

The Many Functions of MicroRNAs in Glioblastoma

E. Antonio Chiocca and Sean E. Lawler

Recent years have seen an explosion in our knowledge of the functions of a recently discovered class of cellular regulators of gene expression called microRNAs, or miRs. These short, single-stranded ribonucleic acid (RNA) molecules are potent regulators of cell behavior and show distinct patterns of expression during development, in different tissues, and in diseases including cancer. Characteristic miR alterations have been identified in glioblastoma, and these play key roles in the biology of this tumor. This article will discuss the emerging roles of miRs in glioblastoma, and their potential as therapeutic and diagnostic tools.

MicroRNAs, SMALL REGULATORS WITH BIG FUNCTIONS

MiRs are approximately 23-nucleotide-long RNA molecules that typically act by suppressing the translation of messenger RNAs into protein, through binding to complementary sequences in their 3'-untranslated regions (4, 12). MiRs are transcribed from our DNA, and are found either singly or in clusters, and are sometimes associated with the introns of protein-coding genes. The initial primary miR transcript forms a hairpin structure that gets processed into the final single-stranded mature miR in the cytoplasm. The mature miR, in association with specific proteins, then binds to its target sequences to silence gene expression. There are approximately 1000 known miRs in the human genome, and because their target binding is dependent only on a few complementary base pairs they can be very promiscuous. Although the precise number of targets for each miR is not yet known, based on sequence data it is reasonable to suggest that these could number in the hundreds. This means that a single miR can have profound effects on cell biology through targeting multiple components of a single cellular pathway, or components of multiple pathways. miR-target pairs are mostly validated using an assay in which the target sequence is cloned downstream of a luciferase gene. Suppression of luciferase with the corresponding miR is strongly suggestive of direct interaction. This is confirmed by mutating the predicted target sequence and blocking the effect of the miR. At the present time, the vast majority of miR-target pairs within the cell have yet to be characterized.

MICRORNA ALTERATIONS IN GLIOBLASTOMA

Glioblastoma is characterized by the hallmarks of rapid proliferation, angiogenesis, hypoxia and necrosis, heterogeneity, chemoresistance, infiltration of normal brain tissue, and stem cell-like behavior. Many of the major genetic aberrations in glioblastoma are known and result in the activation of characteristic signaling pathways underlying these biological hallmarks (5). Several recent molecular profiling studies have revealed distinct patterns of miR expression in glioblastoma compared with lower-grade astrocytomas, adjacent tissue, or normal brain. These modifications are quite extensive; a comparison of normal brain with glioblastoma tissue shows about 100 significantly differentially expressed miRs. Like other cancer-associated genetic changes, miR alterations can be due to genetic amplification or deletions, methylation events, or altered transcription. Recently, mutations in miR sequences have been identified in some cancer types, and also mutations in their target sequences (2, 14). Such mutations may play key roles in tumorigenesis but have not yet been identified in glioblastoma. Increasing numbers of differentially expressed miRs identified in glioblastoma by profiling methods are being functionally characterized. The best studied of these are shown in **Table 1**. These are emerging as candidate biomarkers and potential therapeutic agents. Specific examples of these are discussed briefly below.

miR-21

miR-21 has emerged as one of the most consistently highly expressed microRNAs in cancer. Inhibition of miR-21 by complementary antisense oligonucleotides in glioma cell lines led to an increase in apoptosis, suggesting that miR-21 acts as an oncogene in glioblastoma by suppressing apoptosis (1). miR-21 antagonism also represses glioma formation in vivo (3). miR-21 has been shown to target other genes such as PTEN (phosphatase and tensin homolog) (21), which may also contribute to its effects in glioblastoma. miR-21 targets a network of p53, transforming growth factor- β (TGF- β), and mitochondrial apoptosis factors in glioblastoma (16) and may also promote invasion by direct targeting and downregulation of anti-invasive proteins including TIMP3 (6). This increasing data supports targeting of miR-21 as a candidate approach for glioblastoma and cancer treatment in general.

