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Chapter 18

MicroRNAs Regulated Brain Tumor Cell Phenotype and Their Therapeutic Potential

Chunzhi Zhang, Budong Chen, Xiangying Xu,
Baolin Han, Guangshun Wang and Jinhuan Wang

Additional information is available at the end of the chapter

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1. Introduction

MicroRNAs (miRNAs)are short 18–25 nucleotide small non-coding RNA molecules that function to silence gene expression via sophisticated post-transcriptional regulation[1]. Since their discovery in the early 1990s, these small molecules have been shown to play an important regulatory role in a wide range of biological and pathological processes. Over 30% of human messenger RNAs (mRNAs) are regulated by miRNAs[2]. miRNAs generated by the canonical biogenesis pathway are transcribed as precursor RNAs from intergenic, intronic or polycistronic genomic loci by RNA polymerase II (Pol II). The primary miRNA (pri-miRNA) transcript forms a stem–loop structure that is recognized and processed by the Drosha and DGCR8 RNase III complex or the spliceosome apparatus in the nucleus. In the non-canonical miRNA pathway, miRNAs are transcribed directly as endogenous short hairpin RNAs (endo-shRNAs) or derive directly through splicing from introns that can refold into hairpins (mirtrons). The trimmed precursor (pre-miRNA) hairpins from both canonical and non-canonical miRNA pathways are then transported by an exportin 5 and RAN-GTP-dependent process to the cytosol, where they are typically further processed by the Dicer and transactivation-response RNA-binding protein (TRBP) RNase III enzyme complex to form the mature double-stranded ~22-nucleotide miRNA. Argonaute proteins (for example, AGO2)

then unwind the miRNA duplex and facilitate incorporation of the miRNA-targeting strand (also known as the guide strand) into the AGO-containing RNA-induced silencing complex (RISC). The RISC– miRNA assembly is then guided to specific target sequences in mRNAs. The initial recognition of mRNAs by the RISC–miRNA complex is driven primarily by Watson–Crick base-pairing of nucleotides 2 to 8 in the mature miRNA (termed the seed

sequence) with specific mRNA target sequences chiefly located in the 3′ untranslated region, and additional base-pairing affords greater affinity and targeting efficiency(Figure1)[3].

Given the pivotal regulatory role of miRNAs in a broad range of biological processes, it is not surprising that miRNAs play a role in human cancers, including brain tumor. First, about 50% of human miRNAs are located in cancer-associated genomic regions and fragile sites, suggesting that they might be the target genes underlying such aberrant intervals [5]. Second, the advent of high-throughput detection method has promoted expression profiling of miRNAs in normal and tumor tissues. Compared to normal tissues, anomalous levels of miRNA subsets have been found in almost all tumor types examined [6, 7]. Third, miRNAs with tumor-suppressive gene and oncogene-like properties have been described.

Since the first description of aberrant miRNA expression in glioblastomas and pituitary adenomas in 2005 [8, 9], there have been increasing reports each year about miRNA deregulation and function in various brain tumors. In this chapter, we summarize the current findings of miRNA study in brain cancers and discuss the diagnostic and therapeutic potential of miRNAs, mainly in glioma.

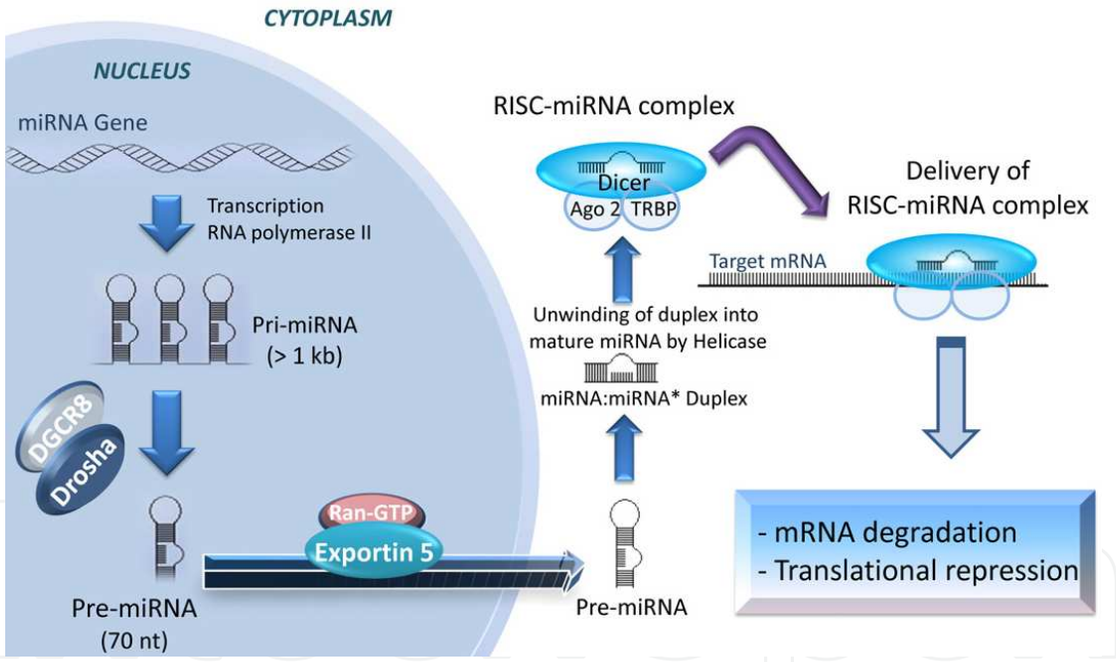

Figure 1. The miRNA biogenesis pathway[4]

2. MiRNAs profile in brain tumor

CBTRUS obtained incidence data from 48 population-based cancer registries that include cases of malignant and non–malignant (benign and uncertain) primary brain and central nervous system tumors in the United States in 2004-2007. The most frequently reported histology is the

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predominately non–malignant meningioma, which accounts for 34% of all tumors, followed by glioblastoma (17%). The predominately non-malignant pituitary and nerve sheath tumors account for 13% and 9% of all tumors, respectively. Acoustic neuromas (defined by ICD-O-3 site code C72.4 and histology code 9560) account for 63% of all nerve sheath tumors(Figure2).

Only recently, the miRNAs attracted increasing attention as potential diagnostic or even therapeutic tools in brain tumors. Profiling techniques to identify global expression patterns of miRNAs in brain tumors have been widely used to uncover aberrantly expressed microRNAs in tumor genomes. Ciafrè et al. found nine (miR-10b, miR-130a, miR-221, miR-125b-1, miR-125b-2, miR-9-2, miR-21, miR-25, miR-123) and four miRNAs (miR-128a, miR-181c, miR-181a, miR-181b), respectively, out of 245 miRNAs to be up-/down-regulated in human glioblastoma samples, and nine (miR-221, miR-23a, miR-24-2, miR-24-1, miR-23b, miR-21,miR-222-prec, miR-191, miR-220) and seven miRNAs(miR-181a, miR-181b, miR-128b, miR-197, miR-181c,miR-125b-2, miR-125b-1), respectively, to be up-/down-regulated in human glioblastoma cell lines[10]. Chan et al.[8] demonstrated five (miR-21, miR-138, miR-347, miR-291-5′, miR-135) and three miRNAs (miR-198, miR-188,miR-202), respectively, out of 180 miRNAs to be up- and downregulated in glioblastoma samples. Sasayama et al.[11] found miR-10b, miR-21, miR-183, miR-92b and miR-106b to be up-, and miR- 302c*, miR-379, miR-329, miR-134 and miR-369-3p to be downregulated in human glioblastoma samples. Other studies reported several miRNAs to be significantly deregulated in glioma samples of Chinese patients (including miR-34a, miR-15b, miR-200a and miR-146b) [12], or miR-29b, miR-125a and miR-149 to be downregulated in glioblastomas [13]. In an array study with 192 miRNAs, 13 miRNAs (miR-101, miR-128a, miR-132, miR-133a, miR-133b, miR-149, miR-153, miR-154*, miR-185, miR-29b, miR-323, miR-328, miR-330) were found to be downregulated and three miRNAs to be upregulated (miR-21, miR-155, miR-210) in glioblastoma multiforme [14]. Another microarray study identified 55 miRNAs out of 756 miRNAs to be upregulated and 29miRNAs to be downregulated in malignant astrocytomas (primary and secondary glioblastoma and anaplastic astrocytoma, respectively) compared to controls [15].

