



MicroRNA in Brain Neoplasia: A Review

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Abstract

MicroRNAs (miRNAs) are small RNAs involved in regulation of several cellular processes and are involved in the silencing of cell's message in a variety of ways. In cancer, miRNAs can be involved in the regulation of important genes involved in tumorigenesis, tumor development, and angiogenesis. For these reasons, miRNAs could have considered oncogenic-miR (miRNA with oncogenic roles) or oncosuppressor-miR (miRNA with tumor suppressor roles). MiRNAs may alter the expression of genes involved in cell cycle, regulation of cell proliferation, apoptosis, angiogenesis, immune response, tumor invasion and metastasis, and genomic instability of cancer cells. Deregulation of miRNA expression has been demonstrated in each cancer type studied (e.g. breast and ovarian carcinomas, non-small cell lung cancer, pancreatic cancer and brain tumors). Moreover, miRNA expression signatures could be associated with well-defined clinicopathologic features and disease outcome and prognosis. This review focuses on the studies from 2006 to 2013 investigating miRNAs differentially expressed in different type of brain tumors.

Keywords

MicroRNA, Glioblastoma, Medulloblastoma, Brain neoplasia, Review

Introduction

MicroRNAs (miRNAs) are small RNAs, about 21 nucleotides in length (19–23 nucleotides), involved in regulation of proliferation, differentiation, and apoptosis during normal development. MiRNAs are involved in the silencing of the cell's own message in a variety of ways, as translational repression and mRNA cleavage (Figure 1) [1-3]. MiRNAs may also act by fine-tuning target activity, simultaneously modulating multiple targets and deregulating targets for rapid reactivation. More than a thousands of annotated miRNA sequences in human genome are available in the latest update of miRBase (June 2013, <http://www.mirbase.org/>) and they are thought to regulate more than one third of human genes [4,5].

Aberrant miRNA expression has been demonstrated in each cancer type studied [6], as breast cancer [7], ovarian carcinomas [8], non-small cell lung cancer [9] or pancreatic cancer [10].

Brain tumors are one of the top 10 causes of cancer deaths and an early and accurate diagnosis is essential for disease management. Brain tumors could be classified into glial tumors (e.g. glioblastoma, astrocytomas, oligodendroglial tumors, ependymal tumors), embryonal tumors (e.g. medulloblastomas), tumors of the the meninges (e.g. meningiomas), tumors of the hematopoietic system and tumor of the sellar region [11]. Introduction of molecular markers as 1p/19q codeletion [12], O6-methylguanine methyltransferase (MGMT) methylation status [13] or isocitrate dehydrogenase (IDH1 or IDH2) mutations [14] have provided new therapeutic or prognostic tools but other molecules are needed to improve management of these tumors. From here, it is gathered the necessity to discover new molecules as potentially useful parameters for prognosis, diagnosis or target therapy strategies and miRNAs could represent important molecular biomarkers.

This review focuses on the studies investigating different miRNAs expression profiles in different type of brain tumors from January 2006 to June 2013.

Starting Material and Techniques

Starting material

Cell Lines: Cell lines are one of the most used starting materials for expression analysis. This is due to the evidence that extracting RNA from cell lines allows to obtain a good quality and quantity of the nucleic acid. However, considering that miRNAs are more resistant than mRNA to degradation, the need of high-quality RNA is less crucial for miRNAs analysis. Moreover, it should be considered that miRNA expression is highly variable and results obtained in cell lines in particular from immortalized ones, could provide results far away from those obtained in "ex vivo" samples. This could be due to changes in cultured cells, but also to the fact that cell lines are not

Citation: Visani M, Acquaviva G, Marucci G, Ragazzi M, Franceschi E, et al. (2015) MicroRNA in Brain Neoplasia: A Review. Int J Brain Disord Treat 1:002

Received: July 30, 2015; **Accepted:** August 25, 2015; **Published:** August 27, 2015

