The many roles of microRNAs in brain tumor biology

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MicroRNAs (miRNAs) are now recognized as the primary RNAs involved in the purposeful silencing of the cell's own message. In addition to the established role of miRNAs as developmental regulators of normal cellular function, they have recently been shown to be important players in pathological states such as cancer. The authors review the literature on the role of miRNAs in the formation and propagation of gliomas and medulloblastomas, highlighting the potential of these molecules and their inhibitors as therapeutics. (DOI: 10.3171/2009.10.FOCUS09207)

KEY WORDS • microRNA • glioma • medulloblastoma • cancer stem cell

MICRORNAs are now recognized as the primary endogenous small RNAs, about 21 nucleotides in length (21–23 nucleotides), involved in the purposeful silencing of the cell's own message in a variety of ways, including translational repression, mRNA cleavage, and deadenylation. They were first described in *Caenorhabditis elegans* with the discovery that *lin-4* coded for an RNA transcript capable of modulating the protein expression of *lin-14*.⁴² After their initial discovery in 1993, miRNA research proliferated and a variety of screening efforts have led to the discovery of hundreds of miRNAs. MicroRNAs are now considered fundamental to normal cellular function in eukaryotes, and the alteration of miRNA expression and activity has been implicated in a variety of pathological processes.

Biogenesis

MicroRNAs are stem-loop structures encoded by a cell's own genome. They interact with complementary mRNA leading to the disruption of target protein expression. MicroRNAs are generated by a multistep process (Fig. 1). Each miRNA can be transcribed separately from an individual transcriptional unit, or each transcriptional unit can encode a cluster of distinct miRNAs; miRNAs

can also be processed directly from other RNA species, such as introns by Dicer.² The primary miRNA transcript (often abbreviated pri-miRNA) is typically transcribed from the genome by RNA polymerase II and is subsequently capped and polyadenylated.⁴⁰ The primary miRNA transcript folds into a stem-loop structure, which is essential for the maturation process. In animals, the primary miRNA transcript is then cleaved in the nucleus by Drosha, an RNase III endonuclease, in association with the double-stranded RNA-binding domain protein DGCR8/Pasha in a protein complex referred to as the microprocessor complex.¹⁷ Drosha cleaves both strands of the stem at sites near the base of the primary stem-loop,⁴³ generating an intermediate known as the miRNA precursor (sometimes abbreviated premiRNA or pre-miRNA). The miRNA precursor is then exported out of the nucleus by Exportin-5 into the cytosol where the RNase III domain-containing nuclease, Dicer, cleaves the terminal loop to generate the ~ 22 nucleotide mature miRNA. Dicer functions with the double-stranded RNA-binding domain proteins TRBP and PACT.⁴⁶ Drosha and Dicer cleave with great precision, generating very exact ends, and it is this feature that is likely responsible for the high specificity of miRNA with their target mRNA.⁸

Posttranscriptional Gene Silencing

Immediately after formation of the mature miRNA, the duplex is unwound and loaded onto the RNA-induced silencing complex, which ultimately carries out the silencing of target mRNA. The RISC is a trimeric complex composed of Dicer, TRBP, and a protein of the

Abbreviations used in this paper: miRNA = microRNA; npBAF = neural-progenitor-specific BAF; REST = repressor-element-1-silencing transcription factor; RISC = RNA-induced silencing complex.

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FIG. 1. Long primary miRNAs (pri-microRNAs) are processed in the nucleus into stem-loop precursors by the RNase III endonuclease Drosha and Pasha. The products of the enzymatic reaction are transported into the cytoplasm by exportin 5 and Ran-GTP, and these precursors are further processed into small RNA duplexes of approximately 22 nucleotides by the Dicer RNase III enzyme and Logacious (Loqs). The microRNA duplex is then loaded onto the RNA-induced silencing complex (RISC). The microRNA guides the RISC to the target mRNA for translational regulation.