In additional to gliomas, miRNA profiling also had been discovered in some other brain tumors. Ferretti et al were the first to identify signatures of a set of 248 miRNAs in a panel of primary medulloblastomas and normal cerebellar controls using high throughput expression profiles. They showed different expression profiles between normal brain and tumor and between distinct tumor histotypes. In particular, they detected an upregulation of mir-21 and miR-17-92 cluster (miR-17-5p, miR-20a and miR-19a) and a downregulation of miR-128a/b, let-7, miR-124a, miR-103, miR-134, miR-138, miR-149, miR-181b, miR-9 and miR-125a, most of them previously reported to be dysregulated in other brain tumor cell lines or nervous system cancers [16]. Moreover, Recent microarray data reported a possible role of miRNAs in pituitary adenomas. The first connection between pituitary adenomas and miRNAs was established by Bottoni et al in 2005, that showed a downregulation of mir-15a and miR-16-1 in GH-secreting and in PRL-secreting adenomas compared to normal pituitary tissue[8]. In 2007 Bottoni et al explored the miRNAome of pituitary tumors by microarray. They found that 30 miRNAs are differentially expressed between normal pituitary gland and pituitary adenomas. Among them, miR-150, miR-152, miR-191, and miR-192 were found to be upregulated in pituitary

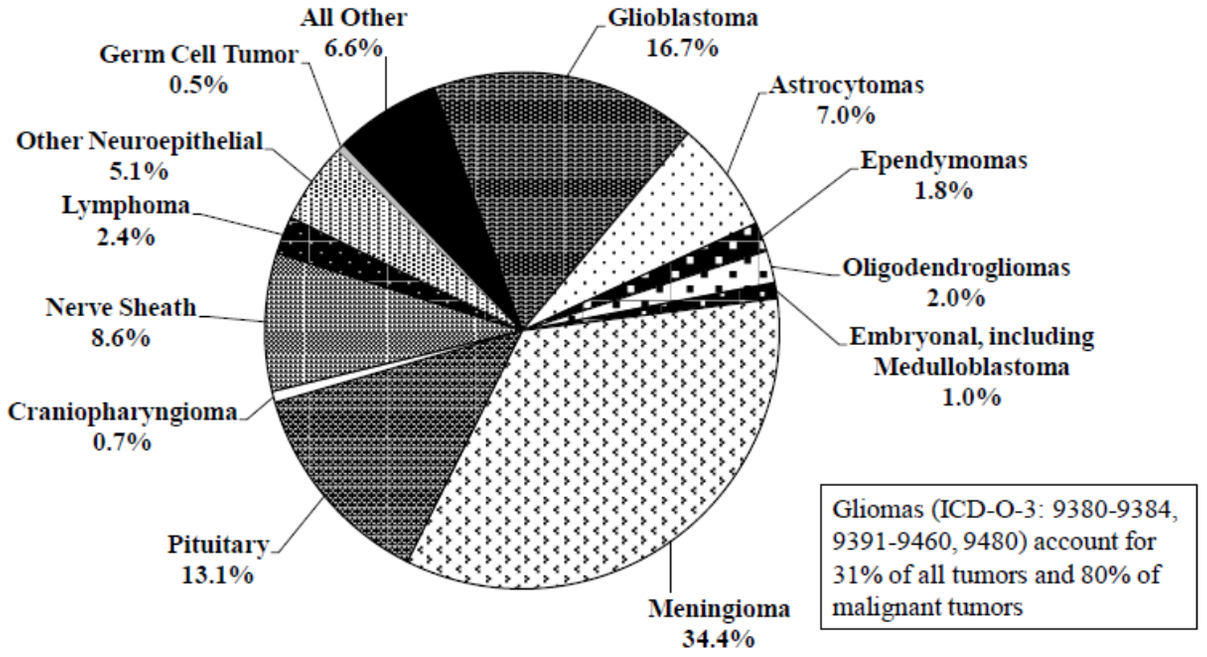

Figure 2. Distribution of All Primary Brain and CNS Tumors by Histology (N=226,791) CBTRUS Statistical Report: NPCR and SEER Data from 2004-2007

adenomas, and miR-132, miR-128a, miR-136, miR-16-1, and let-7 are downregulated[17]. As for meningioma, Feng Zhi et al found a list of 14 miRNAs that were differentially expressed in meningioma compared to normal adjacent tissue(NAT)samples. Twelve miRNAs, including miR-17-5p, miR-22-3p, miR-24-3p, miR-26b-5p, miR-27a-3p, miR-27b-3p, miR-96-5p, miR-146a-5p, miR-155-5p, miR-186-5p, miR-190a and miR-199a were shown to be upregulated by a factor greater than twofold, whereas two miRNAs, including miR-29c-3p and miR-219-5p, were significantly downregulated[18].

3. Abberant miRNAs as prognostic and/or diagnostic marker for brain tumor patients

The use of miRNAs as tumor biomarkers has gained growing interest in the last few years. Accumulating evidence indicates that miRNA expression can be used as a prognostic and/or diagnostic marker for brain tumor patients. Niyazi et al. separated 35 glioblastoma patients into long- and short-term survivors. Then, they found that some miRNAs were significantly different in two group, including miR-3163, miR-539, miR-1305, miR-1260 and let-7a. this is the first dataset defining a prognostic role of miRNA expression patterns in patients with glioblastoma[19]. Moreover, Ma also identified that MiR-196b is overexpressed and confers a poor prognosis via promoting cellular proliferation in glioblastoma patients[20]. Costa et al identified miRNAs that are over-expressed in ependymomas, such as miR-135a and miR-17-5p, and downregulated, such as miR-383 and miR-485-5p. We have also uncovered

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associations between expression of specific miRNAs which portend a worse prognosis. For example, we have identified a cluster of miRNAs on human chromosome 14q32 that is associated with time to relapse. We also found that miR-203 is an independent marker for relapse compared to the parameters that are currently used. Additionally, we have identified three miRNAs (let-7d, miR-596 and miR-367) that strongly correlate to overall survival[21].

4. Deregulated miRNAs regulated brain tumor cell phenotype

Increasing evidence supports the supposition that miRNAs play an important role in different types of cancers and in various aspects of cancer biology. Abnormal miR levels in tumors have important pathogenetic consequences: miRNAs may act as oncogenes or suppressor genes[22]. For examples, Calin et al. [23] identified two clustered miRNAs (miR-15a and miR-16-1) on the minimal deletion region of chromosome 13q14 and showed that the levels of these miRNAs were significantly reduced in the majority (68%) of B-cell chronic lymphocytic leukemia (CLL). Both miRNAs target the anti-apoptotic gene BCL2, which is frequently overexpressed in CLL [24]. These findings indicate that downregulation of miR-15a and miR-16-1 elevates BCL2 level, contributing to CLL formation and suggesting a tumor-suppressive role for both miRNAs. In contrast, miR-17-92 showed marked upregulation in B-cell lymphomas. Enforced expression of miR-17-92 cluster acted with c-myc to accelerate the onset of B-cell lymphoma in a mouse model [25]. The data suggest that miR-17-92 cluster is a potential oncogene.

Recent evidence supports the ability of miRNAs to regulate brain tumor cell phenotype. We had done some work to identified that miR-221 and miR-222 regulated their target expression to co-modulated glioma cell phenotype, including proliferation, cell cycle, apoptosis and invasion(Figure3)[26-28], and so on.

MiRNAs also regulated radio- and chemo-resistence of glioma. Hierarchical clustering analysis of expression 1100 miRNAs in three glioma cell lines treated with clinically relevant doses of radiation (2Gy) revealed significant (3-4 fold) up-regulation of several miRNA that are implicated in stimulation of survival and proliferation of tumor cells [29]. The set of up-regulated miRNAs includes miR-1285, miR-151-5p, and miR-24-1 that display beneficial effects on tumors by inhibiting the core tumor suppressor p53 (miR-1285) and supporting migration, local metastasis (miR-151-5p), and antiapoptosis (miR-24-1) [30]. Overall, activation of these miRNAs might possibly increase tumor radioresistance in subsequent radiotherapy sessions and stimulate motility of cancer cells thereby at least partially explaining the evidence on enhanced migration of malignant glioma cells in response to radiotherapy [31]. The radiation treatment of glioma cell lines with normal capacity to repair radiation-induced double strand breaks (DSB) of DNA caused activation of let-7 [29], a family of miRNA that suppresses proliferation of gliomblastoma cells [30] (Fig. 4). In contrast, in the radiosensitive human glioma cell line M059J that is deficient in DNA-dependent protein kinase (DNA-PK) and has a low activity of ATM, two key members of the non-homologous end joining pathway of DNA-DSB repair, let-7 miRNA was down-regulated [30].