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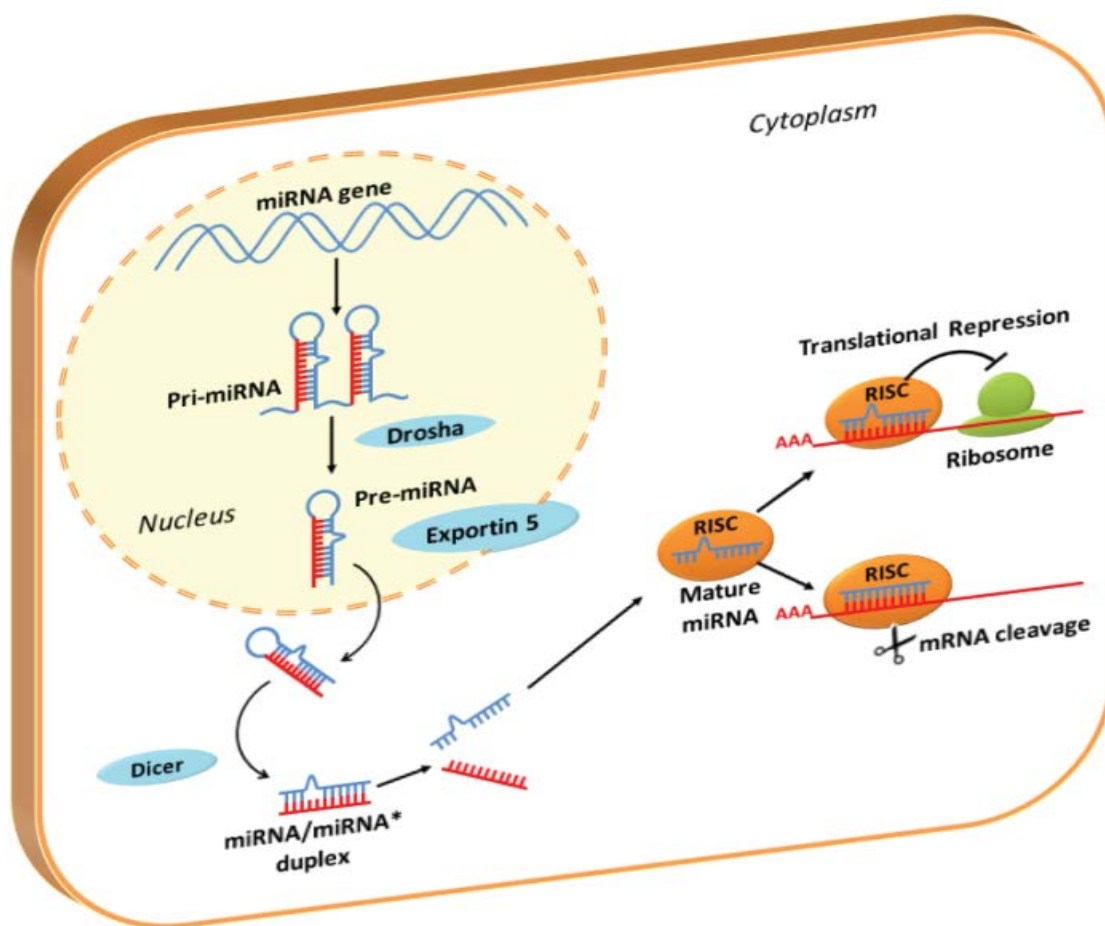


Figure 1: Pathway of Translational Silencing by miRNAs

In the nucleus miRNAs sequences are transcribed by RNA polymerase (mainly RNA polymerase II): the long, stem-loop primary transcript (pri-miRNA) is further processed in ~60-70nt pre-miRNA by nuclear Drosha RNase III endonuclease. Pre-miRNAs are transported into cytoplasm by Exportin-5 system: once in the cytoplasm, Dicer RNase III endonuclease, process miRNA precursors into mature 19-23nt miRNA duplexes. These duplexes comprise the mature miRNA and complementary fragment derived from the opposing arm of the pre-miRNA (miRNA*). Only the strand with the less stable hydrogen bonding at its 5'-end is selected (mature miRNA), while the complementary miRNA* is degraded. Mature miRNA is incorporated into ribonucleoprotein complexes known as RNA-induced silencing complexes (RISC). The major components of miRNPs are proteins of the Argonaute family: these proteins play a central role in binding the mature miRNA and drive it to mRNAs target recognition.

