

Chapter 2

miRNA Expression and Functions in Glioma and Glioma Stem Cells

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1 Introduction

Gliomas are the most common form of primary brain tumors, accounting for 80 % of malignant CNS tumors [1]. These tumors exhibit a marked malignant progression characterized by high level of infiltration throughout the brain, resistance to traditional chemotherapy and radiotherapy and high level of angiogenesis [1, 2]. Malignant gliomas are classified on the basis of histopathological features, clinical presentation and genetic alterations as astrocytomas, oligodendrogliomas, or tumors with morphological features of both astrocytes and oligodendrocytes, termed oligoastrocytomas [3]. Astrocytic tumors are graded based on the WHO consensus-derived scale of I to IV according to their degree of malignancy as judged by various histological features accompanied by genetic alterations [3, 4]. Grade I tumors are biologically benign and can be cured if they can be surgically resected; grade II tumors are low-grade malignancies that may follow long clinical courses, but their infiltration into the surrounding brain parenchyma renders them incurable by surgery; grade III tumors exhibit increased anaplasia and proliferation and grade IV tumors (glioblastoma, GBM), exhibit more advanced features of malignancy, including vascular proliferation, necrosis and resistance to radio and chemotherapy [5]. On the basis of clinical presentation, GBMs have been

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further subdivided into primary or secondary subtypes [6]. Primary GBMs account for the great majority of tumors in older patients, while secondary GBMs are less common and tend to occur in patients under the age of 45. Remarkably, primary and secondary GBMs are morphologically and clinically indistinguishable as reflected by a similar poor prognosis when adjusted for patient age. However, although these GBM subtypes share a common phenotypic endpoint, recent genomic profiles have revealed strikingly different transcriptional patterns and DNA copy number aberrations between primary and secondary GBMs [6]. Based on these studies, a recently expanded group of novel markers has been identified, which allow a better classification of gliomas and can serve as novel prognostic markers [7–9]. In addition, specific miRNAs have been also identified as diagnostic and prognostic markers in GBM and as novel therapeutic targets [10, 11].

1.1 The New Classification of GBM

A recent study by Phillips et al. [12] reported the identification of three high-grade astrocytoma subsets, identified by differential expression of markers associated with outcome, and named them proneural, proliferative and mesenchymal in recognition of the key features of the molecular signatures associated with each group. This study showed that the proneural subtype is distinguished by markedly better prognosis and expresses genes associated with normal brain and the process of neurogenesis. The two other subtypes, the proliferative and mesenchymal, are characterized by a resemblance to either highly proliferative cell lines or tissues of mesenchymal origin and show activation of gene expression programs indicative of cell proliferation or angiogenesis, respectively.

More recently, using an unsupervised approach to classify data from The Cancer Genome Atlas (TCGA) project, Verhaak [13] reported the existence of four GBM subtypes, termed proneural, neural, classical and mesenchymal. The four GBM subtypes have distinct molecular markers and three of the subtypes identified in this study were demonstrated to have a strong association with specific genomic alterations; the proneural subtype exhibiting IDH1 mutations and/or PDGFRA amplification, the classical subtype exhibiting amplification and/or mutation of EGFR, and the mesenchymal subtype showing loss and/or mutation of NF1.

While additional subtypes likely exist, only the proneural and the mesenchymal GBMs have been consistently identified in both supervised and unsupervised classification of GBM and have been reported to have both prognostic and predictive values [12, 13]. Recent studies also identified specific miRNAs that are characteristic of the mesenchymal GBM [14, 15].

