibit proliferation via repeat-binding factor 2 (*TERF2*) inhibition in U87MG and T98G glioma cell lines (Vinchure et al., 2021).

Another study showed that members of the miR-181 family, namely, miR-181a, miR-181b and miR-181c, acted as tumor suppressors both in GBM tissues and GBM cell lines (Ciafre et al., 2005; Conti et al., 2009; Corsten et al., 2007). By performing clustering analyses, studies revealed that the expression of many miRNAs, including the miR-376 family, miR-7, miR-95, miR-128, miR-139, miR-411, miR-451, miR-381, miR-379, and miR-873, was downregulated in glioma tissue or cells (Skalsky and Cullen, 2011). Wu et al. found that miR-7 overexpression decreased invasion and migration by targeting focal adhesion kinase (*FAK*) in human GBM tissues and cell lines (U251 and U87) (Wu et al., 2011). Nan et al. reported decreased expression of miR-451, and knockdown of miR-451 inhibited growth, induced G0/G1 phase arrest and promoted cell apoptosis in glioma cell lines (U251 and U87). Overexpression of miR-451 targeted calcium binding protein 39 (*CAB39*) to reduce the epithelial-mesenchymal transition (EMT) and metastasis of glioma cells by suppressing the phosphatidylinositol-4, 5-bisphosphate 3-kinase (*PI3K*)/AKT serine/threonine kinase/snail family transcriptional repressor 1 (*SNAI1*) signaling pathway in cell lines and xenograft models (Nan et al., 2021). MiR-30c has been confirmed to be a tumor suppressor by directly or indirectly targeting various signaling pathways (McCann et al., 2019; Hojbjerg et al., 2019; Wu et al., 2015). Liu et al. reported that miR-30c was expressed at low levels in GBM tissues and U251 and U87 cell lines and that upregulating the expression of miR-30c with mimic oligonucleotides suppressed the proliferation, migration and invasion of glioma cells by binding SRY-box transcription factor 9 (*Sox9*) to downregulate its protein levels (Liu et al., 2019a). Table 2 summarizes the abovementioned downregulated miRNAs that function as tumor suppressors in the development of GBM.

3.2. MiRNA in EVs-based, immune, the hypoxic and reverse pH microenvironment of GBM

GBM shows high inter- and intratumor heterogeneity, which associates with an intense network of interactions among nontumor cells, tumor cells, EVs with their multifunctional cargos (such as miRNAs), immune cells, the hypoxic and acid-base properties (Zhao et al., 2020; Chen and Hambardzumyan, 2018). This complex multilevel communication and interaction contains multiple nontumor cells, such as infiltrating and resident immune cells, vascular cells, and nontumor glial cells, which play important roles in controlling the progression of pathology (Charles et al., 2012; Cole et al., 2020). The major immune cells are innate immune cells, particularly tumor-associated macrophages (TAMs), which account for approximately 30%–40% of immune cells in

Table 2
Downregulated tumor-suppressive miRNAs and their functions in GBM.

miRNA	Targets	Functional Assays	Tumor Grade	Sources	References
miR-519a	*STAT3, Bcl-2*	Cell proliferation, migration, invasion, apoptosis	GBM	Cell line, tissue, xenografts	(Hong et al., 2016; Li et al., 2018)
miR-758-5p	*ZBTB20*	Cell migration, invasion, proliferation	GBM	Cell line, tissue, xenografts	Liu et al. (2018)
miR-125b	*CDK6, CDC25A, MMP2/9*	Cell invasion, cell cycle, apoptosis, stemness, resistance to TMZ	Grade III-IV	GSCs, tissue	(Shi et al., 2010, 2012a, 2012b)
miR-124/137	*CDK6*	Cell cycle, proliferation	Grade III-IV, GSCs	GSCs, tissue	Silber et al. (2008)
miR-490	*TERF2, TNKS2, SMG1*	Cell proliferation, telomere maintenance	GBM	Cell line	Vinchure et al. (2021)
miR-181	*Bcl-2, CCNB1*	Cell proliferation, apoptosis, invasion, angiogenesis, radio-chemosensitivity	Grade III-IV	Cell line, tissue	(Ciafre et al., 2005; Conti et al., 2009)
miR-7	*FAK*	Cell invasion, migration	Grade III-IV	Cell line, tissue	Wu et al. (2011)
miR-451	*CAB39, PI3K/Akt/SNAI1*	Cell proliferation, apoptosis, cell cycle, EMT	GBM and GSCs	Cell lines, xenograft	Nan et al. (2021)
miR-30c	*Sox9*	Cell proliferation, migration, invasion	GBM	Cell line, tissue	(Wu et al., 2015; Liu et al., 2019a)

Abbreviations: STAT3 (signal transducer and activator of transcription 3), Bcl-2 (B-cell lymphoma 2), ZBTB20 (zinc finger and BTB domain containing 20), CDK6 (cyclin-dependent kinase 6), CDC25A (cell division cycle 25A), MMP2/9 (matrix metalloproteinase 2/9), GSCs (glioma stem cells), TERF2 (telomeric repeat-binding factor 2), TNKS2 (tankyrase 2), SMG1 (SMG1 nonsense mediated mRNA decay-associated PI3K-related kinase), CCNB1 (cyclin B1), FAK (focal adhesion kinase), CAB39 (calcium binding protein 39), PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinase), Akt (AKT serine/threonine kinase), SNAI1 (snail family transcriptional repressor 1), Sox9 (SRY-box transcription factor 9).

the GBM (Chen and Hambardzumyan, 2018; Charles et al., 2012). And the tumor cells, including cancer stemlike cells, contribute significantly to GBM initiation and development. Besides the nontumor cells and tumor cells, the distinct pathophysiology of GBM also dependents on the unique surrounding brain microenvironment which includes a network of interactions among EVs/miRNAs, immune cells, the hypoxic and reverse pH properties characterized with hypervascularization and necrosis mainly caused by hypoxia and a reversed pH between the intracellular and extracellular regions, namely, an alkaline intracellular pH and an acidified extracellular environment (Domenech et al., 2021; Macharia et al., 2021). Disordered miRNAs result in dysregulation of hypoxia-inducible factors (*HIFs*), reversed pH and the polarization of TAMs and thus contribute to tumor initiation, angiogenesis, proliferation, and invasion *in vivo* or *in vitro* (Takkar et al., 2021; Zhao et al., 2022). In addition, the disordered hypoxic-hypoglycemic microenvironment may lead to the opposite roles for miRNAs in the same GBM cell lines. For instance, the expression of miR-451 is decreased in central tissue regions and higher in peripheral tissue regions due to the heterogeneous microenvironment of GBM tumors. The decreased miR-451 expression regulates the activity of adenosine 5′-monophosphate (AMP)-activated protein kinase (*AMPK*), the switch between mammalian target of rapamycin (*mTOR*) and Rac family small GTPase 1 (*Rac1*) activation to subsequently suppress tumor cell proliferation via a low level of activity of the *CAB39/AMPK/mTOR* pathway and enhance migration via a high level of *Rac1*/cofilin (*CFL1*) pathway activation, enabling cells to adapt to microenvironmental conditions in the tumor (Zhao et al., 2017).

3.2.1. *The miRNAs in the EVs-based microenvironmental of GBM*

Although many studies have detected the roles of miRNAs in GBM cells, the miRNAs communication via EVs between different cells involved in GBM phenotype has not been adequately studied. Large numbers of miRNAs are detected intracellularly, and many miRNAs have been observed outside of cells, which adds additional layers of complexity and underscores the importance of miRNAs communication between different cells. The ‘secreted’ factors must be important. Many ‘secretomes’ consist of multiple EVs with their multifunctional cargo, such as genomic DNA and various RNAs (mRNA, miRNAs, and long noncoding RNAs), and have emerged as important mediators that transfer bioactive molecules between cells and tissues (Bronisz et al., 2014). Although the individual EV/miRNA interaction may be weak, the combinatory regulatory effects of multiple mRNAs targeted by one miRNA in neighboring or even distant cells result in considerable changes in the tumor EVs-based microenvironment (Godlewski et al., 2015).