MiRNA-RISC complexes recognize and link miRNA complementary regions in 3'-UTR of target mRNAs. According to the grade of homology with the mRNAs, miRNAs could act in two ways: a perfect complementarity leads to mRNA cleavage, while an imperfect complementarity represses mRNA translation. RISC: RNA-Induced Silencing Complex.

influenced by microenvironment as happened in *ex vivo* specimens. Several analysis of miRNA expression in brain cell lines have been performed [15-20], however these results should be confirmed in *ex vivo* specimens for determining brain tumor miRNAs profile (Figure 2).

“Fresh/Frozen” specimens: The limitation of “fresh” brain tissue for analyzing miRNAs as biomarkers is the extremely invasive procedure for collecting material. In fact, if on the one hand non-formalin fixed specimens allow obtaining a huge amount of miRNA, on the other their availability from surgical samples is limited, considering that material for diagnostic evaluation needs to be preserved. For this reason, the analysis of miRNAs starting from formalin-fixed paraffin-embedded (FFPE) specimens could provide greater amount of material for research purposes. Due to the good amount of good quality material obtained from fresh/frozen specimens, several papers have investigated miRNAs expression starting from this type of samples (Figure 2) [15,21-56].

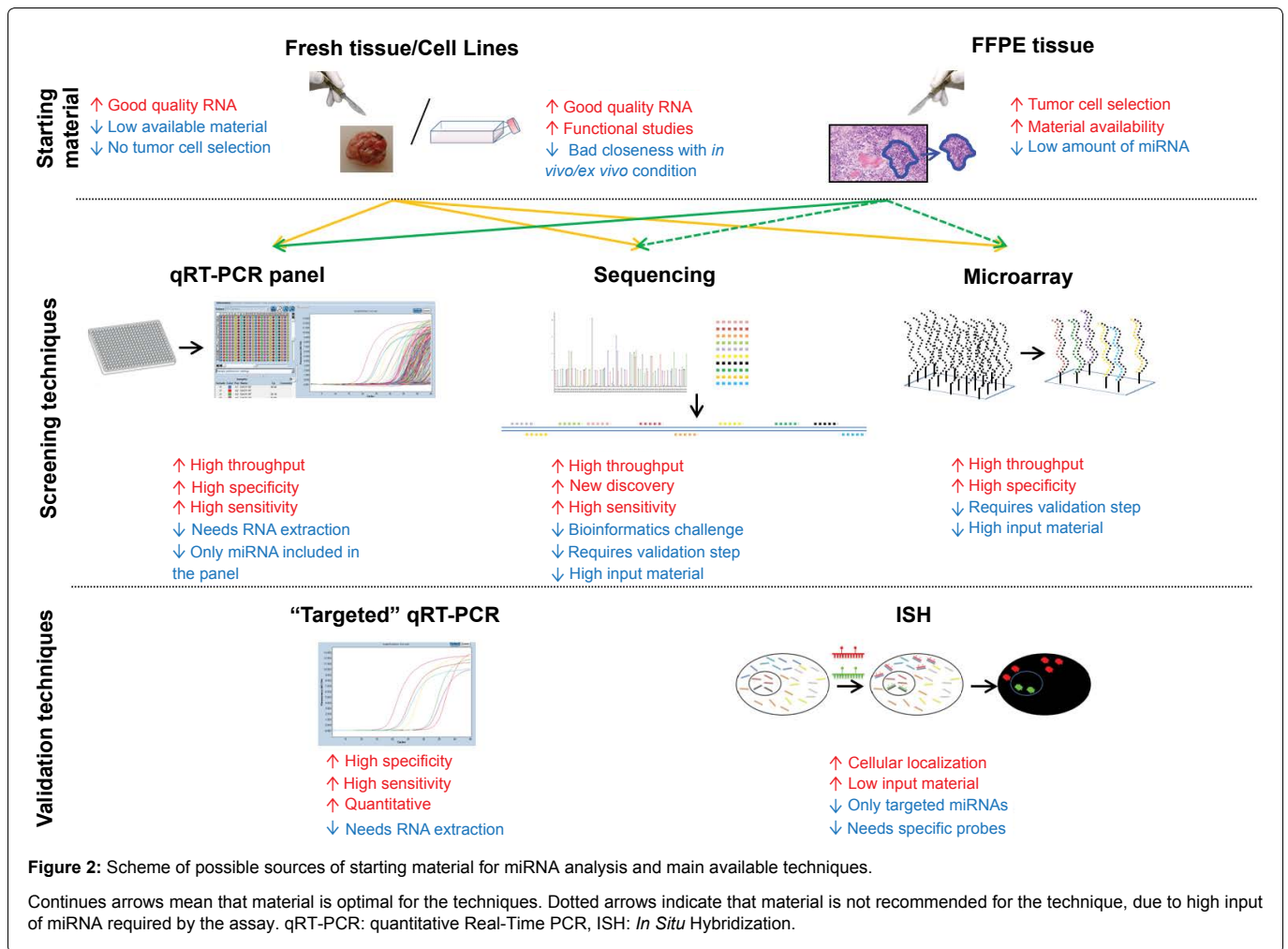
FFPE samples: Starting from formalin fixed and paraffin embedded (FFPE) samples might be of great usefulness for miRNAs expression studies. Due to their short length, the mature miRNAs seem not to be influenced by nucleic acid degradation caused by formalin fixation [57], as happened on the contrary for long RNA or DNA. Some papers reported the feasibility of miRNAs expression