1.2 The Mesenchymal Transformation of GBM

The epithelial to mesenchymal transformation (EMT) is a process that allows polarized epithelial cells to undergo multiple biochemical changes that enable them to acquire a mesenchymal cell phenotype which includes enhanced migratory capacity, invasiveness and increased resistance to apoptosis [16]. Activation of EMT is important for cancer cell dissemination and for the promotion of tumor metastasis in epithelial tumors [17]; however, the importance of this process in the neuro-epithelial context is much less characterized. The results from recent studies suggest that primary GBM, GSCs and glioma cells can undergo mesenchymal transformation [18–21]. During this process, which strongly resembles EMT of epithelial tumors, glioma cells acquire a mesenchymal stem cell-like properties which include increased ability to migrate, upregulation of mesenchymal markers and the ability to differentiate to the mesenchymal lineage.

The clinical relevance of the mesenchymal transformation of GBM is of utmost importance, as several studies showed that GBM patients whose tumors have a proneural phenotype have better survival compared with those that have mesenchymal phenotype [12, 13] and upon recurrence, tumors frequently transform toward the mesenchymal phenotype which is characterized as the most aggressive and therapy resistant subtype [12, 20, 22, 23].

1.3 The Factors Underlying the Mesenchymal Transformation of GBM

Using gene regulatory network analyses, the transcription factors signal transducer and activator of transcription 3 (STAT3) and CCAAT enhancer-binding protein β (C/EBP β) have been recently identified as synergistic initiators and master regulators of the mesenchymal transformation in glioma [24]. Analysis of regulatory networks of available expression microarray data sets of GBM, the transcriptional co-activator TAZ has been identified as another major regulator of mesenchymal differentiation in malignant glioma [25]. In addition, a recent study reported that the TNF-1/NF-KB pathway activated by macrophages/microglia, also plays a role in the mesenchymal transformation of proneural glioma stem cells [23]. Similarly, specific miRNAs have been also identified as important regulators of the EMT process [26] and of the mesenchymal transformation of GBM [27, 28].

2 Cancer Stem Cells (CSCs)

2.1 General Aspects of Cancer Stem Cells

CSCs represent a subset of tumor cells that has the ability to self-renew, generate the diverse cells that comprise the tumor and to continually sustain tumorigenesis. CSCs share important characteristics with normal tissue stem cells, including self-renewal (by symmetric and asymmetric divisions) and differentiation capacity, albeit in an aberrant mode [29]. The first evidence for the existence of CSCs came from acute myeloid leukemia in which a rare subset comprising 0.01–1 % of the total population could induce leukemia when transplanted into immunodeficient mice [30]. CSCs are distinct from the cell of origin, which specifically refers to the cell type that receives the first oncogenic hit(s). Moreover, CSCs do not necessarily originate from the transformation of normal stem cells but may arise from restricted progenitors or more differentiated cells that have acquired self-renewing capacity [31]. One implication of this model is that there are mechanistic parallels between the self-renewal programs of normal stem cells and CSCs. It has been presumed in many cases that the cells in which cancer originate share committed cells that have undergone some degree of differentiation [32].

2.2 Glioma Stem Cells (GSCs)

Glioma stem cells (GSCs) were one of the first CSCs isolated from solid tumors. GBM contain a small subpopulation of self-renewing and tumorigenic CSCs which are implicated in tumor infiltration, resistance to conventional therapies and tumor recurrence [33–38]. Interestingly, GSCs isolated from human tumors and cultured in vitro showed remarkable similarities to normal neural stem cells (NSCs), expressing neural stem/progenitor markers such as Nestin, Sox2, and Olig2 and upon induction, could be differentiated to cells expressing neuronal or glial markers [36, 39]. Transplantation of GSCs into immunodeficient mice yielded tumors that shared similar histology and global gene expression patterns with their parental tumors [40]. Understanding the mechanisms associated with the stemness and oncogenic features of these cells is essential for the development of therapeutic approaches that can eradicate GSCs and may provide the basis for the development of novel therapeutic approaches for GBM patients [41–43].