Argonaute superfamily (Ago2 in humans).³⁰ It identifies target mRNA based on complementarity with the associated single-stranded miRNA and results in either mRNA cleavage or translational repression.² An estimated one-third of all mRNAs are thought to be susceptible to post-transcriptional gene silencing by miRNAs.⁶⁶

MicroRNA and Neuronal Development

One of the most defining moments in the development of the nervous system is when neuroprogenitors lose multipotency at mitotic exit and begin to develop their terminal cell fate. In the case of neurons, it means they commit to their final topographical position and established stable connections that will persist for the lifetime of the organism. This transition is in large part accompanied by a switch in chromatin-remodeling/regulatory complexes such as in the exchange of the BAF53a and BAF45a subunits within Swi/Snf-like npBAF complexes. The subunits of the npBAF complex are essential for neural-progenitor proliferation. The exchange of the BAF53a and BAF45a in the neuron-specific BAF complex promotes postmitotic neural development and dendritic morphogenesis (Fig. 2). MicroRNA (miR-9* and miR-124) has been implicated in this process by regulating the chromatin architecture and dynamics.⁷² Interestingly, miR-9* and miR-124 are repressed by the REST, which mediates cell type- and developmental stage-specific gene repression, gene activation, and long-term gene silencing for protein-coding genes resulting in postmitotic neurons. The expression and function of REST are tightly regulated by context-specific transcriptional and posttranscriptional mechanisms including bidirectional feedback loops with various miRNAs. It is therefore not surprising that deregulation of REST and miRNAs are both implicated in the pathogenesis of various brain tumors.

FIG. 2. The subunits of the npBAF complex are essential for neural-progenitor proliferation. The exchange of the BAF53a and BAF45a in the nBAF (neuron-specific BAF) complex promotes postmitotic neural development and dendritic morphogenesis.

MicroRNA Function and Cancer

Following the discovery of miRNAs and other small RNAs, a wealth of data were rapidly generated, which revealed a novel mechanism for the modulation of gene expression. As gene-finding computational models have evolved from simple homology-based searches to more complex multifactorial models, the number of identified miRNAs has continued to grow.⁴⁷ Over recent years, there has been a significant push to better understand how they function in both normal and pathological states.

Though miRNAs have been demonstrated to modulate genes involved in a variety of cellular processes, a significant proportion of miRNAs regulate genes associated with cellular fate. It is now well accepted that miRNAs are fundamental to the regulation of proliferation, differentiation, and apoptosis during normal development. It has been shown that miRNAs have a predilection in targeting developmental genes. Genes involved in functions common to all cells, such as in maintenance and general activities, have very few miRNA target sites, and seem to be under selection to avoid targeting by miRNAs.⁶¹ Furthermore, alterations in the expression of miRNAs are seen in a variety of pathological processes, including cancer. Aberrant miRNA expression has been demonstrated in essentially every cancer type studied, including breast^{58,64,68,73} and ovarian carcinomas,^{48,74} pancreatic cancer,^{4,27} non-small cell lung cancer,^{41,56} leukemia,⁵⁻⁷ and brain tumors (Table 1).^{52,66} MicroRNA expression can be altered in cancer through a variety of mechanisms including chromosomal changes, epigenetic defects, mutations, and alterations in the machinery involved in miRNA biogenesis.⁶⁶

Beyond mere biomarkers, the altered expression profiles of miRNA implicate them as key regulators of tumorigenesis. The miR-17-92 polycistron, located on chromosome 13q32-33, was the first example of miRNAs acting as mammalian oncogenes.³⁵ This region of the chromosome is amplified in several types of cancer and can be activated by *c-Myc*, a well-established protooncogene overexpressed in different cancer types.^{51,66} The first individual miRNA assigned an oncogenic role was *miR-155*,¹⁸ which when overexpressed in a transgenic mouse model, led to the development of B-cell leukemia and high-grade lymphoma.¹⁵

There is also evidence accumulating that miRNAs are involved in cell-cycle checkpoint regulation. Using a mutated version of dicer-1, Hatfield and colleagues³³ dem-

TABLE 1: MicroRNAs implicated in glioma and medulloblastoma biology

Tumor	miRNA	Cellular Role
GBM	miR-7	suppresses EGFR expression; independently inhibits Akt pathway
	miR-10b	may promote invasion; found to be increased in invasive high-grade gliomas
	miR-15b	results in cell cycle arrest
	miR-21	anti-apoptosis; suppresses tumor suppressors
	miR-26a	targets PTEN; enhances Akt pathway
	miR-124	inhibits proliferation by inducing G0/G1 cell cycle arrest via CDK6 inhibition
	miR-128	expression decreases levels of Bmi-1
	miR-137	inhibits proliferation by inducing G0/G1 cell cycle arrest via CDK6 inhibition
	miR-181a	tumor suppressor
	miR-181b	tumor suppressor
	miR-221	unknown; found to be increased in invasive high-grade gliomas
	miR-425	upregulated in non-cancer stem cells; promotes differentiation
	miR-451	upregulated in non-cancer stem cells; promotes differentiation
	miR-486	upregulated in non-cancer stem cells; promotes differentiation
MB	miR-let7g	upregulated in anaplastic MB; differentially expressed in desmoplastic MB
	miR-9	regulates proliferation, apoptosis
	miR-19a	over-expressed in hedgehog-dependent MB; upregulated in anaplastic MB
	miR-20	over-expressed in hedgehog-dependent MB
	miR-92	over-expressed in hedgehog-dependent MB
	miR-106b	upregulated in anaplastic MB; differentially expressed in desmoplastic MB
	miR-124	regulates cell cycle via CDK6
	miR-125a	regulates proliferation, apoptosis
	miR-125b	hedgehog dependent proliferation
	miR-191	upregulated in anaplastic MB
	miR-199b-5p	expression correlates with decreased metastatic potential; associated with survival
	miR-324-5p	hedgehog dependent proliferation
	miR-326	hedgehog dependent proliferation