from FFPE specimens in different tissues as kidney [58], prostate [59] or breast [60]. Several studies have analyzed miRNA expression profile in FFPE specimens compared with non-neoplastic or benign tissue, obtaining robust and reliable results [26,37,61-66]. It should be considered that: using FFPE samples, a neoplastic cells enrichment step (micro or macro-dissection) was feasible; FFPE samples could be easily retrieved from the archives of anatomic pathology institutes and, furthermore, the results displayed a good correlation of miRNA expression values obtained in FFPE specimens and in the corresponding fresh/frozen material (Figure 2) [26,67].

Another important aspect to consider in miRNA expression analysis is the selection of control group. There are different specimens that could be used as normal reference for miRNAs analysis in brain samples as the normal area adjacent the tumor, a commercial reference or cell lines. As previously demonstrated by our group, the selection of control group is a crucial step in miRNA analysis; in fact, it is possible to obtain discrepant results in miRNA expression data simply due to different choice of non-neoplastic control [68].

Techniques

Analysis of miRNAs with high specificity and sensitivity is technically challenging as mature miRNAs are short molecules, differ in GC (guanine-cytosine) content, and with single nucleotide



difference within miRNA families. Several techniques based on extracted RNA (e.g. deep sequencing, microarrays, quantitative Real-Time Polymerase Chain Reaction - qRT-PCR), or in situ hybridization assays (ISH) have made possible to profile miRNAs in brain lesions with high accuracy (Figure 2).

Microarray: Microarray analysis is a high-throughput assay that allows monitoring the expression of thousands of miRNAs in a single experiment. In the detection of miRNAs by microarray, appropriate probe design is crucial. In fact, capture probes must have a high specificity and affinity for individual transcripts. Because miRNAs are small molecules, the probe design possibilities are limited on the miRNA sequence. The microarray techniques should not be used to make quantitative statements, but rather to determine the relative change in expression between two states (e.g. tumoral vs. non-tumoral) [69]. Moreover, it should be considered that another limitation of microarray analysis is that the results need to be validated using a targeted technique, as qRT-PCR or ISH. Several papers have performed a genome-wide microarray analysis of miRNA expression of neoplastic brain specimens to detect expression signatures and associate this profile with characterization of the tumor [15-18,20,22,24,27,28,31,34,35,37,39,41,43-45,49,50,55,62,63] (Figure 2).

Next Generation Sequencing: Next generation sequencing (NGS) platforms became available for the sequencing sRNAs, including miRNAs, and allows to analyze thousands sequence in parallel from a given sample. Moreover NGS could highlight both miRNAs present in few copies and discover novel miRNAs, without the limitation of microarray probe selection. The high sensitivity of NGS offers an advantage over microarray assays, because the high variability of miRNA expression complicates the detection of miRNAs expressed at low copy numbers. However, because each NGS experiment produces several giga base pairs (Gbp) of sequence data, this technique needs massive bioinformatics challenges to analyze and handle sequence information. Due to relative novelty of this approach and

to the bioinformatics challenges to analyze the output data, NGS has been used less than other techniques for miRNAs analysis in brain neoplasia (Figure 2) [17,19,33,52].