Tumor cells have the capacity to both adapt to the environment and change it to benefit their own growth. EVs may be avidly taken up by tumor cells in the stroma, where they directly or indirectly reprogram the translational, transcriptional, and proteomic profiles of the target cells. Some studies focused on the delivery of tumor-derived EVs carrying oncogenic and tumor-suppressive miRNAs functions in recipient cells (Skog et al., 2008). An oncogenic miRNA, miR-21, strongly overexpressed in GBM and in EVs from GBM cell lines (SNB19, U251, SF767, A172, U87, U373, LN229, LN428, LN308, 1123, 326, 83, 30, AC17, AC20, 84, BT70, and CMK3), suggesting that GBM cells actively secrete miR-21-containing EVs (Chan et al., 2005; Gaur et al., 2011; Akers et al., 2013). Furthermore, the cerebrospinal fluid (CSF) levels of miR-21-containing EVs in patients with GBM were, on average, 10-fold higher than the levels of miR-21-containing EVs isolated from non-oncologic patients (Akers et al., 2013). Exosome-mediated transfer of another oncogenic miRNA, miR-124a, is involved in neuron-to-astrocyte signaling via significantly and selectively targeting the excitatory amino acid transporter 2 (*EAAT2*, rodent analog *GLT1*) protein, but not its mRNA, in cultured astrocytes. Consistent with the *in vitro* findings, an intrastriatal injection of specific antisense against miR-124a substantially reduced *GLT1* protein expression without affecting its mRNA levels in adult mice (Morel et al., 2013). Skog et al. found that a subset of 11 miRNAs (let-7a, miR-15b, miR-16, miR-19b, miR-21, miR-26a, miR-27a, miR-92, miR-93, miR-320 and miR-20) are abundant in microvesicles from two different primary glioblastomas (GBM1 and GBM2) (Skog et al., 2008). Other novel oncogenic miRNAs, such as miR-4301, miR-4454 and miR-5096, were detected at high levels in both U251 glioma cells and their microvesicles (MVs) (Li et al., 2013). In addition to oncogenic miRNAs, aberrant expression of tumor suppressors, such as miR-1, orchestrates EV function and GBM growth and invasion partially by altering EV molecular cargo, which is involved in the blocking the growth, neovascularization and invasiveness of intracranial xenografts. Annexin A2 (*ANXA2*), one of the most abundant GBM-derived EV proteins, was found to be the direct target of miR-1. EV-derived miR-1, along with other *ANXA2* EV networking partners, targeted multiple pro-oncogenic signaling pathways in cells and impaired EV-based microenvironmental communication, including a reduction in phospho-AKT serine/threonine kinase (*AKT*) and phospho-c-Jun N-terminal kinase (*JNK*) levels, which were consistently reduced; repression of the glioma stem cell marker CD133, the self-renewal factor and proto-oncogene BMI1 proto-oncogene, polycomb ring finger (*BMI1*) and SUZ12 polycomb repressive complex 2 subunit (*SUZ12*), which increased the levels of the differentiation marker glial fibrillary acidic protein (*GFAP*) in stem-like culture

conditions. However, under neurosphere conditions, pro-MET proto--oncogene (receptor tyrosine kinase), MET and phospho-MET levels were significantly reduced, as along with epidermal growth factor receptor (*EGFR*) levels in U87 cells and X12 cells, suggesting cell type-specific effects and a multimodal mechanism (Bronisz et al., 2014).

3.2.2. The miRNAs in the immune microenvironment of GBM

In GBM, the proangiogenic and inflamed tumor microenvironment results in a highly permeable BBB, leading to immune cells exiting from the blood flow and migrating to the extracellular matrix to form the tumor immune microenvironment. Bioinformatics analysis of 22 immune cell types in the tumor immune microenvironment showed that the proportions of several immune cell types are increased in GBM samples compared to nontumor samples, especially type-2 polarized macrophages (Wang et al., 2018). According to recent research, the majority of TAMs (more than 85%) are infiltrating bone marrow-derived monocytes/macrophages, and a small number of TAMs are resident microglia (less than 15%) (Chen et al., 2017). Because of the immunosuppressive and proangiogenic functions and enormous amounts of TAMs in the tumor immune microenvironment, these cells contribute to the proliferation of glioma cells (Wang et al., 2018). Many miRNAs target some macrophage genes and regulate the polarization of TAMs. On the other hand, M2-like TAMs secrete miRNAs via exosomes to regulate the growth of GBM (Zhao et al., 2022).

Many miRNAs act as tumor suppressors by inhibiting the differentiation of TAMs or activating TAMs in GBM. Liu et al. showed that increasing the expression of miR-340-5p inhibits tumor growth and recurrence by suppressing the differentiation of TAMs into polarized M2 cells by directly targeting periostin (*POSTN*) and latent transforming growth factor beta binding protein 1 (*LTBP-1*) *in vitro* and *in vivo* (Liu et al., 2019b). According to Liu et al., high expression of miR-181b antagonizes the expression of vascular cell adhesion molecule-1 (*VCAM-1*), a cytokine-induced adhesion molecule expressed on the surface of cancer cells that correlates with TAM adhesion to GBM by targeting protein phosphatase 2A (*PP2A*) (Liu et al., 2017). Moreover, accumulating evidence supports the hypothesis that the tumor immune microenvironment of exosome-targeted miRNAs contributes to the occurrence and development of GBM and the phenotypes of glioblastoma stem cells (GSCs). Hong et al. found that an antitumor miRNA, miR-124, was loaded into HEK293T-derived EVs (miR-124 EVs) and secreted into the extracellular matrix, which possessed high antitumor efficiency and inhibited M2 microglial polarization via the *STAT3* signaling pathway in both U373MG GBM cells and microglia with a three-dimensional (3D) microfluidic device (Hong et al., 2021). Zhao et al. found that exosomal miR-27b-3p from M2-like TAMs were transferred into GSCs and maintained the stem-like properties of GSCs by activating the lysine methyltransferase 2B (*MLL4*)/PR/SET domain 1 (*PRDM1*)/interleukin 33 (*IL-33*) axis in GBM (Zhao et al., 2022). Qian et al. found that hypoxic glioma-derived exosomes (H-GDEs) induced the secretion of miR-1246, which induced the proliferation, migration and invasion of GBM cells by targeting telomeric repeat binding factor 2, interacting protein (*TERF2IP*) to facilitate M2 macrophage polarization via exosomes *in vitro* and *in vivo* (Qian et al., 2020). Natural killer (NK) cells, which are mainly innate immune cells, are highly heterogeneous in the tumor immune microenvironment. Briand et al. showed that exosomal miR-378a-3p inhibits the cytotoxicity of NK cells by downregulating the expression of granzyme-B (*GZMB*) in GBM (Briand et al., 2020).

3.2.3. The miRNAs in the hypoxic and reverse the pH microenvironment of GBM

A subset of GBM cells, known as GSCs, possess the ability to form tumors. These GSCs settle in various locations and form a unique niche. Uribe et al. showed that hypoxia and a reversed pH form part of the GSG niche (Uribe et al., 2017). Patients with GBM have a lower median partial pressure of oxygen (6–9 mm Hg) and an acidic pH ($\leq$6.8) compared to healthy people (Boyd et al., 2021). The hypoxic cells generate energy and essential precursors (nucleic acids, amino acids, and lipids) via anaerobic glycolysis. Accordingly, anaerobic glycolysis generates large amounts of acidic metabolites, such as lactic acid, and further lowers the extracellular pH (Garcia-Bermudez et al., 2018). Therefore, hypoxia and a reversed pH microenvironments of GBM are two markers that simultaneously contribute to various pathways of tumorigenicity, such as therapeutic resistance, patient survival, and tumor invasion (Evans et al., 2010).