2.3 miRNAs in Cancer

miRNAs are 19–25 nucleotide non-coding small RNAs that can play important regulatory roles in animals and plants by targeting mRNAs for cleavage or

translational repression [44–46]. miRNAs induce gene silencing by partial sequence homology and thus a single miRNA can have hundreds of targets and therefore regulate diverse cellular functions such as pathways involved in stem cell function, cell proliferation, migration and oncogenic transformation [47, 48]. Indeed, aberrantly expressed miRNAs have been described in various types of tumors including GBM [49–51]. The expression and function of specific miRNAs in astrocytic tumors have been studied with regards to mechanisms of gliomagenesis, patient prognosis and their use as novel therapeutic targets. [52, 53].

Mechanisms of miRNA deregulation have been described at various levels including genetic, epigenetic, transcriptional, and processing levels [54]. Amplification and deletion of many miRNAs are associated with their location at regions that are either amplified or deleted in human cancers [55]. Repression of some miRNAs is mediated by CpG hypermethylation in different tumors [56], whereas other miRNAs are regulated by transcription factors such as p53, E2F, STAT3 or via deregulation of Dicer or Drosha as has been observed in many cancers [57]. Various studies documented that miRNAs can act as tumor suppressor or oncogenes based on the function of their major target genes [58]. In addition, they have been shown to regulate a variety of cellular functions such as cell proliferation, cell death and apoptosis, cell migration and invasion, metastasis, angiogenesis, tumor microenvironment, tumor immunology and chemoresistance as well as many aspects of cancer stem cell biology [48–52, 59].

2.4 miRNAs as Biomarkers in GBM

Various studies have been performed to identify biological markers for the detection and risk stratification of gliomas. miRNA expression and signature have been also associated with the diagnosis and prognosis of patients with different types of tumors [60–62]. Genome-wide profiling studies indicated that the miRNA signature can contribute to the distinguishing of different types of cancers, the identification of tissue of origin of purely characterized tumors or of metastasis of undefined origin and the classification of tumor histological subtypes [63, 64]. In addition, specific miRNAs have been demonstrated to have great potential as early minimally invasive biomarkers in different tumors due to their stability, abundance and accessibility in various fixed and fresh tissues, different biologic fluids and circulating exosomes [65, 66].

Various gene profiling studies suggested the existence of multiple GBM classes but their characteristics are not fully defined. Recent studies employed also the use of miRNAs as biomarkers in GBM [66]. A study by Kim et al., analyzed 261 miRNA expression profiles from TCGA and identified five clinically and genetically distinct subclasses of glioblastoma based on their miRNA signature. These subgroups were related to different neural precursor cell types resembling those of radial glia, oligoneuronal precursors, neuronal precursors, neuroepithelial/neural crest precursors, or astrocyte precursors [11]. In addition, this study also

identified specific miRNAs as potent regulators of subclass-specific gene expression networks in GBM. One of the miRNAs, miR-9, was found to suppress the mesenchymal differentiation of glioblastoma by inhibiting JAK kinases and of STAT3 [11].

To identify significant miRNA-mRNA correlations in gliomas, a study by Ma et al. [67] analyzed the miRNA and mRNA signatures and the relationship between them in 82 glioma specimens. Statistical analysis showed that expression of miR-128a, -504, -124a, and -184 was negatively correlated with the expression of mesenchymal markers in GBM. Functional analysis of miR-128a and -504 demonstrated that mesenchymal signaling in GBM may be negatively regulated by miR-128a and -504 [67].

In another study, Li et al., described an integrated approach to identify miRNA functional targets during glioma malignant progression by combining the paired expression profiles of miRNAs and mRNAs across 160 glioma patients. They identified miR-524-5p and miR-628-5p as protective factors and their expression decreased during glioma progression [68]. miRNA profiling of glioma was also analyzed in cerebrospinal fluid (CSF) in a study by Baraniskin et al. [69] which identified miR-21 and miR-15b as potential markers in CSF for glioma that can distinguish these patients from normal individuals and from patients with brain lymphoma and metastatic brain tumors.