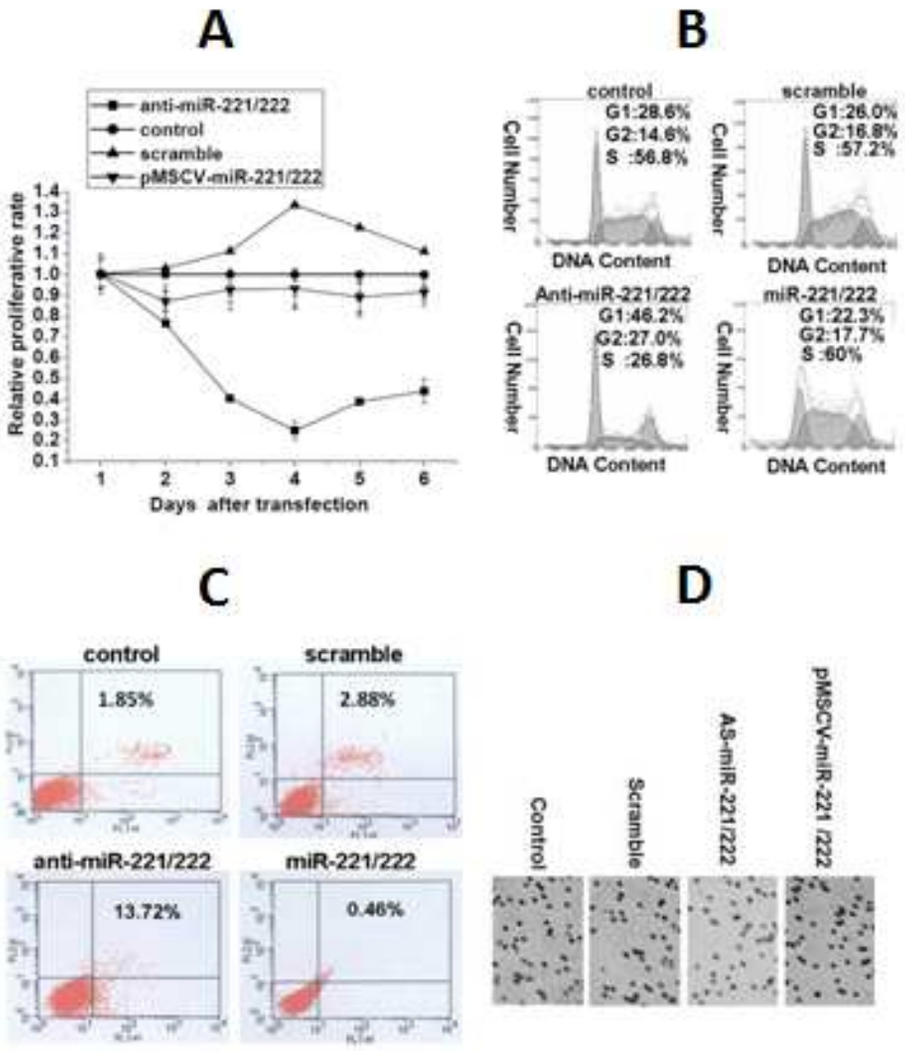

Figure 3. miR-221 and miR-222 regulated their target expression to co-modulated glioma cell phenotype. (A: MTT assay show that knockdown of miR-221/222 lowed proliferated rate of glioma cell; B: knockdown of miR-221/222 induce G1 arrest in glioma cell ;C: Annexin V analysis showed that knockdown of miR-221/222 significantly increased glioma cell apoptosis; D: The transwell assay revealed that knockdown of miR-221/222 significantly decreased glioma cell invasion.

Moreover, MiRNAs were shown to be also involved in the regulation of chemoresistence of glioma by modulating some relevant proteins that are involved in drug metabolism and drug transporter. MiRNAs also affect chemotherapeutic agents induced DNA damage repair (Fig. 4).

5. MiRNAs in gliomas and glioblastoma

MiRNAs affect biological character of varies cells by regulating their targets. So, deregulated miRNAs regulated brain tumor cell phenotype by regulating their targets, including proliferation, cell cycle, apoptosis, invasion, and so on.

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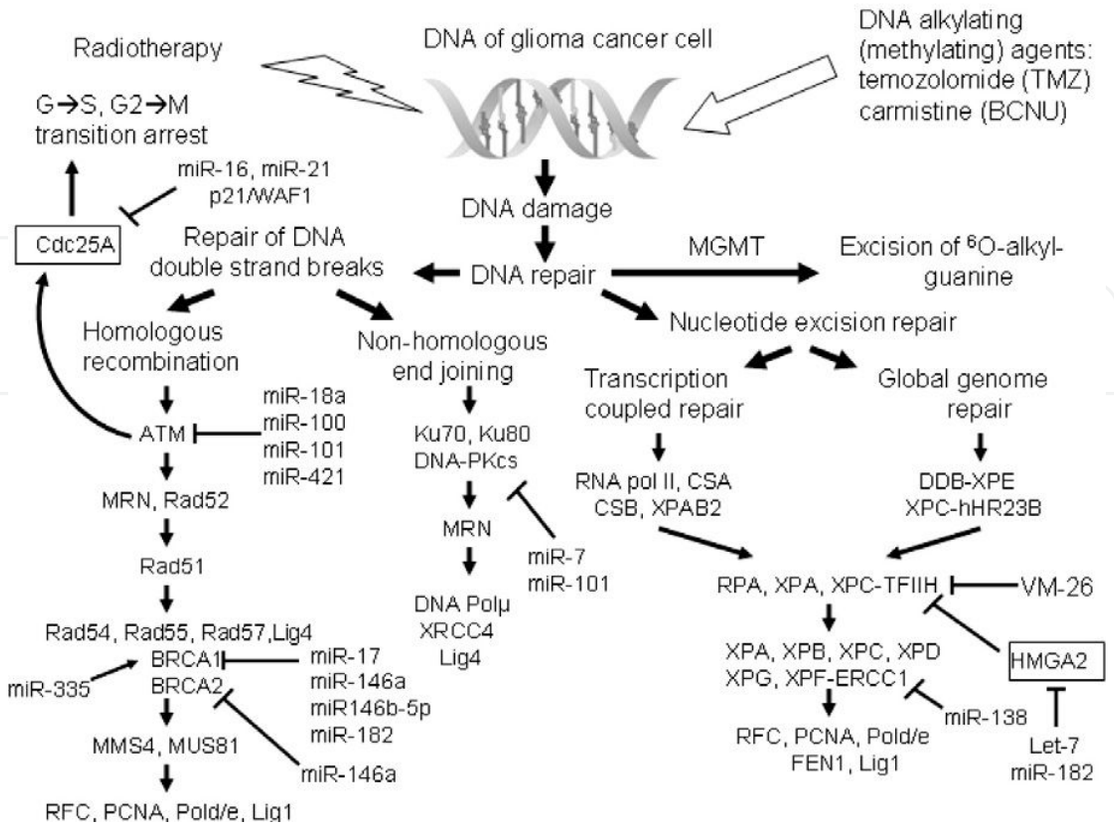

Figure 4. MicroRNAs are involved in the regulation of DNA repair in cancer cells after treatment with ionizing radiation (radiotherapy) and DNA alkylating drugs such as temozolomide and carmistine[30].

5.1. MiR-221 and miR-222

MiR-221 and miR-222 are both up-regulated in several glioma derived cell lines and in glioma samples [26-28]. They are clustered within a 1-kb genomic interval on chromosome X, so they could be generated from the same primary transcript [26].

Two members of the Kip/Cip family of CDK inhibitors and key negative regulators of the cell cycle, p57 and p27, have been identified as putative direct targets of these miRNAs. According to this theory, the 3'-UTR of p57 harbors one site expected to be recognized by both miR-221 and miR-222, whereas the 3'-UTR of p27 contains two sites for both miRNAs. Transfection of both miR-221 and miR-222 greatly reduce the levels of p57 and p27 proteins, while downregulation of either microRNA 221 or 222 in glioblastoma cells caused an increase in p27 levels and enhanced expression of the luciferase reporter gene fused to the p27 3'-UTR. These results suggest that miR-221 and miR-222 could promote cell proliferation and S-phase entry together with further cooperative events and that miRNA 221 and 222 inhibition may be a potential therapeutic target to reduce the aggressive growth of glioblastoma by restoring normal levels of their target proteins [32]. The inverse correlation between mir-221/222 and p27 levels, the impairment of growth potential and the G1 to S shift in the cell cycle of glioma cells after inhibition of mir-221/222, were later confirmed by Zhang et al. They extended their study to in vivo trials and showed that miR-221/222 knocked-down through antisense oligonucleotides

strongly reduced glioma subcutaneous mice xenografts growth through up regulation of p27 [26] and enhanced radiosensitivity of glioblastoma cell lines [33]. Further evidence showed that knockdown of miR-221/222 induced a change of mitochondrial membrane potential and caspase-mediated apoptosis on glioblastoma cells. Indeed, mir-221/222 directly downregulate the proapoptotic protein PUMA, which leads in turn to decrease Bcl-2 and increase BAX. these results confirm the oncogenic role of mir-221/222 on glioblastoma [27]. Our recent studies identified that mir-221/222 increased cell invasion and reduced gap junction intercellular communication (GJIC) by regulating TIMP3 and Cx43[28,34].