onstrated that miRNAs modulated stem cell division in *Drosophila* by bypassing the G1/S checkpoint. This work suggested that miRNAs may make stem cells insensitive to environmental stimuli and that a similar mechanism may be implicated in tumorigenesis.¹⁶ More recently, specific miRNAs capable of regulating cellular checkpoints in cancer have been described, including miR-221, miR-222, and miR-27a.^{45,49,67}

In addition to their involvement in cancer formation, miRNAs have also been associated with tumor progression and metastatic potential.³ Although still not well characterized, specific miRNAs—such as miR-10b, miR-21, miR-30a, miR-30e, miR-125b, miR-141, miR-200b, miR-200c, and miR-205—have been suggested to play an important role in tumor invasiveness and metastasis.¹ Some of the targets of these miRNAs have recently been elucidated and include tumor suppressor genes such as *tropomyosin 1* and other targets with metastatic potential such as *PDCD4* and *maspin*.⁷⁶

MicroRNAs and Gliomas

Gliomas are tumors arising from glial cells, the neuroepithelial support cells of the CNS. Gliomas are the most common primary tumor of the CNS comprising over 50% of primary brain tumors.⁶² Glioblastoma is the most

common and deadliest glioma with approximately 10,000 new cases every year and a median survival of only 14 months even with the most current therapies.⁶² Gliomas, like tumors in other parts of the body, develop because of fundamental genetic alterations that cause the formation of a tumor stem cell population that divides without regard to normal physiological biochemical signaling.^{11,65} It is no surprise then that the same miRNAs that are involved in stem cell regulation and differentiation are also implicated in tumor stem cell biology of gliomas.

Microarray studies of glioma tissue have implicated a number of miRNAs involved in glioma formation and propagation. In their quantitative reverse transcriptase polymerase chain reaction analysis of high-grade astrocytomas in humans (glioblastomas and anaplastic astrocytomas), Silber et al.⁵⁹ found 35 different miRNAs that were significantly deregulated compared with control brain tissue. Those miRNAs that have been shown to play a role in human glioma include miR-7, -10b, -15b, -21, -26a, -124, -128, -137, -181a, -181b, -221, -451, and others. The majority of miRNAs are underexpressed in proliferating glioma cells with the important exception of miR-10b, -21, and -221.

MicroRNAs are key regulators of tumor suppressor genes. The overexpression of miR-21, a recently described

antiapoptotic factor, appears to be vitally important in not only glioma but also cancer arising from other parts of the body.⁹ Chen and coworkers¹⁰ used bioinformatics analysis to screen and identify various genes with miR-21 binding sites. Using the glioblastoma cell line T98G, they confirmed that miR-21 binds and silences the tumor suppressor gene *PDCD4*. Corsten et al.¹⁴ transfected human glioma cells with anti-miR-21 oligonucleotides and simultaneously implanted neural precursor cells expressing a secretable variant of the cytotoxic agent TRAIL (S-TRAIL). They found a synergistic effect of knocking down miR-21 levels and adding the apoptotic agent S-TRAIL evidenced by increased caspase activity and decreased cell viability in the human glioma cells in vitro. They then found complete eradication of tumor cells in a murine model transfected with anti-miR-21 and then exposed to S-TRAIL in vivo.

MicroRNAs regulate oncogenes implicated in brain tumor formation. The expression level of miR-128 is inversely correlated with expression level of transcription factor E2F3a, a protein that promotes cell entry into S-phase and implicated in cancer of the bladder and prostate.⁷⁵ Godlewski and colleagues²⁸ found that miR-128 levels were downregulated in glioma cells compared with normal brain tissue. They reported that increasing miR-128 expression leads to a decrease in the expression of the oncogene *Bmi-1*. Overexpression of miR-128 in glioma neurosphere cultures specifically blocks glioma self-renewal consistent with *Bmi-1* downregulation.