A recent review that summarized the expression of specific miRNAs in GBM based on the recent literature identified 253 upregulated and 95 downregulated miRNAs. Both of the oncogenic and tumor suppressor miRNAs were found to target genes involved in cell migration, invasion, angiogenesis and proliferation [28].

In addition to studies aiming at analyzing the miRNA signatures of GBM, there have been multiple studies reporting the expression of specific miRNAs in different tumor grades. miR-21 has been shown to exhibit both diagnostic and prognostic values in different tumors including gliomas [70]. The expression of miR-26a has been associated with poor prognosis [71]. The decreased expression of miR-328 is associated with unfavorable prognosis in glioma [72] and miR-181d expression acted as a predictive biomarker for temozolomide response [73]. We recently reported that both miR-145 [74] and miR-137 [75] were downregulated in glioblastoma as compared to normal brain specimens and that the decrease of miR-137 was attributed to hypermethylation of pre-miR-137 promoter [75].

2.5 miRNA Functions in GBM

In addition to their diagnostic application, specific miRNAs have been also implicated as potential therapeutic targets and tools [76, 77]. The most appealing advantage of using miRNAs for the treatment of cancer therapy is their ability to affect multiple target genes in the context of a network, making them suitable especially for the treatment of glioma that is a complex heterogeneous tumor. The

miRNAs that are overexpressed in a deregulated manner in tumors are considered as oncogenes and are called oncomiRs [71, 78]. They are associated with the inhibition of tumor suppressor genes or those which control cell proliferation, differentiation, migration and apoptosis.

Various studies have highlighted the importance of miRNA deregulation in several aspects of the pathogenesis of GBM including cell cycle control, invasion, migration, resistance to chemotherapy and radiotherapy and cell apoptosis [79–81]. Specific miRNAs control some of the core signaling pathways in GBM such as EGFR signaling and those related to p53, PTEN/PI3K/AKT and the Notch pathways. Moreover, several in vitro and pre-clinical studies demonstrated the therapeutic benefits of either expressing tumor suppressor miRNAs or inhibiting OncomiRs [82–85].

Various modification of anti-miRNA oligonucleotides have been reported to successfully decrease miRNA expression in pre-clinical models, including conjugation to peptides, addition of 2-O-methoxyethyl and locked nucleic acid (LNA)-oligonucleotides anti-miRNA oligonucleotides [86, 87]. In addition, various reports described the simultaneous silencing of multiple miRNAs that share the same seed by using seed-targeting tiny LNAs [88]. Similarly, miRNA sponges, which are transcripts with repeated miRNA antisense sequences that contain tandem siRNA binding sites can also sequester miRNA from their endogenous targets and can block an entire miRNA seed family [89].

The delivery of miRNA mimics can be used for increasing the expression of downregulated or tumor suppressor miRNAs [90]. Studies using adeno-associated virus, lipid-based nanoparticles, and the combination of miRNA expression vectors and lipid-based nanoparticles, demonstrated effective biological impact in various xenograft systems [91]. However, difficulties in the delivery of miRNA mimics may be due to difficulties in targeting a specific tissue and the fate and function of the passenger strand that sometimes acts as an anti-miRNA.

2.5.1 Oncogenic miRNAs in GBM

Different oncogenic miRNAs have been described in GBM. This paragraph highlights some of these miRNAs and their cellular functions.

The miR-21 – miR-21 is located on chromosome 17 within TMEM49, a transmembrane protein that is overexpressed in various tumors [70] and is also associated with the malignancy of glioma [92]. miR-21 is considered an oncogenic miRNA and it regulates cell migration by inhibiting RECK and TIMP3 [93] and cell apoptosis [94]. Silencing of miR-21 decreases glioma cell migration and growth, induces cell apoptosis and inhibits the growth of glioma xenografts [95].

miR-26a – miR-26a was reported to be amplified in glioma and to promote glioma cell growth and transformation. MAP3K2 and MEKK2 have been implicated as potential targets of miR-26a that eventually lead to cell

proliferation and inhibition of cell apoptosis via targeting of the RBI, Pi3K/AKT and the JNK pathways [95].

miR-221/222 – These two miRNAs are highly expressed in GBM and regulate glioma cell proliferation by targeting p27 and cell apoptosis by targeting the survivin 1 homologs BIRC1 and NIAP. These miRNAs also promote resistance of glioma cells to cytotoxic T cells by targeting ICAM-1 [96].