5.2. MiR-21

Microarray profiling studies, further validated by Northern blotting qRT-PCR and real-time PCR analysis, showed that miRNA-21 is strongly elevated in glioma and glioblastoma tumor samples and glioma cell lines when compared to non-neoplastic control samples, and upregulation is particularly prominent in grade IV astrocytomas [35-37]. High expression of miR-21 was significantly associated with poor patient survival, suggesting that, in combination with other aberrant expressed miRNAs (low expression of miR-181b or miR-106a), miRNA-21 profiling has potential application as novel diagnostic and prognostic indicator [37].

Suppression of mir-21 in glioblastoma cells decreased the metabolic activity of cell culture and cell number, and was associated with a marked increase in apoptosis and caspase activation [35]. MiR-21 knockdown also leads to a considerable reduction of glioma volumes in mouse xenografts [38]. Knockdown mir-21 inhibited proliferation and cell invasion and induced apoptosis through the activation of caspase-3 and -9 in glioma cell lines, which may be related to a mir-21-dependent modulation of multiple potential target genes, such as TIMP3 [39,40].

From an in silico constructed study of miR-21 predicted targets and further pathway analysis of computer generated lists of the identified target genes, emerged that mir-21 targets multiple components of the p53, transforming growth factor-β (TGF-β) and mitochondrial apoptosis pathways, that contribute to its tumor promoting and antiapoptotic activity. The phenotypic effects observed upon downregulation of miR-21 in glioblastoma cells, reflect the repression of these pathways and result in significant increase in apoptotic cells, reduced growth and cell cycle arrest at G0-G1 [41]. Further bioinformatics analysis evidenced that multiple genes involved in apoptosis pathways (such as PDCD4, MTAP, and SOX5), carry putative miR-21 binding sites. PDCD4 mRNA is a direct functional target of miR-21 and its expression inversely correlates with mir-21 in a number of glioblastoma cell lines (T98G, A172, U87, and U251). Consistent with these observations, downregulation of mir-21 restored protein level of PDCD4 while, ectopic overexpression of mir-21, inhibits PDCD4-dependent apoptosis [42]. Mir-21 controls tumor invasiveness and microvascular proliferation by regulating the expression of two of the major inhibitors of matrix metalloproteinases (MMPs): RECK and TIMP3. Mir-21 knockdown decreases RECK and TIMP3 protein levels and MMPs activity both in vivo and in vitro leading to a reduction of glioma cell motility and invasion [43]. Although PTEN tumor suppressor gene has been validated as a miR-21 target by computational analysis, down-regulation of mir-21 leads to an inhibition of tumor growth in glioblastoma cell lines and xenograft tumors independently of PTEN mutational status [44,45]. MiRNA expression profile

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scanning data after inhibition of miR-21, strongly indicated that BID, FAS, PRS6, and SOCS4 tumor suppressor genes were upregulated and evidenced a suppression of EGFR, activated Akt, cyclin D, and Bcl-2. This study highlighted that miR-21 targets multiple pathways responsible of inhibition of glioma growth in absence of PTEN [46].

Further findings strengthened the hypothesis that miR-21 represents a promising target to improve the efficacy of chemotherapy. In a recent study mir-21 was shown to play a key role in promoting human glioblastoma cells U87MG resistance against the antitumoral agent temozolomide (TMZ). Indeed, ectopic overexpression of mir-21 significantly reduced TMZ-induced apoptosis in this cell line through a suppression of Bax/Bcl-2 ratio and caspase-3 activity. These results confirm the hypothesis that mir-21 overexpression during glioma progression could be responsible of clinical resistance to chemotherapy with the promising alkylating agent TMZ [47]. Li et al demonstrated that mir-21 is also involved in glioblastoma cell chemoresistance to the chemotherapeutic agent VM-26, as shown by the observation that miR-21 knockdown sensitized GBM cells to VM-26. This is likely to happen because mir-21 regulates and inhibit LRRFIP1 gene expression by direct interaction [48]. This gene encodes a protein, also known as the TRAF-interacting protein (TRIP), that inhibits NF-κB signaling, a pathway that is known to be responsible for protection against apoptosis [49] and tumor chemoresistance [50]. Additionally, suppression of miRNA-21 expression in glioblastoma cell lines enhances sensitivity of cancer cells to antineoplastic cytotoxic therapy with neural precursor cells (NPC) expressing a secretable variant of the cytotoxic agent tumor necrosis factor related apoptosis inducing ligand (S-TRAIL). The synergistic effect was observed both in vitro, through an increased caspase associated cytotoxic death, and in vivo, where miR-21 knockdown and NPC-mediated S-TRAIL reduce cancer growth in tumor xenografts [38]. A recent study that evaluated the potential role of mir-21 as a therapeutic tool to enhance the cytotoxic effect of standard chemotherapy showed that co-delivery of miR-21 and 5-fluorouracil (5-FU) by using a poly(amidoamine) (PAMAM) dendrimer as a carrier, significantly improved the cytotoxicity of the antitumor agent leading to an higher apoptosis rate and a reduction of the migration ability of tumor cells [51]. Finally, downregulation of miR-21, contributes to the antitumor effects of IFN-β on glioma cell and intracranial tumor xenografts and the activation of the transcription factor STAT3 may have a key role in the IFN-β mediated suppression of mir-21 [52].

5.3. Mir-34a

Mir-34a, transcriptional target of p53 located within chromosome 1p36, has been proposed as a potential tumor suppressor. MiR-34a is also downregulated in glioblastoma tissues and cell lines compared to normal brain tissues and is markedly reduced in p53-mutant cells compared to cells expressing wild-type p53 [53,54].

MiR-34a possesses hundreds of predicted mRNA targets which could mediate its inhibitory effects on tumor growth. A few of these have been experimentally verified and include oncogenes such as MYC, CCND1, CDK6, SIRT1 [55] and c-Met [56]. It was shown to directly inhibit c-Met in glioma and medulloblastoma cells and Notch-1/Notch-2 in glioma cells and stem cells by binding to the 3'-UTRs of their mRNA. Furthermore, mir-34a inversely correlates

with c-Met, Notch1, Notch2 and CDK6 protein expression in glioma cells. Transfection of microRNA34a in brain tumor cells strongly inhibits cell proliferation, cell cycle progression, cell survival, cell invasion, and in vivo glioma xenograft growth but do not affect cell cycle or death when transfected to human astrocytes, which showed normal expression levels of microRNA 34a. Restoration of c-Met or Notch-1 and Notch-2 expression by constructs lacking 3'-UTR regions partially reverted cell cycle arrest and apoptosis induced by miR-34a in glioma cells or stem cells, confirming the hypothesis that the antitumor effects of miR-34a are achieved via targeting of multiple oncogenes [53]. Interestingly, mir-34a also affect glioma stem cell differentiation and growth. Luan et al recently reported silent information regulator 1 (Sirt1) as a direct negative target of mir34a in glioma cell lines at a posttranscriptional level [54]. Sirt1 is a NAD-dependent deacetylase that regulates apoptosis in response to oxidative and genotoxic stress, and has recently been involved in tumorigenesis acting as an oncogene [57].