Interestingly, other than serving as key regulators of oncogenes and tumor suppressors, microRNAs may also dictate the invasiveness and aggressiveness of tumors. Conti et al.¹³ found both miR-21 and miR-221 upregulated in glioma samples; however, they noted that whereas miR-21 was elevated in all gliomas, high levels of miR-221 were only found in high-grade gliomas. MicroRNA-10b has similarly been associated with high-grade tumors. Overexpression of miR-10b may promote glioma invasion via the RhoC and urokinase plasminogen activator receptor (uPAR), which have been implicated in other cancers as prometastatic and proinvasive factors.⁵⁵ Huse and colleagues³⁷ studied the overexpression of miR-26a in a subset of high-grade glioma, most often associated with monoallelic loss of phosphatase and tensin homolog (PTEN). Studying 3 human glioblastoma samples, they found that miR-26a directly targets PTEN and enhances the Akt pathway. By transfecting miR-26a into a murine model, they were able to show that overexpression of miR-26a enhances gliomagenesis. Because miR-26a overexpression appears in only a subset of glioma with monoallelic loss of PTEN, they hypothesized that this overexpression functionally substitutes loss of heterozygosity at the PTEN locus.

MicroRNAs have also been shown to be important regulators of cellular proliferation/differentiation and the cell cycle. Silber and colleagues⁵⁹ were the first to discover that miR-124 and miR-137 are downregulated in high-grade gliomas compared with normal controls. These microRNAs are also upregulated during adult neural stem cell differentiation. The upregulation of miR-124 and miR-137 in tumor stem cell populations promotes

neuronal differentiation of the tumor stem cells and inhibits proliferation of the tumor cells by inducing G0/G1 cell-cycle arrest. The mechanism of action is via the inhibition of CDK6 expression, a protein essential for cell-cycle progression.⁵³ Kefas et al.³⁹ studied miR-7, another miRNA that is downregulated in glioma. They found that miR-7 suppresses EGFR expression and independently inhibits the Akt pathway in glioblastoma. Transfection of glioblastoma with miR-7 decreased viability and invasiveness of primary glioblastoma cell lines and increased the apoptotic fraction of cells. Overexpression of miR-15b, another differentially expressed microRNA, results in cell-cycle arrest while suppression of miR-15b results in more cells in S-phase.⁷⁰ Gal and colleagues²³ examined glioblastoma stem (CD133-positive) and nonstem (CD133-negative) cells and found that miR-451, -486, and -425 were significantly upregulated in CD133-negative cells compared with CD133-positive cells. Transfection of glioblastoma cells with these miRNAs inhibited neurosphere formation, and transfection with miR-451 resulted in neurosphere dispersion and inhibited glioblastoma growth. Gal and colleagues found that combining miR-451 transfection with Imatinib mesylate treatment had a cooperative effect in dispersal of glioblastoma neurospheres.

MicroRNAs have recently been shown to function as bona fide tumor suppressors. Shi et al.⁵⁷ reported on downregulated miR-181a and miR-181b involved in glioma formation. Their study showed that these miRNAs functioned as tumor suppressors. Transfection of these miRNAs into glioblastoma cells inhibited proliferation in vitro, resulted in loss of anchorage-independent growth, induced apoptosis in glioma cell lines, and depressed the invasion of glioma cells in vitro.

MicroRNAs and Medulloblastoma

Medulloblastoma is the most common malignant brain tumor in children with an incidence of approximately 2 per 100,000. It appears to arise from stem cells and from granule neuron precursors in the external granule layer of the cerebellum²⁶ or multipotent precursors in the ventricular zone of the cerebellum.⁴⁴ About 70% of cases occur before the age of 16 years. Approximately one-third disseminate in the CSF and up to 5% spread systemically. Medulloblastoma treatment most often involves a combination of surgery and radiation therapy. Chemotherapy is usually reserved for children younger than 3 years of age or for recurrent tumors. Five-year survival rates for medulloblastoma have been estimated to range from 35 to 75%.¹⁹

Evidence for a role of miRNA in medulloblastoma tumorigenesis has only very recently emerged. Pierson and associates⁵⁴ were the first to report the involvement of miRNA in medulloblastoma by demonstrating that miR-124 modulates cell-cycle regulation in medulloblastoma cells. They showed that miR-124 expression is significantly decreased in medulloblastoma and that augmentation of miR-124 levels can slow tumor cell growth by targeting CDK6.