qRT-PCR: Although high throughput assays (e.g. microarray or NGS) are useful tools to provide a broad overview of the miRNA expression, these data need to be confirmed by "miRNA specific" approaches. Quantitative real-time reverse transcriptase polymerase chain reaction is a method frequently used either to assessing expression in brain neoplasia of specific miRNAs and to confirm data obtained with broad range techniques [21,23,25,26,29,30,32,36-38,40,42,45-47,51-54,56,61,64-66]. Several approaches to analyze miRNAs continue to be based on single-target assays; however, high-throughput qRT-PCR technologies can be employed to identify differences in miRNA expression levels. For example, TaqMan low density arrays (TLDA) and Locked Nucleic Acid (LNA) array platform have been introduced for miRNA detection (Figure 2).

In situ Hybridization (ISH): A limitation of previously described techniques is that they are based on extracted miRNA and then do not provide information regarding the cell type present in the sample and the specific site of expression of a particular miRNA. Thus, the use of an ISH/IHC assay (in situ hybridization/immunohistochemistry) allows rapid and direct evaluation of changes of miRNA expression within the lesional cells. In situ techniques are used either as main assay for evaluating miRNA expression in pancreatic lesions visualized at cellular/subcellular level or to confirm results obtained by qRT-PCR or high-throughput technique (e.g. miRNA microarray or real-time array, see above) (Figure 2) [62,64].

MiRNA Expression in Glioblastoma

Glioblastoma multiforme (GBM) is the most malignant and the most frequent brain tumor of the adult, accounting for about 50% of diffuse gliomas [11,70]. It is a pleomorphic astrocytic tumor,