5.4. Mir-128

Mir-128, a brain enriched microRNA, is downregulated in glioma cell lines and tissue when compared to normal brain samples [58]. Mir-128 overexpression decreased glioma cell proliferation in vitro and glioma xenograft growth in vivo, and is inversely correlated to Bmi-1 expression. Mir-128 directly targets and downregulates Bmi-1 by binding its 3'-UTR region [59] and leads to a concomitant overexpression of p21CIP1 and a decrease in phosphorylated Akt [58]. Bmi-1 expression is a critical factor of normal stem cell maintenance and glioblastoma self-renewal and loss of Bmi-1-mediated self-renewal of neural stem cells has been shown to be associated with upregulation of p21CIP1 [60] and decreased Akt activation [61]. Consistently with these observations, miR-128 overexpression in human glioma neurosphere cultures possessing a glioma 'stem-like' cell phenotype, evidenced that miR-128 specifically blocked glioma self-renewal reducing neurosphere number and size. In conclusion, mir-128 downregulation is likely to enhance glioma tumorigenesis by promoting an undifferentiated phenotype and self renewing state through Bmi-1 increased expression [58]. By bioinformatical analysis, E2F3a, a transcription factor involved in cell cycle progression [62], has been identified as a direct target of this miRNA. Indeed, Mir-128 and E2F3a levels are negatively correlated and mir-128 overexpression has similar inhibitory effects on proliferation of glioma cell lines as E2F3a knocking down Ectopic overexpression of E2F3a partially reversed the effects of mir-128, suggesting that mir-128 could exert its antitumor effects at least partially by inhibiting E2F3a expression. Among its target genes angiopoietin-related protein 5 (ARP5) was identified with bioinformatical tools and then confirmed to be inversely correlated to mir-128 levels in glioblastoma cells and tissue and downregulated after ectopic overexpression of this miRNA [58]. ARP5 seems to be a key regulator of cell proliferation, remodeling and regeneration [63] and could be a member of a group of genes regulated by mir-128 that coordinately contribute to glioma and GBM pathology [59].

5.5. Mir-451

Mir-451 was found to be upregulated in glioma samples compared to normal brain on microarray-based miRNAome profiling [64]. MiR-451 showed a striking spatial distribution,

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with groups of positive cells concentrated around a subset of blood vessels. Moreover, high levels of mir-451 are correlated to a poor prognosis in glioma patients. Mir-451 could regulate the balance of proliferation and migration in glioma cells in response to changes in glucose levels and metabolic stress. It regulates 5'-adenosine monophosphate activated protein kinase (AMPK) pathway in response to glucose levels in glioma cells, through the modulation of the activity of its upstream activator LKB1 [65]. Activation of LKB1 is a potent anti-proliferative signal, and also influences cell polarity, a process known to affect cell motility [66].

MiR-451 regulates LKB1 activity by directly targeting CAB39, a component of the active LKB1complex, leading to a downregulation of CAB39 protein levels. Down-regulation of mir-451 levels in response to glucose deprivation leads to intense activation of LKB1 and downstream pathways, allowing cells to survive metabolic stress and promoting cancer cell migration. Conversely, when glucose is sufficient, elevated miR-451 levels lead to reduced LKB1/AMPK pathway activation. This facilitates cell proliferation but makes cells more sensitive to glucose deprivation. Thus mir-451 could be a key regulator of adaptive response of cancer cells to altered energy availability [65].

In partial contrast with these studies a recent report found mir-451 to be downregulated in glioma cell lines. Transfection of mir-451 to glioma cell lines was able to inhibit cell growth, induced G0/G1 phase arrest, increased cell apoptosis and most notably diminished the invasive capacity of these cells as evidenced by transwell invasion assay. These tumor suppressive effects may be due to modulated expression of a panel of proteins including CyclinD1, p27, MMP 2/9 and Bcl-2, probably via regulation of the PI3K/AKT signaling pathway. Indeed, expression of Akt1 protein decreased after mir-451 transfection [67].

5.6. Mir-7

Mir-7, a miRNA modulated during neural differentiation of embryonic stem cells is a putative tumor suppressor gene in glioblastoma. It inhibits EGFR gene expression by directly binding its mRNA at 3'-UTR. Furthermore, miRNA-7 suppresses the activation of Akt pathway and reduces phospho-Akt levels by directly targeting its upstream regulators IRS-1 and IRS-2 [68]. They also identified Raf1 as a direct target of miR-7 by microarray analysis, Western blot and luciferase reporter assays of glioblastoma cells transfected with miR-7 [69]. Inhibition of Akt activation seems to occur independently of mir-7-mediated repression of EGFR pathway. Consistenly with these findings miRNA-7 was markedly downregulated in glioblastoma tissue when compared to normal brain. The proposed mechanism that explains the lower expression of this miRNA is a processing defect in generating pre-miR-7 from pri-miR-7 in glioblastoma. Pre-miRNA-7 transfection decreased viability and invasiveness of glioblastoma lines and glioblastoma derived stem cells and led to an increase in the sub-G0 apoptotic fraction, a decrease in the S fraction and determined a G2-M arrest, most likely by affecting EGFR and Akt pathways, which have an established key role in gliomagenesis [68]. Indeed, EGFR is frequently amplified and highly expressed in glioblastomas [26]. In a recent report mir-7 was also shown to directly inhibit p21-activated kinase 1 (Pak1) in non-brain cancer cells [70]. Pak1 is a widely upregulated signaling kinase in multiple human cancers, and it is involved in the regulation of many signaling pathways, including EGFR and Akt, thus

interfering with cell motility, proliferation, and apoptosis [71]. Furthermore, in glioblastomas, PAK1 upregulation is a negative prognostic marker and its knockdown results in impaired cell invasion [72]. These preliminary results suggest mir-7 could have a potential and promising therapeutic role in brain tumors.

5.7. Mir-181

Mir-181a and mir-181b are well known brain-enriched miRNAs [73]. Downregulation of mir-181a and mir-181b was detected in both glioma samples and cell lines [74]. Transfection of both these miRNAs on glioma cell lines results in cell growth inhibition, invasion reduction and apoptosis induction, miR-181b being more effective than miR-181a [74]. In a recent miRNA expression profiling conducted on a set of 124 astrocytoma samples and 60 normal adjacent tissue samples by qRT-PCR, Zhi et al found that downregulation of miR-181b is significantly associated with poor patient survival independently of other clinicopathological factors. Thus, mir-181b has a great potential for being used as a diagnostic and prognostic marker and to better select patients for adjuvant therapy [39].

In a recent study MiR-181b and miR-181c downregulation was found to be significantly associated to a positive response to concomitant chemoradiotherapy with temozolomide in glioblastoma patients, suggesting they could be used as predictive factors for therapy efficacy [75].

5.8. Mir-124/137

MiRNA-124 and miRNA-137 are downregulated in anaplastic astrocytomas and glioblastoma multiforme relative to non-neoplastic brain tissue [9-10]. Consequently, growth factor signaling could promote brain tumor formation through suppression of miR-124 and/or miR-137 expression and inhibition of neural stem cells/tumor stem cell differentiation. Transfection of mir-124 and mir-137 to neural stem cells and tumor-derived stem cells leads to induction of cellular markers of differentiation, G1 cell cycle arrest and reductions of the expression of the cell cycle regulator CDK6, a direct target of both miRNAs. These changes are accompanied by reduced self-renewal and tumorigenicity. Overexpression of miR-124 or miR-137 also reduced the expression of phosphorylated RB, a downstream target of CDK6 [76]. These results suggest that ectopic expression of miRNA-124 and miRNA-137 could represent a valid therapy for glioblastoma multiforme treatment inducing differentiation of tumor cells and cell cycle arrest.

5.9. MiR-125

Mir-125b downregulated in glioma cell lines after treatment with ATRA (all-trans-retinoic acid), a regulator of neural differentiation and proliferation. Ectopic overexpression of mir-125b stimulated glioma cell proliferation, partially recovering the cell growth inhibition induced by ATRA treatment, while mir-125b knockdown promoted ATRA-mediated cell apoptosis. Furthermore, Bmf was identified as a direct target of miR-125b, and they are inversely correlated [77]. Bmf interacts with the prosurvival Bcl-2 proteins, regulating cellular apoptotic pathways [78]. Indeed, transfection with miR-125b increase BCL-2 levels in glioma

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cells, and expression of BCL-2 was significantly decreased when cells were transfected with miR-125b inhibitor. Thus, Bmf may play an important role in the process of miR-125b influencing cell apoptosis [77].

Recently, Cortez et al identified that mir-125a downregulated in glioblastomas and particulary in glioma stem cells CD133+. Overexpression of mir-125a inhibits invasion properties of glioblastomas, probably through a direct downregulation of podoplanin (PDPN), a membrane sialo-glycoprotein related to invasion and malignancy [79].

5.10. MiR-326

Kefas et al evidenced a feedback loop between miRNA-326 and Notch pathway, frequently deregulated in gliomas. Notch-1 knockdown induced mir-326 upregulation in glioma stem cells, and ectopic overexpression of mir-326 decreased Notch pathway members level and activity. Forced expression of mir-326 also inhibited cell proliferation, viability and invasiveness and induced apoptosis in both established and stem cell-like glioma lines, these effects are at least partially explained through downregulation of the Notch pathway. Moreover, it reduced tumorigenicity in mouse glioma xenografts. Together with the observation that mir-326 is downregulated in glioblastomas, these findings suggest that miR-326 is a potential tumor suppressor in glioma cells and that reversing Notch/miR-326 axis toward miR-326 prevalence appears to be a potential therapy for brain tumors [80].