As shown in a study by Hu et al., hypoxia induces the transformation of pre-miR-215 into mature miR-215, which reprograms glioma-initiating cells (GICs) by targeting lysine-specific histone demethylase 1B (*KDM1B*) in GBM xenografts (Hu et al., 2016). Agrawal et al. found that miR-210 overexpression promotes the growth of GBM cells by targeting tumor-inhibiting factor neuronal differentiation 2 (*NEUROD2*), a neuronal basic helix-loop-helix transcription factor, in the hypoxic microenvironment of GBM samples and anaplastic astrocytoma (Agrawal et al., 2018). Under the conditions of hypoxia and a low pH, *HIF-1α* induces the migration and infiltration of GBM cells; however, therapies targeting *HIF-1α* have not achieved satisfactory results against GBM. Wang et al. reported that the combination of *HIF1α/HIF2α*-targeted therapies with TMZ effectively inhibits the malignant progression of GBM. Under hypoxic conditions, miR-210-3p regulates GBM cell proliferation, dedifferentiation and chemoresistance by regulating the expression of epidermal growth factor (*EGF*) via *HIF-1α/HIF-2α* (Wang et al., 2020). The hypoxic GBM microenvironment effectively suppresses the curative effect of TMZ; however, the underlying mechanisms are poorly understood. Ge et al. showed that *HIF-1α* induces the protective response of mitochondria by upregulating the expression of miR-26a, an oncogene in the hypoxic microenvironment. Further research indicated that high expression of miR-26a negatively correlates with the expression of Bcl-2 associated X, apoptosis regulator (*Bax*) and Bcl-2 associated agonist of cell death (*Bad*) (Ge et al., 2018). A recent study by Li et al. showed that hypoxia-induced miR-137 methylation prevented miR-137 from binding target genes, resulting in chemoresistance to TMZ by directly targeting low-density lipoprotein receptor-related protein 6 (*LRP6*) *in vitro* and *in vivo* (Li et al., 2020). In addition, autophagy facilitates the survival of GBM cells in a hypoxic microenvironment. Based on the results reported by Guo et al., miR-224-3p inhibits autophagy by suppressing the expression of two autophagy-related genes (ATGs). Further research showed that hypoxia inhibits the expression of miR-224-3p and eliminates the inhibitory effect of miR224-3p on autophagy (Guo et al., 2015). Table 3 summarizes the abovementioned miRNAs that function in the EVs-based, immune and hypoxic and reverse the pH microenvironment during the development of GBM.

4. Application of miRNAs for the treatment of GBM

Various miRNAs play roles in the pathogenesis and development of GBM, and many miRNAs have been confirmed to be associated with the inhibition of GBM (Costa et al., 2015b). At present, due to various uncertain factors (such as drug safety and efficacy), therapeutic measures to target specific miRNAs remain to be explored in glioma cell and xenograft models (Yan et al., 2020). Many studies still need to be conducted prior to their application in the clinic.

4.1. Clinical prognostic indicators of miRNAs in GBM

The expression of miRNAs is usually unregulated during GBM initiation and development, suggesting that miRNAs may be used as important genetic markers of the biological activities of GBM cells, such as proliferation, migration, invasion, conventional radiotherapy and chemotherapy resistance (Acunzo et al., 2015), as well as biomarkers of GBM prognosis. Tian and his colleagues found that the abnormal expression of miR-9 in glioma correlated with the grade of glioma in seventy-one glioma samples. Namely, the higher the grade is, the higher the expression of miR-9, indicating that miR-9 may be deemed a

Table 3
miRNAs and their functions in the EVs-based, immune and hypoxic and reverse the pH microenvironment of GBM.

miRNA	Expression	Targets	Functions	Source	Grade	Reference
The miRNAs in the EVs-based microenvironmental of GBM						
miR-21	Upregulation	*CASP3, PDCD4*	Apoptosis, cell proliferation and invasion	Cell line, tissue and xenograft	Grade II-IV	(Chan et al., 2005; Gaur et al., 2011; Akers et al., 2013)
miR-124a	Upregulation	*EAAT2/GLT1*	Neuron-to-astrocyte communication signaling	Cell line, mouse model	GBM	Morel et al. (2013)
miR-1	Downregulation	*ANXA2*	Tumorigenicity, growth, angiogenesis and invasion	Cell line, tissue, mouse model	Grade III-IV, GSCs	Bronisz et al. (2014)
The miRNAs in the immune microenvironment of GBM						
miR-340-5p	Downregulation	*POSTN, LTBP-1*	Tumor growth and recurrence, TAM recruitment and M2-TAMs polarization	Cell line, tissue and xenograft	GBM	Liu et al. (2019b)
miR-181b	Downregulation	*PP2A*	Tumor growth and invasion, monocyte adhesion	Cell line, tissue	Grade I-III	Liu et al. (2017)
miR-124	Downregulation	*STAT3*	Cell proliferation, metastasis, chemosensitivity, EMT, M2 microglial polarization	Cell line	GBM	Hong et al. (2021)
miR-27b-3p	Upregulation	*MLL4*	M2-like tumor-associated macrophages transmit exosomal, stem-like properties of GSCs	Cell line, tissue and xenograft	GBM, GSCs	Zhao et al. (2022)
miR-1246	Upregulation	*TERF2IP*	Cell proliferation, migration and invasion, M2 macrophage polarization	Cell line, tissue and xenograft	LGG, GBM	Qian et al. (2020)
miR-378a-3p	Upregulation	*GZMB*	Cytotoxic activity of natural killer (NK)	Cell line, tissue, mouse model	GBM	Briand et al. (2020)
The miRNAs in the hypoxic and reverse the pH microenvironment of GBM						
miR-215	Upregulation	*KDM1B*	Reprogram GICs, Tumorigenic Abilities of GICs under hypoxia	GICs, tissue and xenograft	GBM, GICs	Hu et al. (2016)
miR-210-3p	Upregulation	*NEUROD2, HIF-1α/HIF-2α*	Cell proliferation and migration, apoptosis, dedifferentiation, chemoresistance	Cell line, tissue, mouse model	Grade II-IV	(Agrawal et al., 2018; Wang et al., 2020)
miR-26a	Upregulation	*Bax, Bad*	Temozolomide (TMZ) resistance, apoptosis	Cell line, tissue, mouse model	GBM	Ge et al. (2018)
miR-137	Downregulation	*LRP6*	Cell invasion, EMT, chemosensitivity to temozolomide (TMZ)	Cell line, tissue and xenograft	Grade II-IV	Li et al. (2020)
miR-224-3P	Downregulation	ATG5, FIP200	Cell proliferation, hypoxia-induced apoptosis	Cell line, tissue, mouse model	Grade I-IV	Guo et al. (2015)

Abbreviations: CASP3 (caspase 3), PDCD4 (programmed cell death 4), EAAT2/GLT1 (excitatory amino acid transporter 2, rodent analog GLT1), ANXA2 (annexin A2), GSCs (glioblastoma stem cells), POSTN (periostin), LTBP-1 (latent transforming growth factor beta binding protein 1), PP2A (protein phosphatase 2 phosphatase activator), STAT3 (signal transducer and activator of transcription 3), EMT (epithelial-mesenchymal transition), MLL4 (mixed linked leukemia 4), TERF2IP (telomeric repeat binding factor 2 interacting protein), LGG (low-grade glioma), GZNB (granzyme B), KDM1B (lysine demethylase 1B), GICs (glioma-initiating cells), NEUROD2 (neuronal differentiation 2), HIF-1α/HIF-2α (hypoxia inducible factor 1 subunit alpha/hypoxia inducible factor 2 subunit alpha), Bax (Bcl-2 associated X, apoptosis regulator), Bad (Bcl-2 associated agonist of cell death), LRP6 (LDL receptor related protein 6), ATG5 (autophagy related 5), FIP200 (FAK family-interacting protein of 200 kDa).

biomarker for the prognosis of gliomas (Tian et al., 2020). As shown in studies by Luo et al., the expression of miR-524-5p, miR-586, miR-433, miR-619, miR-548d-5p, miR-525-5p, miR-301a, miR-10b-5p, miR-210, miR-15b-5p and miR-182 increased significantly and the expression of miR-124, miR-128, miR-146b and miR-218 was downregulated in patients with glioma, and these changes were associated with a poor prognosis (Luo et al., 2020). Hu et al. conducted a meta-analysis and found that increased miR-210 expression contributed to shorter overall survival (OS) of patients with gliomas (Hu et al., 2020). Lai et al. also observed significantly higher expression of miR-210 in advanced pathological grade glioma tissues than in normal brain tissues, leading to shorter progression-free survival (PFS) and OS. These findings suggest that miR-210 expression might be used as a predictor of shorter survival in patients with glioma (Lai et al., 2014).