Table 1: MiRNAs profiling studies in glioblastoma "ex vivo" specimens

Number of Human miRNAs analyzed	Samples setting	Methods	Up-regulated miRNAs	Down-regulated miRNAs	References
245	9 fresh GBMs vs 9 ANBs	Microarray	-10b , -130a, -221 , -125b-1, -125b-2, -9-2, -21 , -25 , -123	-128a , -181c , -181a , -181b	[15]
180	3 fresh GBMs vs 8 NBs	Microarray	-21 , -138, -347, -291-5', -135	-202	[24]
534	240 GBMs vs 10 NBs (data from TCGA)	Microarray	-15a, -15b , -16 , -17-5p , -25 , -92, -93 , -106a , -106b , -320, -502, -532, -590, -660	-487a, -323 , -329 , -218	[28]
474	3 fresh GBMs vs 2 NBs	Microarray	-10b , -182, -10a , -183, 21 , -148a , -503, -505, -451 , -144, -542-5p, -542-3p, -26a, -142-3p, -199b, -15b , -550, -25 , 9*, -92b , -142-5p, -27a, -23a , -486 , -362, -532, -93 , -106b , -223	None found	[34]
756	26 fresh GBMs vs 13 AAs	Microarray	-16 , -21 , -22, -24, -34a, -126, -142-5p, -143, -146b-5p, -155 , -193a-3p, -199a/b-3p, -335, 376c, -381, -451 , -509-3-5p, -513a-5p, -552, -886-3p/5p	-128 , -219-2-3p	[43]
127	3 fresh GBMs vs ANB	Microarray	-10b , -21 , -183, -92b , -106b	-302c, -379 , -329 , -134, -369-3p	[44]
305	222 GBMs vs 10 NBs (data from TCGA)	Microarray	-20a , -106a , -17-5p , -148a , -146b, -200b, -193a	-221 , -222 , -31	[49]
NS	261 GBMs vs 10 NBs (data from TCGA)	Microarray	-21 , -10b , -210 , -27a, -25 , -15b , -23a , -92b , -106b , -92, -155 , -93 , -339, -494, -565, -130b, -34a, -16 , -20a , -320, -106a , -19b, -100, -17-5p , -424	-124a, -139 , -134, -7 , -218 , -338, -129, 2-19, -128b, -128a , -136, -410, -138 , -29b, -29c, -491, -132 , -379 , -377, -149, -769-5p, -330 , -323	[50]
1145	82 fresh GBMs vs 5 NBs	Microarray	-518b, -566	-181d, -524-5p, -1227	[55] †
282	12 fresh GBMs and 12 fresh O vs 4 fresh NBs	Microarray and qRT-PCR	-21 , -155 , let-7f, let-7a, -16 , -15b , -9, -210	-132 , -128 , -7, -330-3p, -139-5p, -124	[37]
8	10 fresh GBMs, 10 AAs, 8 LGAs, vs 4 NBs	qRT-PCR	-21 , -221	-181b	[25]
365	8 fresh GBMs vs 4 AAs	qRT-PCR	-15b , -21 , -135b, -196a, -196b , -363	-105, -128b, -184, -302b, -302d, -367, -383, -504, -517c, -601	[32]
875 §	3 fresh GBMs vs 3 NBs	NGS	-10b , -96, -10b*, -182, -135a*, -21* , -21 , -542-3p, -148a , -92b	-433, -7-1*, -129*, -628-5p, -935, -218 , -31 , -876-3p, -1258, -132	[33]
157	4 fresh secondary GBMs vs LGGs	qRT-PCR	-9, -15a, -16 , -17, -19a, -20a , -21 , -25 , -28, -130b, -140, -210	-184, -328	[40]
192	4 fresh GBMs, 4 AAs vs 4 NBs	qRT-PCR	-10b , -21 , -155 , -210	-7 , -31 , -107 , -124 , -124b, -129, -137 , -138 , -139 , -187, -203, -218 , -101, -128a , -132 , -133a, -133b, -149, -153, -154*, -185, -29b, -323 , -328, -330	[47]
Whole-genome small RNA sequencing (according to miRBASE v 16.0)	6 fresh GBMs vs 3 NBs	NGS	-10b*, -10b , -891a, -93 , -196b , -21*, -320d, -217, -4448, -25 , -660, -21 , -320c, -296-3p, -92b , -10a , -92a, let-7i*, -148a*	-124 , -95, -132 , -139-5p, -7, -543, let-7d, -323-3p, -128 , -598, -103a, -103b, -139-3p, -487b, -873, -323b-3p, -1381*, -301b, -107 , -411-3p, -124*, -342-3p, -379*, -212, let-7g, -153, -181d, -22, -889, -885-5p, -379 , -138 , let-7e, -218 , -221 , -136, -24, -4787-3p, -126, -548i, -382, -1270, -495, -2392, -1273d, -767-5p, -770-5p, -504, -490-3p	[48]
>1000	35 FFPE GBMs: short-term vs long-term survivors	Microarray	miR-3163	-539, -1305, -1260, let-7a	[63]
8	22 FFPE-dissected GBMs vs 6 NBs	qRT-PCR	-21 , -125b	-128a , -222 , -221 , -181b , -181c , -181a	[65]
8	38 FFPE GBMs vs 6 NBs and 4 commercial RNAs	qRT-PCR	-196a, -196b , -21	-128a , -221 , -195, -222 , -181c	[66]

Only miRNAs showed a fold change greater than or equal to two-fold, were considered. Studies performed on cell lines were not reported GBM: Glioblastoma, AA: Anaplastic Astrocytoma, NB: Normal Brain, ANB: Adjacent Normal Brain, LGA: Low Grade Astrocytoma, LGG: Low Grade Glioma, O: Oligodendroglioma, DA: Diffuse Astrocytoma, NS: Not Specified, NGS: Next Generation Sequencing, TCGA: The Cancer Genome Atlas (TCGA) Datasets, FFPE: Formalin-Fixed Paraffin-Embedded. In bold, miRNAs reported up- or down-regulated in at least 3 studies. § Only the most 10 up- or down-regulated miRNAs are reported, † The authors considered a cut-off fold change >1.5.

characterized by poor cell differentiation, high cell density, marked nuclear atypia and high mitotic activity. Moreover, microvascular proliferation and necrosis are peculiar features, in association with a high proliferation rate, marked invasiveness and resistance to conventional therapies [11,70]. Despite progresses in surgical techniques, radiotherapy, chemotherapy and "target therapy", the

prognosis of this neoplasia remains poor [70]. In the last decades several studies have been performed in attempt to identify a specific miRNAs expression pattern of GBM and a small subset of consistently deregulated miRNAs were further functionally characterized for their activities and downstream targets possibly involved in this tumor [34,44,71-74].