5.11. Mir-26a

Mir-26a up-regulated in high-grade gliomas. MiR-26a is a direct negative regulator of PTEN and significantly represses PTEN expression. MiR-26a overexpression was attributed to the amplification of miR-26a-2 locus (chromosome 12q), one of the two miR-26a loci present in the human genome (mir-26a-1 and miR-26a-2) and is correlated with monoallelic PTEN deletion. It was suggested a temporal sequence to the molecular evolution of miR-26a-amplified gliomas, with PTEN loss most likely preceding miR-26a-2 amplification and, concordantly, miR-26a overexpression in genetically engineered PTEN+/- mice precluded loss of their remaining PTEN allele. Amplification of mir-26a is likely to promote the silencing of the remaing PTEN transcript in PTEN+/- tumors, event analogous to a loss of heterozygosity. Furthermore, in a murine glioma model, PTEN repression mediated by overexpression of miR-26a, enhanced de novo tumor formation. Akt pathway activation and suppression of its negative regulator PTEN are particularly important and frequently occur in glioma development. These observations document a new epigenetic mechanism for PTEN regulation in gliomagenesis, further highlighting that dysregulation of Akt signaling is crucial to the glioma development and could be modulated through manipulation of miRNA expression [64]. Moreover, MiR-26a is a frequent target of the 12q13.3-14.1 amplicon that also contain CDK4 and CENTG1, two oncogenes that regulate the RB1 and PI3 kinase/AKT pathways, respectively. The presence of this amplification is negatively correlated to patient survival. PTEN, RB1, and MAP3K2/MEKK2 were detected as functional targets of mir-26a in glioblastoma. Ectopic overexpression of miR-26a increased GBM cell growth, decreased apoptosis and enhanced gliomagenesis in vivo. Thus, miR-26a decreases PTEN expression, activates AKT,

and promotes tumor growth. Using human U87 GBM cells that lack functional PTEN, mir-26a overexpression increased cell growth and decreased apoptosis despite the absence of PTEN, most probably by downregulating RB1 and MAP3K2, a gene that encodes MEKK2[81]. MEKK2 is a mitogen-activated protein kinase kinase involved in JNK activation pathway that promote apoptosis in GBM cells [82].

5.12. Mir-10b

Mir-10b is upregulated in glioma samples and glioma cell lines compared to non-neoplastic brain tissues. RhoC and urokinasetype plasminogen activator receptor (uPAR), which are known to contribute to glioma invasion and migration, were correlated with mir-10b expression in gliomas, probably via inhibition of the translation of the mRNA encoding homeobox D10 (HOXD10], which in turn represses the expression of these genes [83]. These results suggest that mir-10 could be involved in regulation of the invasion and migration abilities of gliomas.

5.13. Mir-15b

Mir-15b was shown to be elevated in a panel of glioma cell lines. Transfection of glioma cells with miR-15b significantly increased G0/G1 cells and decreased the percentage of cells in S phase, while inhibition of mir-15b increased G0/G1 cell amount. Thus, miR-15b regulates cell cycle progression in glioma cells by targeting cell cycle-related factors as CCNE1 (cyclin E1], a validated downstream target of mir-15b [84].

5.14. Mir-146b

Mir-146b, a miRNA found to be downregulated in human glioma tissue, could exert an antitumor activity by reducing the expression of a matrix metalloproteinase gene, MMP16, one of its direct downstream targets. Indeed, transfection of U373 glioma cells with miR-146b precursor decreased tumor cell migration and invasion, while inhibition of miR-146b by LNA modified anti-miR-146b produced the opposite effect, without affecting cell proliferation [85].

5.15. Mir-296

Recent studies support a role of mir-296 in promoting angiogenesis in gliomas. When brain microvascular endothelial cells were co-cultured with U87 glioma cells or when vascular endothelial growth factor (VEGF) was added to cultured human brain microvascular endothelial cells, miR-296 was upregulated. Moreover, mir-296 levels were higher in endothelial cells isolated from human brain tumors compared to normal brain endothelial cells. Downregulation of miR-296 in endothelial cells resulted in the inhibition of morphologic characteristics associated with angiogenesis and reduced angiogenesis in glioma xenografts in vivo. This probably happens through the downregulation of the hepatocyte growth factor-regulated tyrosine kinase substrate (HGS), a validated target of mir-296, thus leading to a reduced HGS-mediated degradation of the platelet derived growth factor receptor (PDGFR) and vascular endothelial growth factor receptor (VEGFR). This result points out an interesting feedback loop, where VEGF indu-

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ces miR-296 expression, which in turn increases cell response to VEGF. Consequently, manipulating mir-296 expression, could enable to control a key step in cancer angiogenesis [86].

5.16. Mir-29

Mir-29b was found to be significantly downregulated in glioblastoma samples and cells and CD133+ tumor stem cells. Forced overexpression of this miRNA inhibited invasion and proliferation and induced apoptosis in glioblastoma cells. Mir-29b as well mir-125a directly targets podoplanin (PDPN), a putative marker of neural stem cells, related to invasion and malignancy in glioblastoma [13].

5.17. MiR-17-92 cluster

MiR-17-92 cluster, located within the locus 13q31-q32, encloses five miRNAs (miR-92-1, miR-19a, miR-20a, miR-19b, miR-17-5p), which have been frequently involved in tumorigenesis [87]. This cluster was detected to be amplified in glioblastoma samples. Furthermore, expression of miR-17-92 cluster is downregulated upon induction of differentiation of GBM spheroid cultures by using ATRA. Induction of differentiation leads to deregulation of most of the key pathways associated with self-renewal such as insulin-like growth factor 1 signaling, vitamin D receptor/retinoic acid X receptor activation, Wnt/β-catenin signaling and retinoic acid receptor activation. Inhibition of miR-17-92 cluster leads to decreased cell viability and decreased cell proliferation probably through a de-repression of CDKN1, E2F1, PTEN and CTGF which are upregulated. Particularly, connective tissue growth factor (CTGF) gene was shown to be a direct target of miR-17-92 in glioblastoma spheroids by luciferase reporter assays [88]. CTGF binds vascular endothelial growth factor (VEGFA), which is a central mediator of angiogenesis [89], and inhibits VEGFA-induced angiogenesis [90]. Conversely, VEGFA was shown to inhibit MiR-17-92 [91], thus explaining the concomitant downregulation of VEGFA and miR-17-92 upon induction of differentiation. In conclusion, the interaction between CTGF, VEGFA and miR-17-92 might have a key role in gliomagenesis by targeting multiple regulatory pathways [88].

5.18. Let-7

Let-7 expression is not downregulated in human glioblastoma tissues and cell lines, let-7 ectopic overexpression by transfection on U251 and U87 human glioblastoma cells, reduced in vitro proliferation and migration and also in vivo tumor growth after xenotransplantation into nude mice. Furthermore, let-7 miRNA reduced the expression of total RAS, N-RAS, and K-RAS in glioblastoma cells [92]. These results suggest that let-7 miRNA is able to impair glioblastoma growth and cellular migration via RAS inhibition.

6. MicroRNAs and tumor stem cells

Glioma stem cells (GSCs) have been recently identified [93]. They express common neural stem cell (NSC) markers (CD133, Nestin, Musashi, and Sox2) and display multiple-lineage differ-

entiation potential and a greater tumorigenic activity in rodent xenografts. GSCs also show an increased angiogenic potential through a higher expression of vascular endothelial growth factor (VEGF). GSCs are strongly resistant to radiation [94]and chemotherapy [95].

A recent study by Lavon et al showed a similar expression pattern of mir-21, mir-124, and mir-137 in gliomas and stem cells. Among them we mention the miR-17 family cluster that contains 3 miRNAs upregulated in gliomas and NPCs (mir-17-92, mir-106b-25, mir-106a); the mir-183-182 cluster, also upregulated in gliomas and NPCs; the large 7+46 bipartite miRNA cluster on chromosome 14, as most of miRNAs located within this region have been shown to be downregulated in the same samples [96]. This latter cluster is located within a region which shows a frequent loss of heterozygosity in glioblastomas [97]. Finally, mir302-367 cluster on chromosome 4q25 and mir371-373 cluster on chromosome 19q13, are upregulated in gliomas and NPCs. These data confirm the hypothesis that brain cancers arise from multipotent GSCs, thus explaining the phenotypic heterogeneity of tumors.