Chen et al. compared 122 patients with glioma and 60 healthy individuals, and showed that the expression of miR-720 was significantly upregulated in plasma samples from the glioma group compared to samples from healthy individuals. A higher tumor grade tended to be associated with higher plasma miR-720 expression in the multivariate Cox analysis. The expression of miR-720 in the plasma was associated with the tumor grade, suggesting that high expression of miR-720 may be used as a molecular predictor of a poor prognosis for patients with GBM (Chen et al., 2020). Jia et al. showed that miR-195 acted as a tumor-inhibiting factor in GBM, and increasing the expression of miR-195 significantly increased the median survival time of patients with gliomas by inhibiting the cell cycle and regulating apoptosis-related proteins from 186 patients with glioma. The expression of miR-195 was inversely correlated with pathological grades, and the low-level expression of miR-195 was related to a poor prognosis in patients with glioma (Jia et al., 2020). Taken together, these results indicate that these miRNAs may be used as noninvasive biomarkers and predictors of the prognosis for GBM.

4.2. *Specific miRNAs for the targeted treatment of GBM*

The use of miRNAs as therapeutic interventions is based on the idea that the expression of miRNAs is dysregulated in GBM and that the malignant phenotype can be restored by targeting miRNAs. Both tumor-suppressive miRNAs and oncomirs may be candidates for therapeutic purposes. Given the downregulation of tumor-suppressive miRNAs and the upregulation of oncomirs in GBM, two major approaches could be adopted to develop miRNAs as anti-GBM agents. The most common strategy is to ablate the function of miRNAs by administering single-stranded oligonucleotides with miRNA complementary sequences (gene-silencing therapy); in contrast, restoring the expression of silenced miRNAs with miRNA mimics represents a promising therapeutic strategy (replacement therapy). Both of these approaches may facilitate new discoveries for GBM therapeutics (Ding et al., 2019). With an abundance of in-depth research on the specific miRNAs expressed in GBM, an increasing number of studies have shown that some of the dysregulated miRNAs may have possible implications for aiding in therapeutic decisions for GBM treatment.

A study analyzing 240 tumor and 10 normal samples revealed that the expression of 22 miRNAs affected the development of GBM. Ten of these miRNAs were upregulated, and 12 of these miRNAs were downregulated in tumor tissues, which provided important clues that these miRNAs may play potential roles in targeted treatment of the carcinogenic process in GBM (Dong et al., 2010; Rolle, 2015). Zhi et al. showed

that the expression of miR-520d-5p was significantly downregulated in glioma by directly targeting pituitary tumor transforming gene 1 (*PTTG1*), and activating the expression of miR-520d-5p inhibited the proliferation of glioma cells in tumor samples from a xenograft model (Zhi et al., 2017). Another study showed that miR-520d-5p was significantly overexpressed in low-grade (GII) tumor tissue samples compared with high-grade (GIII and GIV) tissue samples by binding the 3′-UTR of signal regulatory protein alpha (*SIRP alpha*) (Deshpande et al., 2017). By analyzing carcinoma and adjacent tissue in patients with gliomas or GBM cell lines, Dong and his colleagues found that miR-429 was expressed at low levels in glioma tissue compared with noncancerous tissues or GBM cell lines. Increasing the expression of miR-429 downregulated the expression of *SOX2*, thereby inhibiting proliferation, inducing apoptosis, and suppressing the invasion of glioma cells (Dong et al., 2017). In addition, studies of glioma tissues compared with adjacent normal tissues have shown that increased expression of miR-181 inhibits glioma cell proliferation and invasion and induces apoptosis in U251 and SHG-44 cells by targeting the expression of a positive cell cycle regulator, cyclin B1 (*CCNB1*) (Wang et al., 2014). These studies indicated that miR-520d-5p, miR-429 and miR-181 are potential tumor-suppressive miRNAs. Similar studies have shown that miR-194-5p, miR-518b and miR-543 also play roles in the tumor-suppressive effects on GBM proliferation, invasion, and apoptosis (Yan et al., 2020). Therefore, these miRNAs might be potential candidates for the prevention of GBM.

Moreover, miRNAs target or regulate specific genes in a cooperative manner (Ehses et al., 2021). Kosti et al. indicated that the use of individual miRNAs as drug targets for the treatment of gliomas is disadvantageous and proposed a combination therapy that combines 3–4 miRNAs to cooperatively regulate signaling pathways or cancer cells. In GSCs, miR-124, miR-128, and miR-137 synergistically repress the expression of oncogenic factors, influence cancer-related pathways and inhibit the differentiation of GSCs much more effectively than a single miRNA by disrupting cell proliferation and survival, which promotes the differentiation and enhances the response to radiation, revealing the value of combinations of miRNAs to target cancer-initiating cell populations (Kosti et al., 2021). On the other hand, some ongoing clinical trials are incorporating miRNAs used as targets for GBM treatment. Shatsberg et al. showed that the administration of NG-miR-34a nano--polyplexes to human U87 MG GBM-bearing SCID mice significantly decreases tumor growth compared to treatment with NG-negative control miR polyplex or saline (Shatsberg et al., 2016). Anti-let-7a or saline was administered by convection-enhanced delivery (CED) into an orthotopic T87-derived tumor xenograft model, and the expression of its target gene high-mobility group AT-hook 2 (*HMGA2*) was significantly derepressed in the anti-miR-treated animals (Halle et al., 2016).

4.3. Adjuvant therapy with miRNAs in combination with conventional chemo- and radiotherapy for GBM treatment

TMZ is a chemotherapy drug that has shown good efficacy and safety for the treatment of gliomas. Clinicians estimate the TMZ response in patients with gliomas by detecting the level of O6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation (Lee, 2017; Nagane et al., 2007). Chen et al. reported that miR-130a can replace *MGMT* to predict the response to TMZ in patients with gliomas using quantitative real-time polymerase chain reaction (qRT–PCR) (Chen et al., 2015a). However, resistance to TMZ in gliomas is chronic and complicates clinical therapy. In related studies, Areeb et al. detected upregulated expression of miR-221 in TMZ- and radiotherapy-resistant GBM cell lines (U251MG, U87MG and U118MG). The upregulation of miR-221 led to a significant reduction in *EGFR* expression in both cell lines and tumor tissues from patients with GBM, and inhibiting miR-221 expression increased the sensitivity of tumors to chemotherapy and radiotherapy (Areeb et al., 2020). Zhang et al. observed significantly lower expression of miR-625 in human glioma samples and cell lines than in normal brain tissue and human astrocytes, and increased miR-625 expression not only inhibited GBM cell proliferation in a tumor xenograft model but also induced cell cycle arrest and apoptosis by directly regulating the expression of protein kinase 2 (*AKT2*), as well as increasing the sensitivity of tumors to the chemotherapy drug TMZ (Zhang et al., 2017). Sharif et al. revealed that miR-124 exogenously transferred by Wharton's jelly mesenchymal stem cells (WJ-MSCs) via dependent or exosome-independent processes into U87 cells successfully decreased the expression of *CDK6* to enhance the sensitivity of glioma cells to TMZ and decrease the migration of GBM cells (Sharif et al., 2018).