Some of the additional oncomiRs that were described in gliomas include miR-10b, miR-125b, miR-182, miR-296, miR-196a [52].

2.5.2 Tumor Suppressor miRNAs in GBM

Some miRNAs exhibit tumor suppressive activities by targeting oncogenes or signal pathways associated with proliferation, migration or resistance to cell apoptosis. Some of the known glioma tumor suppressor miRNA include the following miRNAs.

miR-181 – miR-181a, 181b and 181c were described as tumor suppressor miRNAs in glioma and overexpression of miR-181a sensitizes glioma cells to radiation by targeting Bcl₂ [97].

miR-124 – miR-124 inhibits glioma cell growth by downregulating SOS1. miR-124 also inhibits the STAT4 signaling pathway and enhances T cell mediated immune clearance of glioma [98].

miR-145 – This miRNA is downregulated in glioma specimens and have been shown to inhibit glioma cell migration by targeting CTGF [74] and NEDD9 [99]. Additional studies demonstrated that miR-145 also targeted Sox9 and adducin 3 in glioma cells [100].

miR-146a – miR-146a is another miRNA that inhibits the migration and invasion of glioma cells probably by targeting MMP16.

Additional miRNAs in this group include, miR-128, miR-137, miR-17 and miR-184, miR-218 and miR-219-5p [52].

2.6 miRNAs in Glioma Stem Cells

Despite the large number of publications related to the expression and function of specific miRNAs in glioma cells, much less is known about the role of miRNAs in GSCs. Three main approaches were undertaken to identify miRNAs important for the function of GSCs, comparative analysis of miRNA expression in GSCs vs. normal neural stem cells (NSCs), comparing subpopulations of GSCs such as CD133+ and CD133– and comparing undifferentiated (neurospheres) vs. differentiated GSCs.

2.6.1 GSCs Versus NSCs

As described in Sect. 2.2, a potential origin of GSCs is attributed to transformed NSCs [39]. Therefore, one of the approaches to identify miRNAs that play a role in the initiation, maintenance and function of GSCs is to study their expression and function in comparison with that of NSCs. The similarity in some of the characteristics of NSCs and GSCs raises the possibility that these two cell types share some common miRNA-based regulatory pathways but differ in others. Indeed, NSCs and GSCs share partially overlapping miRNA profiles in particular those related to self-renewal and proliferation, whereas miRNAs related to differentiation, migration and response to apoptotic stimuli are expected to be quite different. A study that compared the miRNA profiles of glial tumors, embryonic stem cells (ESCs), neural precursor cells (NPCs), and normal adult brain tissues of both human and mouse origin, reported that gliomas display a microRNA expression profile reminiscent of neural precursor cells [101]. Half of these miRNAs were clustered as miR-17-92, miR-106b-25, miR-106a-363, miR-183-96-182, miR-367-302, miR-371-373 and the large miRNA cluster in the Dlk1-Dio3 region [101]. Fifteen miRNAs exhibited disparate expression between stem cells and glioma specimens; ten were associated with stem cell specific clusters and five (miR135b, miR-141, miR205, miR-200c, miR-301c) were associated with malignancy [101].

In a recent study a genomic-wide miRNA expression profiling in GSCs and NSCs using combined miRNA microarray and deep sequencing analysis identified eight miRNAs (miR-10a, miR-10b, miR-140-5p, miR-204, miR-424, miR-34a, miR-193-aP and miR-455-5p) that were up-regulated and two miRNAs (miR-124 and miR-874) that were downregulated in GSCs relative to NSCs. These modified miRNAs inhibited the expression of respective target genes that were involved in either tumor suppression or progression [102].