Many groups investigated the role of miRNAs in GSCs so far: in a first report Silber et al found that mir-124 and mir-137 are reduced in grade III and IV gliomas compared to normal brain. Besides, transfection of these two miRNAs in neural stem cells and glioma cancer cell lines leads to induction of cellular markers of differentiation, G1 cell cycle arrest and reductions of CDK6, thus indicating a hypothetical tumor suppressor role of micro-124 and microRNA-137 in GSCs [29]. As previously mentioned in this review, mir-128 was identified as a negative regulator of glioma self renewal when ectopically overexpressed on human glioma neurosphere cultures [58].

A recent analysis showed that mir-451 is significantly downregulated in CD133+cells. Transfection of miR-451 inhibits proliferation and neurosphere formation in GSCs, highlighting that it can act as a tumor suppressor: two target sites for SMAD protein in the upstream promoter region of miR-451 have been found, leading to draw the conclusion that this miRNA is activated by SMAD pathway. Transfection of SMAD in GBM cells inhibited their growth, suggesting it could induce differentiation of glioma CD133+stem cells through up-regulation of miR-451, thus reducing their tumorigenicity [98].In conclusion, a stable and selective delivery of these miRNAs to GSCs could represent a great advance for brain tumor therapy.

MiR -125b is required for stem cell fission, allowing them to bypass the G1/S checkpoint and making them insensitive to chemotherapy [99]. It has been found to be significantly downregulated in CD133+glioma stem cells compared to CD133-ones, leading to the hypothesis that it may be involved in cell differentiation. As expected, transfection of mir-125b to CD133+cells, decreased the number of proliferating cells and induced G0-G1 arrest: this effect occurs through a mir-125b-dependent downregulation of CDC25A and CDK6, two cell cycle regulatory proteins [47]. Moreover, miR-29b and miR-125a, are under-expressed in glioblastoma CD133+cells compared with their counterpart CD133-cells (see above), suggesting a potential role for these microRNAs in regulation of signaling pathways related to maintenance of stem cell properties and self-renewal of cancer cells [79].

Some miRNAs could have a role in the regulation of key pathways of GSCs. We already mentioned above the existence of a regulatory feedback loop between the tumor suppressor

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mir-326 and Notch pathway, shifted towards a prevalence of Notch activity in brain tumor cells and GSCs [80] Recently, pyruvate kinase type M2 (PKM2) has been identified as a putative target of mir-326, as levels of PKM2 and mir-326 are inversely correlated in glioma cells. PKM2, highly expressed on cancer cells and GSCs, plays the role of mediator in mir-326 metabolic effects: experimental knockdown of this molecule led to an impairment of glioma invasiveness and clonogenicity and decreased ATP levels, suggesting that PKM2 could represent a valid target for glioma therapy [100].

Mir-34a downregulated in gliomas and GSCs. When transfected into GSCs, it decreased the expression of the stem cell markers CD133 and nestin and caused an higher immunostaining for astrocytes and oligodendrocytes markers, besides modestly inhibiting several malignancy end-points (migration, survival, proliferation, cell cycle progression). Remarkably, miR-34a levels in glioma stem cells are significantly lower than in differentiated wild-type p53 glioma cell lines, suggesting that restoration of miR-34a expression for therapeutic purposes could achieve strong anti-tumor effects not only by targeting differentiated glioma cells, but also by inducing glioma stem cell differentiation [56].

7. MicroRNAs in medulloblastoma

Medulloblastoma (MB) is the most common brain malignancy observed in childhood (WHO grade IV) [101]. Ferretti et al were the first showed that miRNAs profiles is different between primary medulloblastomas and normal cerebellar controls. These aberrant miRNAs have a potentially role in MB development: e.g., let-7 microRNAs has been shown to inhibit Ras oncogene expression [102], reported to be a key factor for MB metastatic behavior [103]; miR-17-92 cluster cooperates with myc, frequently overexpressed in MB, to induce neoplastic transformation [104]; miR-9 and miR-125a, both downregulated in MB, are involved in cell proliferation and, when transfected into MB cells, promote apoptosis and impair anchorage-independent growth by downregulating the truncated isoform of the neurotropin receptor TrkC (t-TrkC). T-TrkC expression levels are higher in MB, inversely correlate with miR-9 and miR-125a levels and are responsible of enhanced cell proliferation and worse prognosis. Mir-9 and mir-124 have a common molecular target, REST/NRSF (RE1 silencing transcription factor/ neuron-restrictive silencer factor) complex [105]. These observations suggest that Mir-9 and mir-124 could play an important role in cerebellar tumorigenesis as REST inactivation has been reported to inhibit tumor growth [106] and is overexpressed in many MBs [107]. Consequently, a REST/mir-124 axis shifted towards a prevalence of REST activity, could block neuronal differen-tiation and promote neoplastic transformation [108].

Mir-124 also modulates medulloblastoma growth by targeting CDK6, a key pro-proliferative factor overexpressed in 30% of medulloblastomas, which represents an adverse prognostic marker for clinical outcome [109]. The role of mir-124 in MB development was later confirmed by Li et al who additionally demonstrated that ectopic expression of mir-124 in medulloblastoma cell lines inhibits cell growth by directly targeting SLC16A1, a protein upregulated in MBs, that serve as a carrier to export lactic acid extracellularly, thus maintaining homeostasis

of tumor cells, where aerobic glycolysis is known to be accelerated [110].At the same time, downregulation of miR-218 could account for an overexpression of the pro-oncogene EGFR, which activates MAPK pathway and CTNND2, which in turn encodes the gene of β-catenin that activates the APC/Wnt signal transduction pathway and leads to tumor growth [111].

Hedgehog (Hh) patched (Ptch1) signalling pathway is a key regulator of the development of cerebellar granule cell progenitors and its constitutive activation makes cells susceptible of malignant transformation into medulloblastoma [112]. Hh is a secreted protein that binds to the transmembrane receptor Ptch1 transducing an intracellular signal through the protooncogene Smoothened (Smo) to the downstream transcription factors Gli1, Gli2, and Gli3 [113].

Ferretti et al found that miR-125b, miR-326 and miR-324-5p target and repress activator downstream components of the Hh signalling pathway (Smo and Gli1); consistent with these findings, 50% of MBs showed a downregulation of these miRNAs together with an overexpression of Gli1. Deletion of chromosome 17p, the most frequent mutation in medulloblastoma, could account for several genetic defects, including miR-324-5p, p53 and HIC1 tumor suppressors loss, which cooperate in Hh-dependent tumorigenesis: loss of these genes, together with other molecular events, contribute to a persistent hyperactivation of Hh signalling during cerebellar granule cell progenitors development, leading to higher proliferative activity and susceptibility to malignant MBs [114].

Recently, Hedgehog signalling pathway has been identified as a target of miR-17-92 cluster. Indeed, miRNAs from the miR-17-92 cluster are specifically overexpressed in mouse MB models with specific initiating mutations in Ptch1 and in human MB subgroups with an activated SHH signaling pathway. To evaluate its oncogenic potential, miR-17-92 was retrovirally transduced into mouse granule neuron progenitors cells (GNPs) before orthotopic transplantation into immunocompromised mice. Interestingly, only cells with an SHH signaling defect (Ptc+/-) developed MBs. Tumor cells from this model exhibited markers of activated SHH signaling as elevated Math1 and Gli1 mRNA levels, and lost expression of the wild-type Ptc allele, reinforcing the hypothesis of a functional link between the SHH pathway and this miRNA cluster [115]. Some studies showed that miR-17-92 is most highly expressed in SHH-driven medulloblastomas, and higher levels of miR-17-92 are also related to an overexpression of the oncogene MYC. Moreover, transfection of miR-17-92 maintains mouse CGNP cells in a proliferative state in the absence of SHH, and synergizes with SHH to promote cell growth, while treatment of the same cells with SHH results in upregulation of miR-17-92, confirming that this cooperation is crucial in MB tumorigenesis [116].

Notch pathway plays a key role in regulating granule-cell progenitor differentiation and an increased copy number of Notch-2 was detected in 15% of medulloblastomas [117]. Expression of the downstream effector of Notch, HES1, normally declines during neuronal differentiation, while persistent activation of this factor prevents differentiation of precursor cells [118]. Garzia et al identified mir-199-5p as capable of directly targeting and repress expression of HES1 in MB cell lines. Ectopic expression of this miRNA reduced MB cell proliferation, population expressing stem cell marker CD133 and xenograft tumor growth in mouse models. Their studies also suggest that the documented downregulation of miR-199b-5p in metastatic tumors may be related to epigenetic silencing [119].