News items of interest may be forwarded to:
 Felipe C. Albuquerque, M.D.
 Section Editor, WORLD Neurosurgery News
 E-mail: Felipe.albuquerque@bnaneuro.net



Felipe C. Albuquerque, M.D. Section Editor,
 WORLD Neurosurgery News

Issam Awad, M.D. Section Editor,
 WORLD Neurosurgery News

Table 1. MicroRNAs (miRs) Involved in Glioblastoma

microRNA ID	Expression	Functions	Targets	Refs
miR-21	High	Proliferation (in vitro and in vivo) Apoptosis Invasion Chemoresistance	PTEN, RECK, PDCD4, TIMP3 TPM1, PELI1, MARCKS, CDC25A, TGFB2, BMPR2, LRRFIP1, BTG2, BCL2, SPRY1	(1, 3, 6, 16, 21)
miR-26a	High (genetic amplification)	Transforming Proliferation (in vitro and in vivo) Apoptosis	PTEN MAP3K2 IFNB	(9)
miR-7	Low	Proliferation (in vitro) Invasion Differentiation	EGFR, IRS2, PAK1, SFRS1, SPATA2, SNCA, ABCC1	(11)
miR-124	Low	Proliferation (in vitro) Invasion Differentiation	CDK6, SCP1 PTBP1, ITGB1, LAMC1, NR3C2, EFN1, SOX9 SLC16A1	(17)
miR-34a	Low (reduced by loss of p53)	Proliferation (in vitro and in vivo) Apoptosis Invasion	MET, NOTCH1 NOTCH2, CDK6 MYC, SIRT1	(15)
miR-137	Low (methylation)	Proliferation (in vitro) Neuronal differentiation	CDK6, MITF, EZH2	(17)
miR-128	Low	Proliferation (in vitro and in vivo) Stem cell self-renewal	EGFR, BMI1 E2F3A, NTRK3, SNAP25, TXNIP, DB1, LDLR	(7)
miR-296	High (endothelial cells)	Angiogenesis	HGS	(19)
miR-326	Low (reduced by notch signaling)	Proliferation (in vitro and in vivo) Apoptosis Invasion	CBF1, NOTCH1 NOTCH2, ABCC1 ETS1, SMO	(10)
miR-451	High (glucose regulated)	Switch regulating invasion, growth, and survival in response to metabolic stress Chemoresistance	CAB39, MIF UBE2H, ARPP19 GATA2, ABCC1	(8)

Note: The table shows the miRs for which extensive functional and target-related information is available for glioblastoma. Targets that have been experimentally validated by luciferase assay in glioma cells are shown in bold type. Targets that have been either validated or suggested in other cell types are shown in normal type. Key references are also shown for the glioma studies.

miR-124

miR-124 is the most downregulated miR in glioblastoma comparing tumor and normal brain (7, 17). miR-124 expression increases during neural stem cell differentiation, and glioblastoma-derived stem cells overexpressing miR-124 display a dramatic increase in neural differentiation markers accompanied by reduced self-renewal and tumorigenicity. In normal development, miR-124 engages a feedback loop that is essential for neuronal differentiation (20). Moreover, miR-124 induced G1 cell cycle arrest in glioma cells, associated with decreased expression

of CDK6—which it targets directly (17). These results suggest that low levels of miR-124 in glioblastoma increase tumorigenicity by preventing neuronal differentiation and allowing cell cycle progression.

miR-128

Like miR-124, miR-128 is downregulated in glioblastoma, and miR-128 expression reduces glioma proliferation in vitro and in vivo (7). The known oncogene and stem cell renewal factor Bmir was dem-

onstrated to be an important target of miR-128. In glioma-derived stem cells stably overexpressing miR-128, Bmi1 levels were reduced and self-renewal was severely impaired (7).

miR-451

miR-451 was identified in a microarray screen as a result of its downregulation in migrating glioma cells (8). Subsequent studies showed that its expression suppresses invasion, but increases proliferation. Ultimately, this study showed that in high glucose levels, miR-451 is expressed at a higher level, when it suppresses adenosine monophosphate-activated protein kinase (AMPK) signaling. Low glucose levels reduce miR-451 levels and increases AMPK activation, which allows the cells to adapt to the low energy situation, by counteracting Akt signaling, and increasing migration. Thus miR-451 acts as a switch that regulates glioma growth, invasion, and survival under metabolic stress. Other studies suggest that miR-451 may also be involved in chemoresistance (22).

Several other miRs have recently emerged as functionally important in glioblastoma. Alterations of these miRs under experimental conditions decreases growth, invasion, apoptosis, and angiogenesis. miR-7 was one of the first described downregulated miRs in glioblastoma, where it plays a role by targeting the epidermal growth factor receptor (EGFR) among other genes. miR-7 expression reduced proliferation and invasion in glioma cell lines (11). miR-26a is overexpressed in glioma and contributes to growth by targeting PTEN expression (9). miR-34a is downregulated in glioblastoma as a result of p53 dysfunction. Reexpression of miR-34a slows intracranial tumor growth in vivo (15). miR-326 is also weakly expressed and contributes to Notch signaling (10), and miR-296 is weakly expressed in glioma-associated endothelial cells, where it promotes angiogenesis (19). The impact of these changes on key signaling pathways is shown in **Figure 1**.