2.6.2 miRNAs Associated with CD133+ and CD133– Cells

Another method for identifying miRNAs that play a role in the stemness of GSCs is to compare the expression of miRNA signature between CD133+ and CD133– cells. Using this approach a number of miRNAs were identified including miR-425, miR-451 and miR-486. miR-451 was reported to target the LKB1/AMPK pathway and to sensitize cells to glucose deprivation [52, 103].

2.6.3 miRNAs Associated with the Differentiation of GSCs

One of the approaches to target GSCs is by their differentiation which abolishes their stemness and tumorigenic potential. Therefore differentiation of GSCs in the presence of serum and absence of growth factors can reveal miRNAs that are associated with the stemness inhibition of these cells. Using this approach, Fareh

et al. [104] identified that the miR-302-367 cluster was induced during the differentiation of GSCs and that stable expression of this cluster inhibited self renewal and stemness of GSCs by targeting the CXCR4 pathway upstream of sonic hedgehog (SHH)-GLI-NANOG. Similarly, a recent study analyzing the miRNAs of differentiated GSCs identified miR-1275 as a major miRNA that was downregulated during the differentiation process along with up-regulation of its target gene claudin 11 [105]. miR-137 was also reported to be upregulated during NSC and GSC differentiation along with downregulation of its target gene RTVP-1/GLIPR1 [75].

Additional miRNAs have been identified as important in regulating various functions of GSCs.

miR-7 – miR-7 was reported to affect the proliferation and invasion of a GSC line and to inhibit the expression of EGFR and AKT [84].

miR-9/9* and miR-17 – These miRNAs, in addition to miR-106b, are highly abundant in CD133+ GSCs. Their inhibition decreases neurosphere formation and induces GSC differentiation. Both miR-9/9* and miR-106b target calmodulin-binding transcription activator 1 (CAMTA1) [106]. Interestingly, the inhibitor of differentiation 4 (ID4), induces dedifferentiation of human glioma cells to glioma stem-like cells and enhances SOX2 expression by suppressing miR-9* [107].

miR-124 – miR-124 is one of the major neuronal miRNA and has reported to promote neuronal differentiation by targeting the PTBP1, Sox9 and the REST pathways (27, 114). In addition, miR-124 decreases neurosphere formation, CD133+ population and stem cell marker expression in GSCs by targeting SNAI2 [108].

miR-137 – This miRNA has been also reported to promote the neuronal differentiation of NSCs and GSCs by targeting CDK6 [109]. In addition, we recently reported that miR-137 targets RTVP-1 that regulated the stemness of GSCs upstream of the CXCR4-Gli- pathway [75].

miR-34a – miR-34 which is downregulated in glioma, exerts tumor suppressive effects in GSCs by targeting c-Met, Notch-1 and Notch-2. The Notch pathways plays a major role in the stemness, proliferation and radioresistance of GSCs. miR-34 induced GSC differentiation, and inhibited GSC proliferation, migration and survival [110, 111].

miR-326 – miR-326 is another miRNA that targets the Notch pathway but very interestingly its expression is regulated by its target gene [112]. miR-326 exerted cytotoxic effects on GSCs in vitro and in vivo [112].

miR-17-92 cluster – This miRNA cluster has been implicated in the regulation of GSC apoptosis, proliferation and differentiation. Target genes of the miR-17-92 cluster including CDKN1A, E2F1, PTEN and CTGF have been identified [113].

miR-128 – miR128 has been identified as a tumor suppressor miRNA in glioma [27, 52] and was reported to inhibit the self-renewal and stemness of GSCs by targeting the polycomb transcriptional repressor Bmi [114].

miR-145 and miR-143 – miR145 expression is significantly decreased in GSCs compared to NSCs and it decreases the migration of these cells by targeting CTGF and its downstream signaling protein SPARC [74]. In addition, miR-145 has been reported to target the stemness-related protein SOX2 in GSCs [115]. Similarly, the expression of miR-143 was decreased in GBM and this miRNA inhibited glycolysis and the stemness of GSCs [116].