Studies of GSCs have suggested that the presence of cancer stem cells (CSCs) induces resistance to conventional radiotherapy and chemotherapy (Yin et al., 2014). Asuthkar et al. showed that downregulation of the expression of miR-211, a miRNA targeting *MMP-9*, contributed to the insensitivity of gliomas to conventional radiotherapy and chemotherapy, and the combination of conventional radiotherapy and chemotherapy with a miR-211 mimic effectively induced apoptosis by activating the intrinsic mitochondrial *CASP9/3* cascade reaction in both glioma cells and CSCs, indicating a new therapeutic application for patients with GBM in the future (Asuthkar et al., 2012). In GBM tissues and the corresponding stem cells (GBMSCs), miR-125b-2 is overexpressed, and inhibiting the expression of miR-125b-2 in GBMSCs might cause TMZ to induce apoptosis by inducing the release of cytochrome *c* from mitochondria, inducing apoptotic peptidase activating factor 1 (*APAF-1*) and activating the *APAF1/CASP3/*poly-ADP-ribose polymerase (*PARP*) signaling pathway to resist GBMSC resistance to TMZ (Shi et al., 2012b). According to Cheng et al., overexpressed miR-132 specifically targets and inhibits the expression of tumor suppressor candidate gene 3 (*TUSC3*) and induces the proliferation and enhances the tolerance of gliomas to TMZ and the formation of CSC phenotypes in U87MG cells (Cheng et al., 2017). In addition, miR-34a expression is associated with the malignancy grade of glioma and prognosis of patients with grade IV GBM. Overexpression of miR-34a with exogenous transfection of a miR-34a mimic effectively suppressed the proliferation of cancer cells and induced cell apoptosis by specifically targeting *Bcl-2* in a glioma cell line (U87) (Gao et al., 2013; Duan et al., 2016).

4.4. Auxiliary option of using miRNAs with other drugs for GBM treatment

Peptide nucleic acids (PNAs), which are DNA analogs, are proposed as potential small-molecule drugs for inhibiting the proliferation of glioma cells by targeting specific oncomirs, such as miR-221 (Nielsen et al., 1991; Egholm et al., 1993). Recent studies revealed that a novel series of tubulin polymerization inhibitors possessed the potency of small-molecule drugs to treat glioma. Zurlo et al. showed that an anti-tubulin tetrahydrothieno (2,3-c) pyridine derivative in combination with PNA specifically targeted miR-221 to inhibit antiapoptotic activity in the glioma cell lines U87 and U251 (Zurlo et al., 2021). Research on curcumin, another potential therapeutic drug, showed that upregulating the expression of miR-378 reinforced the effect of curcumin on suppressing the proliferation of U87 cells mainly by altering p38 gene expression *in vitro* and *in vivo* (Li et al., 2015, 2017). Jana et al. explored a novel role for tachyplesin (Tpl), a cell-penetrating antimicrobial peptide that plays an adjuvant role in facilitating the delivery of drugs into glioma cells to inhibit the expression of miR-210, proliferation, migration and spheroid formation ability by upregulating the expression of its targeted tumor suppressor genes *NEUROD2* and *HIF-3α* and inducing apoptosis by increasing the levels of caspase 3/7 (*CASP3/7*) and ROS (Jana et al., 2019).

Tumor necrosis factor-related apoptosis-inductive ligand (*TRAIL*) is a novel drug target for cancer therapy that has entered clinical trials (phases II and III) (Davies, 2014). Wang et al. recently showed that high expression of miR-133a in patients with glioma decreases the sensitivity of glioma to *TRAIL* by inhibiting the activation of death receptor 5 (*DR5*)

and the *NF-κB* signaling pathway *in vitro* and *in vivo*. Reducing the expression of miR-133a in glioma cells significantly enhances the therapeutic effects of *TRAIL* resistance in GBM (Wang et al., 2017). Moreover, the BBB prevents various drugs from successfully entering the brain during GBM clinical treatment. Erythropoietin (EPO), a drug used to treat chemotherapy-induced anemia, has been verified to pass the BBB and accelerate the invasion of glioma cells (Vazquez-Mellado et al., 2017). A new study by the Alural group showed that miR-451 overexpression induced by transfection of its mimic specifically targets and regulates the expression of *MMP2/9* (two genes contributing to the invasion of glioma cells) to significantly reverse the therapeutic effects of EPO, such as increased cell proliferation, migration, invasion, and cisplatin chemoresistance *in vitro* (Alural et al., 2017). These findings suggest that these miRNAs might be useful as adjuvant therapies in addition to chemotherapy and other drugs.

5. Delivery systems associated with the use of miRNAs for the treatment of GBM

As mentioned above, many miRNAs clearly play important roles in GBM pathogenesis, suggesting that some of these miRNAs may become the focus of drug development studies and clinical treatment. However, the development of miRNA-targeted clinical treatment faces several obstacles. One of the greatest challenges is the discovery of highly and ideally safe delivery systems with organ specificity and minimal toxicity and off-target effects for human clinical applications. In particular, GBM presents an even greater challenge due to the BBB, a natural barrier that protects the brain from potentially harmful macromolecules in the blood, which is formed by a tightly packed layer of endothelial cells surrounding brain vessels (Shergalis et al., 2018). Notably, miRNA-based drugs must enter the cell membrane and eventually enter the cytoplasm. Thus, the ideal delivery of miRNA-based vectors to brain tumors must overcome the many biological hurdles associated with the application of oligonucleotides, such as intravascular degradation, low serum stability and innate immune responses, reticuloendothelial system trapping, renal clearance, failure to cross the capillary endothelium and tissue penetrance, ineffective endocytosis by target cells (off-targeting), or ineffective endosome release (Mozhei et al., 2020). As shown in Fig. 2, to date, some delivery systems associated with the use of miRNAs for the treatment of GBM have shown improved bioavailability, including some natural nanomedicines, viral carrier delivery systems and nonviral carrier delivery systems.

5.1. Natural nanomedicine delivery systems for miRNA-based GBM treatment

Many cancer cells and normal cells exchange information through chemical and electronic signals. Recent investigations have revealed that many functional miRNAs packaged into EVs from tumor cells and secreted into the extracellular environment as exosomes exchange information between various cells, such as tumor cells and mesenchymal stem cells, and are involved in tumor initiation and development in different stages (Saadatpour et al., 2016). Exosomes containing miRNAs and their cargos that are released into the circulatory system are stable and enable the activation or deactivation of many recipient cells or molecules, since miRNAs from tumor cells regulate the expression of proteins in the surrounding structure and thus elicit aberrant molecular signals. Briefly, the exosomes secreted by GBM cells can be taken up by normal cells. The molecules contained in exosomes induce tubule formation by endothelial cells to promote proliferation, invasion and migration (Katakowski et al., 2010). Thus, blocking and interfering with the secretion of miRNAs from cells may be a potentially novel therapeutic strategy for GBM. The investigation of the biological functions of exosomes provides a new opportunity for miRNA delivery systems for future GBM clinical therapeutics.

Some studies have described the possibilities of using exosomes and their cargos as miRNA delivery systems in GBM therapeutics. Some specific tumor suppressor miRNAs (ts-miRNAs) have been overexpressed in mesenchymal stem cells (MSCs) or GBM cells *ex vivo*; these modified cells produce many exosomes containing large amounts of the specific ts-miRNA, which are shuttled into the vicinity of the tumor via intercellular communication, leading to the inhibition of tumor growth (Fareh et al., 2017). Together with RNA-binding proteins (such as AGO2, the major functional element of the miRNA-RISC complex), this approach has favorable properties for miRNA delivery and stabilizes many miRNAs in exosomes; hence, this strategy is useful as an miRNA