Ferretti et al.²¹ proposed a role for miRNA in modulating hedgehog signaling, a pathway recently implicated

in tumorigenesis for a subset of medulloblastoma.^{29,32,34} Specifically, they showed that miR-125b, miR-326, and miR-324-5p expression was decreased in medulloblastoma and that the altered expression of these miRNAs led to tumor cell proliferation through a hedgehog-dependent mechanism. Expanding on this work, Uziel et al.⁶³ identified 3 miRNAs overexpressed in hedgehog-active medulloblastomas: miR-92, miR-19a, and miR-20. All 3 miRNAs were encoded by the miR-17/92 cluster, which has been associated with a variety of cancers. Northcutt and colleagues⁵⁰ identified a high-level, focal amplification on chromosome 13q31.3, which mapped to the same miRNA cluster. The expression of miR-17/92 was most elevated in medulloblastomas with activated hedgehog signaling and was also associated with elevated c-Myc and n-Myc. These studies suggest that aberrant expression of miRNAs encoded by the miR-17/92 enhance the growth potential of medulloblastoma and that miRNA-mediated modulation of hedgehog signaling may be an important contributing factor to medulloblastoma pathogenesis.

Ferretti et al.²² used high-throughput screening to examine miRNA expression profiles in 34 patients with medulloblastoma. They identified 78 miRNAs with altered expression in medulloblastoma, compared with normal adult and fetal cerebellar cells. Several of the identified miRNAs have been implicated in other cancer types including glioblastoma.^{12,25} The majority of these miRNAs were downregulated in medulloblastoma, supporting a role for miRNAs as tumor suppressors. Additionally, they found increased expression of miR-9 and miR-125a and that increased expression of these miRNAs was capable of decreasing proliferation, augmenting apoptosis, and ultimately promoting arrest of tumor growth. The proapoptotic effect was mediated by miR-9 and miR-125a targeting of the t-TrkC receptor, which was found in this study to be upregulated in medulloblastoma cells. In this study, miRNA expression patterns were also examined in different tumor subsets. The authors found that miR-let7g, miR-19a, miR-106b, and miR-191 were significantly upregulated in anaplastic compared with desmoplastic medulloblastomas; miR-let7g and miR-106b were differentially expressed in desmoplastic compared with classic medulloblastomas; and miR19a was upregulated in anaplastic compared with classic medulloblastomas.

Changes in expression of Her2 (ErbB2) and c-Myc have been demonstrated to impact biological activity and clinical features of medulloblastoma.^{26,31,36} Ferretti et al.²² examined miRNA expression from medulloblastomas overexpressing either Her2 or c-Myc and identified an miRNA signature in each group of medulloblastomas. Expression of miR-10b, miR-135a, miR-135b, miR-125b, miR-153, and miR-199b was altered in Her2-overexpressing tumors, whereas c-Myc overexpressing medulloblastomas had expression changes in miR-181b, miR-128a, and miR-128b. Additionally, the amount of expression change of 2 miRNAs correlated with disease risk. Though miR-31 and miR-153 were downregulated in all medulloblastomas, the group found that the degree of change was directly proportional to disease severity.

It is well established that the Notch signaling pathway regulates the differentiation of granule neuron precursor

cells and that Notch2 expression is increased in about 15% of medulloblastomas.^{44,60} Expression of the transcriptional repressor HES1, a downstream effector protein of the Notch pathway, normally declines during the process of neuronal differentiation. Conversely, persistent activation of the Notch pathway and HES1 prevents the migration of granule neuron precursor cells out of the ventricular zone and inhibits neuronal differentiation.³⁸ Based on its role in differentiation, it is not surprising that dysfunction of the Notch pathway has been associated with a subset of medulloblastoma with stem cell-like properties.^{20,69,71,77} Garzia et al.²⁴ examined the role of miRNAs in the regulation of Notch/HES1 signaling in medulloblastoma. They found that miR-199b-5p targeted HES1 and that miR-199b-5p-mediated downregulation of HES1 attenuated cellular proliferation in medulloblastoma cell lines. In medulloblastoma patients, increased expression of miR-199b-5p appeared to decrease metastatic potential and was associated with increased survival.

Conclusions

Although the investigation of miRNAs in brain tumors is still in its infancy, there is strong evidence mounting that miRNAs are integrally involved in brain tumor development and progression. It is becoming clear that miRNAs are essential regulators of many of the key pathways implicated in tumor pathogenesis. While adding another layer of complexity, the discovery of the role miRNAs in brain tumors has also revealed a new category of therapeutic targets. As miRNA research continues to evolve, novel therapeutic targets for the treatment of brain tumors will continue to emerge.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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