2.7 miRNA Delivery into Brain Tumors

The potential therapeutic use of miRNAs is hindered by the lack of effective delivery approaches into target tissues. The main challenges are associated with the ability of therapeutic miRNAs to enter the cell cytoplasm without encountering the endosomal vesicles, evading kidney filtration and excretion and removal from the bloodstream by phagocytic cells. This difficulty is further amplified in the case of brain tumors due to the presence of the blood brain barrier (BBB), which prevents the entry of RNA molecules and potential RNA-based therapy. Although the BBB is compromised in areas of tumors, it is still intact in areas of tumor infiltration. Various methods including the use of stereotactic or direct intratumoral injection, convection-enhanced delivery, intrathecal and intra-ventricular injection and intravascular infusion with or without modification of the blood-tumor-barrier were described for the delivery of drugs into the brain [117]. In addition, recent studies demonstrated intranasal delivery of stem cells, exosomes, nanoparticles and viruses to the brain in animal models of brain inflammation and glioma xenografts [117].

2.8 Vectors for miRNA Delivery

Vectors for gene therapy and the delivery of miRNAs can be divided into two categories: viral and non-viral vectors. Viral vectors include adenovirus, adeno-associated virus, lentivirus and retrovirus vectors. Indeed, various reports demonstrated the ability of viral vectors to successfully deliver miRNAs to tumor sites [118, 119]. However, despite multiple reports demonstrating that modified viral vectors are effective in gene delivery, the immune response to adenovirus vectors remains a problem and lentivirus vectors have the potential for insertional mutagenesis, which may be a concern when used for therapy [118, 119]. Therefore, non-viral vectors, which retain biocompatibility, targeting efficacy and enhanced transfection efficiency, are a more suitable alternative to viruses for achieving successful miRNA delivery without side effects [120, 121].

In addition, RNA nanoparticles, in which the scaffold, the ligand and the therapeutic tool are all composed of RNA, have been recently described to specifically target tumors with low toxicity and low immunogenicity [122].

2.9 *Exosomes for miRNA Delivery*

In addition to the biological and chemical vehicles described so far, a recent approach for RNA delivery has been recently explored using extracellular vesicles and in particular exosomes, due to their natural adaptation for the transport of various substances, including nucleic acids [123].

Exosomes are membrane vesicles of endocytic origin, 50–100 nm in diameter, and are secreted by most cells into the extracellular environment [124]. They can impact the function of neighboring cells through intercellular transfer of mRNAs, microRNAs, receptors and enzymes, and are involved in the communication of immune responses [125]. Exosomes have multiple advantages over existing microRNA delivery vehicles such as low immunogenicity since they can be derived from a patient's own cells [126]. More importantly, exosomes are natural carriers for miRNAs, which make them excellent delivery systems for these molecules [127]. We recently showed that synthetic miRNA mimics are delivered by mesenchymal stem cells (MSCs) to glioma cells via exosomes [115]. Similarly, exosomes isolated from MSCs have been reported to deliver miR-146 and miR-9 to glioma cells [128, 129].

These and other studies suggest that the future advances in the manipulation and targeting of miRNA delivery by exosomes may lead to the development of efficient cancer-specific therapy.