THERAPEUTIC AND DIAGNOSTIC APPLICATIONS OF miRS IN GLIOBLASTOMA

The profound effects of miRs on glioblastoma cell biology suggest that they could be harnessed in a therapeutic setting. As shown by the examples cited in this article, miRs may find applications in attacking each of the key hallmarks of malignancy in glioblastoma, through direct effects on growth, migration, differentiation, and treatment resistance. Delivery of miRs remains a key challenge, but given their small size and high potential impact this obstacle may be overcome. Indeed, recently it was shown that systemic miR delivery by an adeno-associated virus reduced tumor growth in a hepatocellular cancer model (13).

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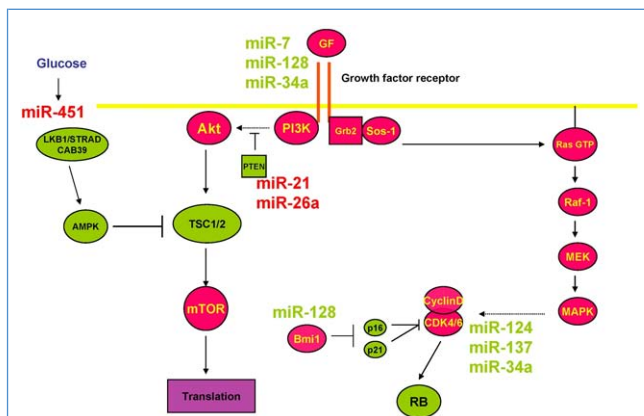


Figure 1. MicroRNA interactions with growth factor signaling pathways in glioblastoma. The figure shows a simplified signaling cascade based on selected downstream events of growth factor tyrosine kinase receptors. The diagram provides an inkling of the key roles played by miRs in fundamental signaling events involved in cancer. Oncogenic alterations are shown in red, and tumor suppressors shown in green. Note how tumor suppressing miRs are predicted to target oncogenes and vice versa.

A detailed understanding of miR mechanisms may lead to the identification of novel targets and pathways that were previously unrecognized. miRs are quite stable and readily detectable through polymerase chain reaction-based methods; therefore, it is a possibility that they may be detectable in patient fluids and used as diagnostic tools or markers of treatment response. Indeed, it has been reported that miRs can be detected in circulating exosomes in the serum of glioblastoma patients (18).

The combined effects of miR alterations in glioblastoma lead to increased proliferation, angiogenesis, chemoresistance invasion, as well as promotion of self-renewal and blockade of differentiation. A challenge will be to determine the appropriate miRs for effective intervention and to deliver these effectively and specifically to tumor cells. However, with the current pace of development in these areas it is possible that in the near future we may be able to harness these activities for improved treatment of brain tumors as well as other diseases of the central nervous system.

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The ISAT. Again . . .

Eberval G. Figueiredo

Treatment of intracranial aneurysms has radically evolved in the past decades. Progresses in surgical treatment, including significant advances in microsurgical and anesthetic techniques, have warranted better surgical outcomes (**Figure 1**). Conversely, a new technique, endovascular coiling, has also been successfully used for the past 15 years. Initially introduced in 1991 as an alternative to surgical clipping, it was approved for clinical application in 1995. Since then, endovascular coiling has proved its usefulness and efficacy in the management of intracranial aneurysms (**Figure 2**). However, thus far few prospective, multicenter, randomized studies have been designed to compare surgical clipping and endovascular coiling.

The International Subarachnoid Aneurysm Trial (ISAT) is the largest randomized, multicenter study to compare the two meth-

ods to date (3-5). It has largely changed the clinical practice in many parts of the world, particularly in Europe. It has been one of the most influential studies in neurosurgery and, at the same time, the most controversial and most criticized study in our field. ISAT results have been sequentially published in 2002, 2005, and 2009 (3-5). The 1-year results of the ISAT study published in 2002 were straightforward: endovascular coiling was superior to microsurgical clipping as it led to lowest rates of death and severe disability, defined as modified Rankin scores 3-6 (3). Criticisms to ISAT have been numerous and very well known (2). Allow us to review some of them briefly.

First, ISAT initially recruited 9559 patients; of these, 7416 patients were excluded, many of them because they were considered as not presenting "clinical equipoise" (3). What was defined