Twenty-four profiling reports have been selected and reviewed for this paper to underline miRNAs found consistently deregulated in GBM samples or in GBM cell lines (Table 1 and Supplementary Table 1). Among them, 16 studies have identified multiple miRNAs that are differentially expressed in GBM compared to normal tissues [15,24,25,28,31,33,34,37,44,47-50,55,65,66]. Three reports have focused on miRNA expression profiles that differentiate GBM (World Health Organization - WHO - grade IV) from Anaplastic Astrocytoma (AA, WHO grade III) or from low grade (WHO grade II or I) gliomas [32,40,43]; in two studies miRNA profile of GBM stem cells was analyzed in comparison with non-stem cells [16,17]; in another one miRNA differences have been analyzed between GBM migrating cells *versus* non migrating cells [18]; finally, Zhou et al. analyzed differences in miRNA expressions in 5 GBM cell lines and 1 AA cell line, compared to a commercial RNA [20] while Nyazi et al. analyzed miRNAs expression in GBM short term and long term survivors [63]. A great variability among the different studies is remarkable, depending from the technique used (microarray, qRT-PCR assay or next generation sequencing), the type or the number of samples analyzed, the number of target miRNAs studied through the expression results obtained.

From this comparison, only few miRNAs (up-regulated: miR-21, miR-10b, miR-15b, miR-16, miR-25, miR-92b, miR-93, miR-106b, miR-155, miR-210, miR-17-5p, miR-106a, miR-148a, miR-196b; down-regulated: miR-132, miR-218, miR-124, miR-128a, miR-323, miR-128, miR-7, miR-181b, miR-221, miR-222, miR-31, miR-138, miR-181c, miR-379) shared the same deregulation pattern in at least 3 studies performed on fresh or FFPE tissues (Tables 1-3 and Supplementary Table 1). Interestingly, miR-221/222, defined as “oncomirs” in GBM and deeply discussed by several works as important miRNAs in GBM pathogenesis [25,75-79], were found either up- [15,25] or down-regulated [48,49,65,66] (miR-221), or only down-regulated (miR-222) [49,65,66] (Tables 1-3 and Supplementary Table 1). Intriguingly, miR-221 and miR-222 were found up-regulated in all studies that have investigated their role in cellular function (Tables 2,3) [80-83]. This discrepancy could be due to several experimental variables, as choice of “non-neoplastic control” or source of the starting material, as previously reported by Visani and colleagues [84].

According to literature data (Tables 1-3 and Supplementary Table 1), it is clear that by now an exhaustive miRNAs profile of GBM is still far to be well defined.

Many other studies were focused only on a single, or on a small subset of miRNAs to analyze their function, genetic regulation and expression in GBM tissues/cell lines. Functional analysis are very important to experimentally validate possible targets of those miRNAs found deregulated in previous studies, clarifying their molecular roles in GBM pathogenesis and giving information above putative prognostic markers or therapeutic targets [16,20,24,31,32,34,44,47,71-74,79-81,83,85-96].

Oncogenic miRNAs in GBM

MiRNAs found consistently up-regulated in at least 3 studies performed on fresh or FFPE tissues are reported in tables 1 and 2 and in supplementary table 1. Several miRNAs found consistently up-regulated in GBM have been intensively investigated with respect to both expression and functionality (Table 2) [20,24,40,44,52,71,77,80-83,97-112]. In particular, miR-21 and miR-10b had been deeply studied in literature. MiR-21 had been found as significantly deregulated in 15 out of 19 profiling studies performed on FFPE or fresh tissues here considered (Tables 1,2 and Supplementary Table 1). In all cases an over-expression in GBM tissues or in GBM cell lines was reported [15,24,25,32-34,37,40,43,44,47,48,50,65,66]. Several functional studies have investigated miR-21 role in several processes involved in malignancy progression and maintenance of GBM. It was demonstrated that miR-21 acts as anti-apoptotic factor blocking the expression of critical apoptosis-related genes. Chan et al. [24] first reported that the forced down-regulation of miR-21 leads to caspase 3/7 activation and associated apoptotic cell death. Chen et al. [71] and Gaur et al. [98] showed that *in vitro* and *in vivo* miR-21 over-expression inhibits the expression of the pro-apoptotic factor PDCD4. MiR-21 also regulates molecules within the p53 and TGFB apoptotic pathways targeting p63 (a homologue of p53), JMY (a p53 activator), TOPORS, TP53BP2 and HNRPK and the receptors TGFBR2/3, respectively [100]. Also DAXX (death associated protein 6), which acts in both pathways stabilizing p53 and mediating TGF-h apoptosis, is regulated by miR-21 [100].