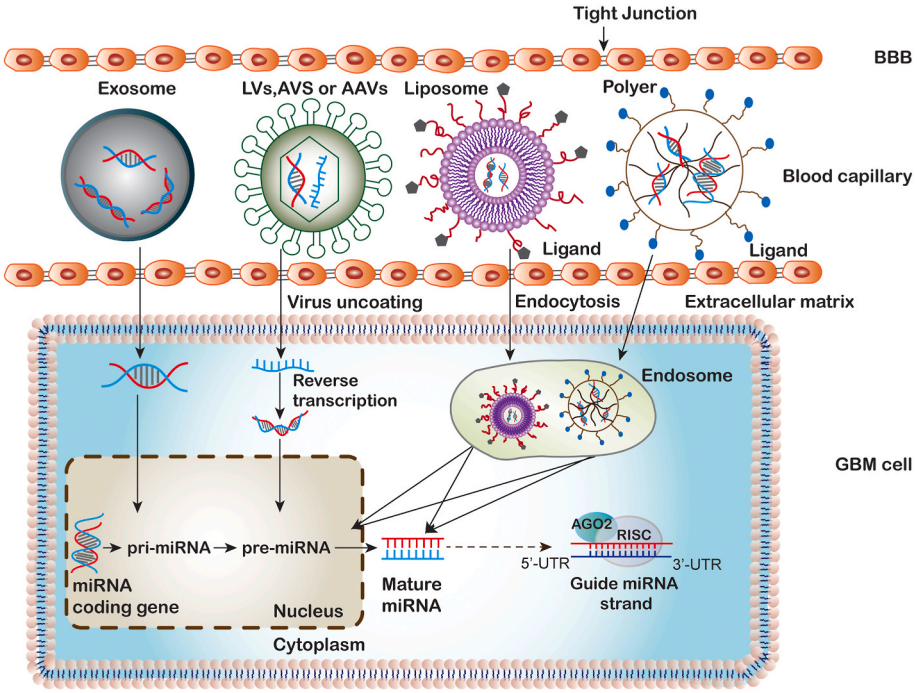

Fig. 2. MiRNA delivery strategies for GBM therapy *in vivo*. Treatment with viral vectors or organic nonviral delivery systems bypasses the BBB and modulates miRNA activity via modified miRNA antagonists or mimics in GBM therapy. The natural delivery system (natural nanomedicine miRNA delivery system) and the virus-based delivery system contain miRNA antagonists or mimics, transporting these nucleic acids into the nucleus. Other nonviral-based delivery systems, including liposome-based nanoparticles and polymer nanospheres loaded with nucleic acids, enter GBM cells and release therapeutics via endocytosis or transcytosis.

delivery tool in many cells through biotechnology and protein engineering (Li et al., 2012). Cerebral arteriovenous malformation (AVM) endothelial cells are favorable for the AGO-miRNA delivery system because they are known to secrete AGO2 compared to other delivery systems whose guide strand must be loaded into endogenous AGO2 to be functional. Ferreira et al. reported that AGO2 increased the miRNA uptake of human endothelial cells (ECs) derived from AVM via normal and glioma endothelium without the need for other transfection agents, which protected miRNAs from degradation after cellular entry both *in vitro* and *in vivo*. The intravenous injection of AGO2-miR-18a complexes inhibits angiogenesis in an intracranial glioma mouse model (Ferreira et al., 2014). Prud'homme et al. further explained this result. They found that neuropilin-1 (*NRP1*), which is highly expressed in vascular endothelial cells and some cancer cells, acts as a receptor involved in the translocation of miRNAs and takes up complexes of AGO2-miRNA, making them an ideal target for the AGO-miRNA delivery system (Prud'homme et al., 2016; Kang et al., 2016). Based on these results, natural nanomedicine miRNA delivery systems, such as the AGO-miRNA delivery system, are high-efficiency systems. The development of a novel delivery system for miRNA-based GBM treatment shows potential in the future. However, the endocytosis and endosomal release pathways for AGO2-miRNA complexes also require further study.

5.2. Viral delivery systems for miRNA-based GBM treatment

Expression cassette-mediated miRNA antagonists or miRNA mimics containing miRNA or its complement have been cloned into viral-based vectors, such as lentiviruses (Kasar et al., 2012), adenoviruses (Idogawa et al., 2009) and adeno-associated viruses (AAVs) (Xu et al., 2021). These viral-based carriers have been used to deliver these vectors into cell nuclei, efficiently express miRNAs and confer protection from nucleases, increasing the half-life of miRNAs in the blood. Viral vectors usually contain strong promoters to ensure the supraphysiological expression of target genes (Chen and Kang, 2015). Moreover, genetically modified viral capsid proteins can be added to the targeting moieties to increase the affinity between viral vectors and cancer-specific cells and aid in the specific delivery into tumors.

Many AVVs packed with protein-coding genes have been used for to treat multiple cancers, including GBM. Crommentuijn et al. showed that intracranial AAV-soluble tumor necrosis factor-related apoptosis-inducing ligand (*sTRAIL*) combined with the TRAIL-sensitizing cardiac glycoside lanatoside C (*lan C*) induced cell death in U87 cells and patient-derived GBM neural spheres and prolonged survival in an orthotopic xenograft mouse model (Crommentuijn et al., 2016). Przystal et al. designed an intravenous bacteriophage (phage) vector, a hybrid AAV phage (AAVP), to deliver a recombinant AAV (rAAV) genome by the capsid of M13 phage for dual targeting GBM therapy displayed to suppress human GBM growth in mice (Przystal et al., 2019). Zhong et al. showed that intracranial delivery of the secreted heat shock protein 70 (*HSP70*)-targeted peptide APOPTIN derived from apoptin to GBM tumors with an AAV vector inhibited glioma development and prolonged mouse survival (Zhong et al., 2017). Maguire et al. found that the transduction of normal cells with an AAV vector encoding interferon-β (*IFN-β*) completely prevented tumor growth in orthotopic xenograft models of GBM, even in the contralateral hemisphere (Maguire et al., 2008). Volak et al. used IV delivery of exosome-associated AAV vectors with green fluorescent protein expressed from specific promoters (NF-κB-responsive promoter and a truncated glial fibrillary acidic protein promoter) to express the toxic antitumor cytokine *IFN-β* in the tumor stroma of a GBM mouse model. This technique significantly prolonged the survival of mice with GBM (Volak et al., 2018). In contrast to gene studies, few studies have explored AVV vector-based miRNA treatment in GBM. Bhere et al. showed that the administration of AAV-miR-7 significantly decreased the volume of GBM tumors, upregulated the expression of *DR5* and activated stem cell (SC)-released *sTRAIL* to eradicate tumors generated from patient-derived TRAIL-resistant GBM stem cells (GSCs) in severe combined immunodeficient (SCID) mice, resulting in significantly improved survival (Bhere et al., 2018). Thus, the use of AAV vectors for miRNA delivery may be a very promising and attractive option for GBM therapy.

5.3. Nonviral carrier delivery systems for miRNA-based GBM treatment

Nonviral vectors may address some issues associated with viral vectors, such as immunogenicity and toxicity. Although nonviral delivery systems usually have a lower transfection efficacy and shorter duration of target gene expression, significant progress in their rational design and modification has led to steady improvements, allowing them to be introduced to miRNA delivery systems and achieving clinically relevant efficiency with encouraging results (Labatut and Mattheolabakis, 2018). Specifically, with the overall progress in nanotechnology for drug delivery, much of the existing knowledge can be applied to miRNA delivery research. NP delivery systems can protect miRNAs from endosomal and/or lysosomal degradation and deliver miRNAs into the target cell cytoplasm or nucleus without causing a strong immune reaction or increasing toxicity (Jena et al., 2020). The key advantage of the NP delivery system is the ability to achieve both controlled drug release and tissue specificity, illustrating that they can be used both in diagnosis and treatment for not only GBM but also other types of diseases (Gutkin et al., 2016). The most widely used NPs for therapeutic or diagnostic modalities are prepared from a variety of materials, including inorganic NPs, polymeric NPs, liposome NPs, polyethyleneimine (PEI)-based NPs, dendrimers, and magnetic NPs.

5.3.1. Inorganic NP delivery systems for miRNA-based GBM treatment

Inorganic NPs contain inorganic molecules such as silica-, iron oxide-, gold-, ceramic-, silver-, and metal sulfide-based NPs. This class of NPs has been used for drug delivery due to their potential biocompatibility, nonimmunogenicity, nontoxicity, and ease of large-scale manufacturing (Pourgholi et al., 2016). Among these different types of inorganic NPs, gold nanoparticles (AuNPs) have been used to effectively deliver carriers to GBM cells, since AuNPs are extremely small in size (a mean diameter less than 15 nm), allowing their uptake through the leaky BBB and brain-tumor barrier (BTB).