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Venkataraman et al recently showed that several miRNAs known to be involved in CNS development, are downregulated in medulloblastoma cell line cultures and tissues. A specific one, miR-128a, is able to decrease tumor growth and proliferation if ectopically re-expressed on MB cells. Bmi-1 has been identified as a putative target of miR-128a, a critical factor in cerebellar development frequently upregulated in MBs. MiR-128a seems to normally down-regulate Bmi-1 oncogene, leading to increased levels of p16, a cell cycle inhibitor. Furthermore, Bmi-1 could be involved in regulating reactive oxygen species by decreasing superoxide generation, thus leading to a lower redox state in cancer stem cells [120]; this event is known to be partially responsible of tumor resistance against common therapies [121].

Finally, mir-34a appeared to be a tumor suppressor gene also in medulloblastomas other than in glioblastomas. Indeed, transient transfection of miR-34a into medulloblastoma cell lines, strongly inhibited cell proliferation, cell cycle progression, cell survival and cell invasion most probably through a direct inhibition of c-Met [56].

8. MiRNAs in pituitary tumors

Pituitary adenomas are benign and frequent neoplasms, accounting for about 15% of primary intracranial tumors [122]. Bottoni et al found that 30 miRNAs are differentially expressed between normal pituitary gland and pituitary adenomas. Furthermore, their expression is inversely correlated with tumor size. They probably act through a negative regulation of RARS (arginyl-tRNA synthetase), which is in turn upregulated in pituitary adenomas and modulates the expression of factors influencing pituitary tumor growth [123]. Mir-15a and mir-16-1 are located within chromosome region 13q14. Interestingly, loss of 13q region of chromosome 13 was frequently detected in pituitary tumors, confirming that this region likely contains tumor suppressor genes [124].

These findings support the hypothesis that miRNA-16-1 plays a key role in tumor growth [195], most probably by interacting with BCL2 [125], which is overexpressed in about one third of pituitary adenomas [126]. MiRNAome could also function as a signature for specific histotypes of pituitary adenomas. Overexpression of miR-23a, miR-23b, and miR-24-2 and lower expression of mir-26b are more typical of GH-secreting and PRL-secreting adenomas, differentiating them from non-functioning adenomas (NFA), characterized by mir-26 upregulation and miRNA-24-2 downregulation. Moreover, NFA express higher concentration of miR-137 and lower of miR-127, miR-129, miR-203, and miR-134 compared to GH-secreting adenomas. Finally, ACTH-secreting adenomas are defined by a strong expression of mir-30 cluster (miR-30a, miR-30b, miR-30c, and miR-30d), supporting the hypothesis that miRNAs could be useful diagnostic markers to distinguish pituitary tumor histotypes [127].More recently, Amaral et al evaluated the expression of microRNAs in ACTH-secreting pituitary tumors: they found that let-7a, miR-21, miR-141, miR-143, miR-145, and miR-150, besides miR-15 and miR-16, are downregulated in corticotropinomas compared to normal pituitary tissue and that a lower expression of mir-141 is linked to a better chance of remission after surgery [128]. Moreover, downregulation of Mir-143 could be involved in tumorigenesis by activating MAPK pathway via ERK5 [129].

Quian et al showed that let-7 expression is decreased in more than one third of pituitary adenomas and is related to higher tumor grade, thus it may act as a tumor suppressor. Levels of let-7 were found to be inversely correlated to HMGA2 expression [130], confirming some previous studies which showed that HMGA2 is repressed by let-7 [131]. High levels of HMGA2 have been detected in most types of pituitary adenomas [132] and they are significantly associated with tumor grade, extent of invasion, tumor size and Ki-67 proliferation index, probably through regulation of E-cadherin, E2F1, cyclin A, and p53 expression. Accordingly, let-7 may be useful as a novel anticancer agent in the future [130]. A recent study on the role of miRNAs in NFAs and GH-secreting pituitary tumors was performed by Butz et al, showing that a group of miRNAs (miR-128a, miR-155, and miR-516a-3p) targets the 3'-untranslated region of Wee1, a nuclear protein kinase that acts as a tumor suppressor, and which was found to be significantly decreased in pituitary tumor samples [133].

9. Therapeutic potential of miRNAs

Current evidence indicates that miRNA deregulation is common in human cancers. The discovery of miRNAs with oncogenic or tumor-suppressive function raises the possibility of exploiting these RNA molecules for therapeutic intervention and the development of novel therapies. The oncogenic miRNAs can be downregulated using antisense miRNA oligonucleotides (AMOs or antagomirs) or miRNA sponges[134,135], whereas tumor-suppressive miRNAs may be targeted by replacement with miRNA mimetics.

AMOs are synthetic oligonucleotides with sequence complementary either to mature miRNA or its precursor and have been widely used to inhibit miRNA activities in vitro and in vivo[136]. A number of chemical modifications to AMOs have been developed to improve specific binding to target miRNAs and to resist nuclease degradation. These include modifications at the 20-hydroxyl position (20-O-methyl (20OMe), 20-Omethoxyethyl(20MOE), 20-fluoro (20F) and locked nucleic acids (LNA)), on the phosphorothioate backbone, and conjugation with cholesterol [137]. Of these modifications,LNA shows high stability, strong affinity for target miRNAs and low toxicity in biological system and has emerged as a strong candidate for targeting miRNAs in vivo. One clinical trial using LNA anti-miRNA in treating human disease has been initiated recently. Using LNA antimiR-21, Corsten et al. demonstrated that miR-21 knockdown in glioma cells led to an increase of apoptotic cell death and a significant reduction of glioma growth in vivo, suggesting that miR-21 is a potential therapeutic target. They further showed that combination of miR-21 suppression and the cytotoxic agent tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) resulted in enhanced caspase activity and synergistic killing of glioma cells in vitro. When these treated glioma cells were implanted intracranially into nude mice, tumor cells were completely eradicated after day 6 of implantation, compared to reduced tumor volume with either treatment alone. This finding suggests that miR-21 knockdown sensitizes glioma cells to apoptosis agent, and that the combined treatment is a promising approach for glioma elimination [138].

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Angiogenesis is a process essential for malignant glioma growth. By co-culturing glioma cells with microvascular endothelial cells, Wurdinger et al. showed that glioma cells could induce changes of miRNA expression in endothelial cells, with miR-296 being significantly upregulated. Enhanced expression of miR-296 was also observed in endothelial cells treated with glioma-conditioned medium or proangiogenic growth factor VEGF or EGF, as well as in endothelial cells isolated from glioma samples. These results suggested a role for miR-296 in angiogenesis. It was further demonstrated that miR-296 contributed to angiogenesis by targeting hepatocyte growth factor-regulated tyrosine kinase substrate (HGS), which controls the levels of growth factor receptors (VEGFR2 and PDGFRb) by directing these macromolecules to lysosomes for degradation. Importantly, intravenous administration of antimiR- 296 AMO significantly reduced angiogenesis in glioma xenografts in vivo[139]. These results suggest that inhibition of angiogenesis by targeting miR-296 in endothelial cells may represent an alternative approach in glioma therapy.

10. Conclusion

MiRNAs are an astonishing new class of gene regulators, and it had been demonstrated that these molecules play a crucial role in cancer development and progression in a variety of malignancies, including brain tumors. Most importantly, in a clinical context, there is first evidence that miRNAs might provide new options to improve diagnostics and therapy in the two most common malignant primary brain tumors in adults (glioblastoma) and children (medulloblastoma). In vitro and in vivo data suggest that miRNAs could be used to discriminate brain tumors from normal brain tissue, and to identify different astrocytoma grades. More important, clinico-pathological features seem to correlate with miRNA expression in these tumors. Furthermore, there is increasing evidence that miRNAs might help to generate targeted therapies and to overcome resistance to conventional anticancer strategies for example in glioblastomas. Of course, it has to be acknowledged at this stage that translation of these preliminary “in vitro data” into “hard clinical facts” is not feasible. But these findings provide a very promising basis for future studies to determine the effect of miRNA modulation on chemotherapy in “in vivo studies”. Although therapeutic delivery of miRNAs is still a developing field, and there is much more work to be done before these molecules can be securely applied in clinical settings, miRNA modulation may one day have a therapeutic application in patients. In summary, the presented data supports the enormous clinical potential of miRNAs in brain tumors, and mandate further intensive investigations in this field.

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Author details

Chunzhi Zhang[1], Budong Chen[2], Xiangying Xu[3], Baolin Han[3], Guangshun Wang[3] and Jinhuan Wang[2]

1 Department of Radiation Oncology, Tianjin Huan Hu Hospital, Tianjin, China

2 Department of Neurosurgery, Tianjin Huan Hu Hospital, Tianjin, China

3 Department of Oncology, Tianjin Baodi Hospital, Tianjin, China

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