2.10 *Stem Cells for the Delivery of miRNAs*

An alternative approach to current therapies of GBM with a potential to target infiltrative tumor cells is the use of stem cells, which exhibit homing to tumor and injury sites in the brain. Indeed, numerous studies demonstrated tropism of NSCs to infiltrating glioma cells in the brain and their therapeutic benefits [130]. Another source of stem cells that exhibit tropism to tumor cells is adult human mesenchymal stromal stem cells (MSCs) that can be obtained from autologous bone marrow (BM) and adipose tissue or from cord or placenta. MSCs exhibit homing abilities, which enable them to migrate to sites of injury, inflammation and tumors [131, 132]. Specifically, MSCs have been shown to cross the blood brain barrier and migrate to sites of experimental GBM when administered intra-arterially and intravenously and can deliver cytotoxic compounds and exert anti-tumor effects [133, 134].

The ability of MSCs to cross the blood brain barrier, to home to tumor cells and deliver therapeutic molecules render these cells excellent delivery vehicles for the targeted therapy of brain tumors. We recently demonstrated the ability of MSCs to deliver miRNA mimic to glioma cells and GSCs in vitro and in vivo. The delivery of the miRNA mimic was mediated by exosomes and had an impact on the expression of target genes, cell migration and invasion [115]. In addition, recent

studies also demonstrated the use of MSC-derived exosomes to deliver miRNA mimics to glioma cells [128, 129].

2.11 Pre-clinical Studies in Glioma and GSC-Derived Xenografts

Different viral vectors have been utilized for the treatment of glioma in pre-clinical studies. A recent study combined adenoviral vectors expressing hTERT-targeting ribozyme controlled HSV-tk expression together with the overexpression of miR-145. Intratumoral injection of the adenovirus vector expressing the HSV-tk expression cassette plus miR-145, combined with intraperitoneal injection of ganciclovir increased animal survival [135]. In another study, Wang et al., employed adenovirus vector expressing siRNAs that silenced the expression of miR-221 and miR-222 in glioma cells and increased the expression of p27kip1 that led to cell cycle arrest in G1 and cell apoptosis [136]. Similarly, we recently demonstrated the effectiveness of lentivirus vectors expressing pre-miR-124, pre-miR-137 and pre-miR-145 in the transduction and function of glioma stem cells [74, 75, 115].

In addition to the use of viral vectors there have been studies describing the use of nanoparticles for the delivery of miRNA mimics or siRNAs. Stable nucleic acid lipid particles (SNALPs), which target GBM cells were generated by covalent coupling of the peptide chlorotoxin (CTX) to the liposomal surface [137]. These CTX-coupled SNALPs efficiently and specifically delivered encapsulated anti-miR-21 oligonucleotides to cultured U87 GBM cells and into established intracranial tumors.

In another study, Yang et al., employed cationic polyurethane (PU)-shortbranch PEI (PU-PEI) to deliver miR-145 into CD133+ GSCs which decreased their oncogenic potential and induced their differentiation into CD133-cells. Intravenous administration of the nanoparticle-formulated miR-145 increased the sensitivity of CD133+ GSC-derived xenografts to temozolomide and radiation and prolonged animal survival [138].

In another study, systemic administration of miR-7 encapsulated in cationic liposomes resulted in the decreased growth of glioma xenografts and metastatic nodules by targeting the EGFR [139].

3 Conclusions

miRNAs have been reported to play major roles in a variety of cellular processes and biological system and their deregulation has been implicated in the pathogenesis of many diseases including cancer. Multiple studies have demonstrated the use of miRNAs as potential diagnostic and prognostic markers in GBM and as important regulators of GSC functions. Moreover, preclinical studies demonstrated their impact on tumor growth and invasiveness and novel approaches have been

developed for the delivery of miRNA mimics or antagonists to tumor sites. However, despite these significant advances, there are still major issues that need to be addressed prior to the clinical application of miRNA-based therapy in GBM. These include specific target validations and prevention of undesired off-target effects, and the development of delivery approaches for the targeting infiltrating glioma cells and GSCs. Nonetheless, the increasing numbers of discoveries and reports contribute to our understanding of the mechanisms involved in the biology of GBM and GSCs and are likely to make a significant therapeutic impact in the near future.

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