Sukumar et al. explored an intranasal (nose-to-brain) approach to directly bypass the BBB for the targeted delivery theranostic polyfunctional gold-iron oxide nanoparticles (polyGIONs) loaded with therapeutic miRNAs (miR-100 and anti-miR-21) in mice and significantly increased the survival of mice (Sukumar et al., 2019). Kouri et al. synthesized miR-182-based spherical nucleic acids (182-SNAs) and gold NPs covalently functionalized with mature miR-182 duplexes as a novel siRNA-based nanotechnological technology for gene silencing biotherapeutics that crosses the BBB/BTB following systemic intravenous administration. The results showed that harnessing the antitumor activities of miR-182 via safe and robust delivery of 182-SNAs reduced the tumor burden and prolonged animal survival (Kouri et al., 2015). Grafals-Ruiz et al. developed gold liposome NPs with oligonucleotide miRNA inhibitors (OMIs) to form spherical nucleic acids (SNAs) and then conjugated them with the brain-targeted peptide apolipoprotein E (ApoE) or rabies virus glycoprotein (RVG) to obtain SNA-liposome-ApoE and SNA-liposome-RVG, respectively, and inhibit the expression of oncogenic miR-92b in U87 cells, which increased their systemic delivery to the brain tumors of GBM syngeneic mice to accumulate at higher levels in brain tumor tissues (Grafals-Ruiz et al., 2020). Another inorganic miRNA delivery system is a silica-based vehicle, which also displays the advantages of biocompatibility, a highly porous structure and ease of application. Silica nanoparticles modified with a disialoganglioside GD2 (*GD2*) antibody specifically deliver miR-34a into neuroblastoma tumors in a murine orthotopic xenograft model, leading to an increase in apoptosis, a reduction in the vascular density of tumors and the inhibition of tumor growth in tumor-bearing mice (Chen et al., 2015b; Tivnan et al., 2012).

5.3.2. Polymeric-based NP (PNP) delivery systems for miRNA-based GBM treatment

Polymeric-based NPs (PNPs) are solid particles derived from natural materials (gelatin, alginate, collagen and chitosan) or synthetic polymers (polyesters, polylactide-coglycolide, and polyethyleneimine). Many studies have documented the ability of PNPs to entrap active ingredients, improve aqueous solubility and deliver miRNAs to the desired tissue *in vivo* (Jativa and Cena, 2017). Lactic-coglycolic acid (PLGA) polymers and polyethyleneimine (PEI) polymers, which are biodegradable, biocompatible, and nonimmunogenic, are commonly used for miRNA delivery. PLGA, a copolymer of lactic acid and glycolic acid linked by an ester bond, is a Food and Drug Administration (FDA)-approved copolyester that is widely used for encapsulating and delivering many different therapeutic agents, such as conventional drugs and biological macromolecules *in vivo*, as well as miRNAs (Castaneda-Gill et al., 2017). However, PLGA does not form complexes with nucleic acid-based drugs via charge–charge interactions alone. Hence, PLGA-NPs have been further modified with PEG, peptides, antibodies, or other positively charged polymers to enable electrostatic loading of negatively charged nucleic acids. PLGA-NPs efficiently escape to the cytosol through the endolysosomal compartment and selectively reverse the surface charge after cellular internalization in the increasingly acidic environment of endosomes, changing the anionic characteristics to cationic properties.

PEI, another widely used synthetically derived cationic polymer material, contains an ethylenediamine monomer unit consisting of multiple positively charged amines and forms a linear or branched structure with various molecular weights. The positive charge of PEI is very suitable for condensing negatively charged miRNAs to form polyplexes through electrostatic interactions within the cytoplasm *in vitro* and *in vivo*. Following the endocytosis of polyplexes into the cell, PEI enters the 'proton sponge effect' and subsequently leads to rupture of the endosomal membrane and release of miRNA-encapsulated NPs into the cytosol to silence the target gene (Ganju et al., 2017). However, its high cytotoxicity restricts its clinical application. The crosslinking of low-molecular-weight PEI through a disulfide linkage has been modified to decrease cytotoxicity (Hwang et al., 2011). Some studies have successfully utilized PLGA/PEI-based NPs for miRNA delivery in human GBM cell mouse models (Tivnan et al., 2017; Malhotra et al., 2018; Ananta et al., 2015; Yang et al., 2012).

5.3.3. Lipid-based NP (LNP) delivery systems for miRNA-based GBM treatment

Lipid-based NPs (LNPs) are defined as bilayered vesicles composed of phospholipid membranes, similar to biological membranes, and possess an aqueous core, which allows them to easily carry water-soluble active compounds, such as miRNAs. LNPs have different forms, such as liposomes, solid LNPs, and nanoemulsions. The most commonly used is liposomes. Given their resemblance to the cell membrane bilayer, liposomes are biocompatible and biodegradable. They have the tendency to pass through the cell membranes and release their cargo into cells through endocytosis. Liposomes protect miRNAs from degradation and increase the stability of miRNAs in blood. However, liposomes have low sensitivity and low specificity and are potentially toxic, which has been addressed by surface modification with neutral polymers, such as PEG (Mattheolabakis et al., 2012), and attachment with lipopeptides. The fusion of liposomes with the cell membrane enhances the delivery of miRNAs into the cytoplasm (Yang et al., 2016). During miRNA delivery, cationic lipid nanocarriers entrap negatively charged hydrophilic miRNAs to form complexes through charge–charge interactions, which increase the uptake efficiencies of the incorporated miRNAs into the target cells (Iturrioz-Rodriguez et al., 2021). Furthermore, lipids can also form pH-sensitive liposomes to resolve problems related to the pH sensitivity of nucleic acids (Chakraborty et al., 2017).

Many investigations have validated that cationic lipids are efficient and safe carriers for gene delivery. Costa et al. performed *in vivo* experiments and showed that intravenously administered chlorotoxin (CTX)-coupled (targeted) stable nucleic acid lipid particle (SNALP)-formulated anti-miR-21 oligonucleotides primarily accumulated in GBM and silenced the expression of miR-21, leading to upregulation of the target gene *RhoB* and the subsequent inhibition of tumor proliferation, enhanced apoptosis activation and improved survival to a lesser extent in GBM-bearing mice (Costa et al., 2015a). Campani et al. showed that hybrid self-assembling nanoparticles (SANPs) with different lipids can be optimized for the delivery of miRNAs such as miR-603 to the brain in mice with orthotopic GBM, which was used to control chemoresistance in GBM (Campani et al., 2020). Taken together, these findings show that lipids have the potential to be a hopefully effective miRNA delivery system that may be used for the development of novel miRNA delivery approaches with reduced toxicity and few side effects. Large amounts of research on liposomal drug delivery exist, facilitating the advancement of miRNA-based cancer therapy toward clinical application. In fact, modified liposomes have been used as carriers for the first miRNA replacement therapy, MRX34, which is being investigated in a clinical trial (Catela Ivkovic et al., 2017).

6. Challenges with/and perspectives on miRNA therapy in GBM

GBM has limited therapeutic strategies available and may be resistant to various therapies. Currently, surgery and postoperative administration of high doses of radiation and concurrent TMZ chemotherapy are the main clinical therapies. However, this therapeutic strategy has a limited contribution to improving the overall prognosis of patients with GBM. The underlying molecular mechanisms associated with the pathogenesis of GBM should be explored to provide new insights into modern therapy. As novel molecules, miRNAs represent an attractive candidate for targeted therapies against GBM because miRNAs direct the post-transcriptional regulation of entire signaling network of genes within the cells involved in various pathogenesis events.

6.1. Novel and unexpected complex mechanisms of miRNA action

MiRNA-based therapies are separated into miRNA replacement therapy and miRNA inhibition therapy (Wang et al., 2015), in combination with chemotherapy and radiotherapy. However, the novel unexpected complex mechanisms of miRNA action should be considered in the development of miRNA-targeting strategies and should be explored in further studies. Hwang et al. showed that miRNAs, such as miR-29, may be localized in the nucleus, increasing the challenge of targeting nuclear miRNAs with miRNA-based therapy (Hwang et al., 2007). Moreover, miRNAs have been shown to target other genetic regions, such as the 5′-UTR of genes, promoter regions, transcribed ultraconserved regions (T-UCRs), and even proteins, in addition to the 3′-UTR (Ling et al., 2013). Thus, miRNAs exert regulatory effects on the whole transcriptome, not just the coding sequences of genes. Orom et al. showed that miR-10a interacts with the 5′-UTR of mRNAs encoding ribosomal proteins, alleviates translational repression of ribosomal protein mRNAs during amino acid starvation, and enhances ribosomal mRNA translation, which induces global protein synthesis and causes the oncogenic transformation of murine NIH3T3 cells (Orom et al., 2008).

Moreover, miRNAs not only downregulate but also upregulate the translation of target genes through diverse mechanisms. Eiring et al. reported that the restoration of miR-328 expression rescues the cell from differentiation and reduces survival by interacting with the translational regulator poly(rC)-binding protein hnRNP E2 and the mRNA encoding the survival factor Pim-1 proto-oncogene (*PIM1*). The interaction with hnRNP E2 increases the translation of CCAAT/enhancer binding protein alpha (*CEBPA*) through hnRNP E2-mediated translational inhibition independent of the miRNA seed sequence in chronic myelogenous leukemia cells. These data reveal the dual ability of miRNAs to post-translationally control biological processes through base pairing with

complementary mRNA targets. A decoy sequence-dependent activity interfered with the mRNA-regulatory function of RNA-binding regulatory proteins (Eiring et al., 2010). According to Vasudevan et al., miR-369-3 directs the association of AU-rich elements (AREs) in the *TNF-α* mRNA and recruits microribonucleoproteins (microRNPs), the protein complex comprising AGO2 and fragile X mental retardation-related protein 1 (*FXR1*), to activate *TNF-α* translation during cell cycle arrest. The authors found that the let-7 miRNAs and the synthetic miRNA miRcxcr4 likewise induced the upregulation of target mRNAs during cell cycle arrest, yet they repressed translation in proliferating cells (Vasudevan et al., 2007). Thus, translational regulation by miRNAs oscillates between repression and activation and is context specific. Therefore, the development of clinically evaluated miRNA-targeting agents should consider not only canonical functional mechanisms but also novel mechanisms of miRNA action.

6.2. Perspectives on miRNA delivery systems for miRNA therapy in GBM

Therapeutic delivery of miRNAs with different types of viruses and NPs to cross or bypass the BBB exerts a more stable effect than other forms of therapies *in vivo* due to their multifactorial effects. Similar to many other gene therapies, the potential toxicity and identification of an effective miRNA delivery system to target tissues that bypasses the restriction of the BBB to reach target tissues are the major challenges in the translation of miRNA therapy to clinical practice. In previous proof-of-principle studies, the development of miRNA therapy tended to focus on viral vectors carrying expressing cassettes encapsulating oligonucleotides due to the strength of gene delivery, long-term efficacy and tolerated cytotoxicity *in vitro* (Shim et al., 2018). Specifically, the approval of AAV for the treatment of other diseases has shown that viral delivery is viable for clinical applications, with a high benefit-to-risk ratio due to the high effectiveness of AAV-mediated RNA interference therapy. Moreover, the baculoviral vector has also shown to be safe and effective for miRNA delivery (Peng et al., 2015). However, viral vectors still present considerable issues, such as safety concerns, limited gene capacity, and difficulty in large-scale manufacturing. The enthusiasm of using a viral delivery system for treating GBM is ebbing because viral carriers are not able to bypass the BBB to reach GBM cells. If viral carriers are delivered prior to miRNA delivery, the potential for immunostimulation and mutagenesis are major obstacles. Hence, localized intrathecal or intratumoral injection may overcome the drawbacks and safety issues identified from miRNA targeting trials.

Nonviral strategies to deliver oligonucleotides via intravenous administration must efficiently target specific tissues to maximize therapeutic efficacy and minimize side effects (Eshraghi et al., 2020). The use of NPs carrying drugs or therapeutic nucleic acids is often called nanomedicine, which creates a new opportunity for the current treatment of GBM. It has the potential to introduce real breakthroughs for GBM treatment. NP carriers, which are commonly designed to be submicron-sized particles ranging from 1 to 1000 nm in diameter, have been used to deliver therapeutic cargos for different drug and gene delivery purposes. Many types of NPs with differing shapes, sizes, charges, compositions, functionalities, customizable surfaces, and solubilities have been developed. They have been designed using a range of techniques, such as nanoprecipitation, double emulsion solvent evaporation, or lithography carrying, to be effective vehicles that penetrate the BBB (Alphandery, 2020). The latest-generation NP delivery system uses "smart" nanoparticles that can be tailored in terms of their size and properties according to the requirements of different microenvironments, conditions or time series. Smart NPs also contain multicomponent and multifunctional carriers to control the release and efficient diffusion of drugs and nucleic acids in tumor tissues (Jnaidi et al., 2020). Nonetheless, NP delivery systems also must be carefully designed and evaluated; for example, the expression or silencing rate of target genes at *in vivo* sites should be quantitated, and the behaviors of therapeutic genes should be further investigated *in vivo*. In addition, off-target effects should be considered during the clinical application of miRNA-based therapies. With advances in materials science and chemical engineering, currently available NP carriers are fabricated from a variety of synthetic materials with low toxicity, low immunogenicity, and the ability to bypass the BBB and include biodegradable and biocompatible polyester polymers, such as polylactic acid (PLA), amphiphilic star copolymers, dendrimers and polycaprolactone (PCL), which can entrap/adsorb miRNAs and improve the delivery efficiency to specific target sites (Wiwatchaitawee et al., 2021). Flexibility in conjugation with antibodies and/or peptides to facilitate BBB crossing has been added to NPs. This property makes the nanocarrier a more promising vehicle for drug delivery, not only by reducing the required therapeutic dosage but also by minimizing systemic and cellular toxicity (Gallego and Cena, 2020). Other novel miRNA delivery systems have also been explored, such as minicells, which are generated by asymmetric bacterial cell division. Minicells have been used to deliver encapsulated nucleic acids to achieve a high yield, stability and specificity with no or low toxicity (MacDiarmid et al., 2007).

7. Conclusions

A large amount of work is still needed for an in-depth understanding of the complexities of miRNAs, especially the biological basis upon which miRNAs contribute to GBM and the lethality of this disease. Research is also needed to explore miRNA-based therapeutic delivery carriers to overcome the BBB. The existing findings raise an issue regarding how this knowledge can be translated into clinical practice. In the future, we propose that miRNA-targeting oligonucleotides will become promising therapeutic cargos and that the BBB will no longer be a barrier for nanocarriers. Moreover, miRNA-based therapies will enter the clinic as next-generation drugs. In particular, personalized treatments for GBM may be designed with specific miRNA mimics or antagonist sets for individuals through analyses of individual miRNA expression profiles. We expect that new miRNA delivery strategies for GBM therapy will overcome the BBB barrier and have the potential to contribute significantly to the future of RNA-based GBM therapeutics.

CRediT authorship contribution statement

Conceptualization: Qingchun Lei, Yongmin Yang, Wenhui Zhou, Wenwen Liu, Lei Ding, Yu Li and Jin Wu. Data collection and/or assembly: Qingchun Lei, Yongmin Yang, Wenhui Zhou, Wenwen Liu, Yixin Li, Nanchang Qi, Qiangfeng Li, Zhonghui Wen, Lei Ding, Xiaobin Huang, Yu Li and Jin Wu. Manuscript writing: Qingchun Lei, Yongmin Yang, Wenhui Zhou, Wenwen Liu, Yu Li and Jin Wu. Final approval of the manuscript: Qingchun Lei, Yongmin Yang, Wenhui Zhou, Wenwen Liu, Lei Ding, Yu Li and Jin Wu. All authors have read and agreed to the published version of the manuscript.

Author agreement

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Declaration of competing interest

None.

Data availability

No data was used for the research described in the article.

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