Table 2: MiRNAs reported up-regulated in glioblastoma samples.

RNAs up-regulated in GBM	Number of profiling studies*	Functions	Targets validated in GBM	References
miR-21	15	Apoptosis	Caspases, PDCD4, p63, JMY, TOPORS, TP53BP2, HNRPK, TGFBR2/3, DAXX	[24,71,98,100]
		Invasion	RECK, TIMP3	[114]
		Cell cycle/	hTERT, PTEN	[20,113]
		Tumor growth		
miR-10b	7	Apoptosis	Bim, caspases, TP53, CYLD, FOXO3	[99,114]
		Invasion	HOXD10, NOTCH1, PAX6	[44,99]
		Cell cycle/Tumor growth	TFAP2C, p16, p21, PTCH1	[99,114]
		Angiogenesis	NOTCH1, CYLD, PAX6	[99]
miR-15b	5	Cell Cycle	CCNE1	[111]
miR-16	5	NA	NA	-
miR-25	6	Cell cycle/apoptosis	Mdm2, TSC1	[108]
miR-92b	5	Proliferation /invasion	NLK	[110]
miR-93	4	Proliferation/ Angiogenesis	Integrin-β8	[105]
miR-106b	4	Cell cycle/proliferation	RBL2, p21	[112]
miR-155	4	Proliferation	GABRA1	[103]
miR-210	4	NA	NA	-
miR-17-5p	3	DNA repair	Pold 2	[40]
		Proliferation	TGFBR2, SMAD4, CDKN1A, PTEN, E2F1	[102]
		Angiogenesis	CTGF	[104]
miR-106a	3	Proliferation/apoptosis	E2F1	[52] †
miR-148a	3	NA	NA	-
miR-196b	3	Proliferation	E2F1	[107]

Only miRNAs reported in at least 3 profiling reports were considered (for more information see Table 1). Studies performed on cell lines were not considered for miRNA profile. *Number of studies reporting the same regulation pattern for each miRNA (see Table 1). †In this study, authors obtained a down-regulation of selected miRNA. NA, Functional studies are not available in literature.

Table 3: MiRNAs reported down-regulated in glioblastoma samples

MiRNAs	Number of profiling studies*	Cellular Functions	Validated targets in GBM	References
miR-128 [^]	8	Proliferation, self-renewal, cell cycle Angiogenesis	ARP5, Bmi1, E2F3A, EGFR, PDGFRA, WEE1 p70S6K1	[31,128,131,132] [133]
miR-218	5	Migration/invasiveness Apoptosis	IKK- β , LEF1 ECOP	[122,125] [129]
miR-132	5	NA	NA	-
miR-7	4	Proliferation Invasiveness	EGFR, IRS1, IRS2 FAK	[72] [127]
miR-124	3	Migration/invasiveness Cell cycle/proliferation	IQGAP1, LAMC1, ITGB1 CDK6, pRB, CDK4,	[120] [47,119]
miR-323	3	NA	NA	-
miR-221	4	Cell cycle Apoptosis Invasiveness	p27/KIP1, p57/KIP2 NIAP, PUMA PTP μ	[80,82]† [81,83]† [78]†
miR-181b	3	Proliferation/invasiveness	IGF1R	[124]
miR-222	3	Cell cycle Apoptosis Invasiveness	p27/KIP1, p57/KIP2 NIAP, PUMA PTP μ	[80,82]† [81,83]† [78]†
miR-31	3	Migration/Invasiveness	RDX	[130]
miR-138	3	Proliferation	Msi1	[126]
miR-181c	3	NA	NA	-
miR-379	3	NA	NA	-

Only miRNAs reported in at least 3 profiling reports were considered (for more information see Table 1). Studies performed on cell lines were not considered for miRNA profile. [^] Five studies were focused on miR-128a. *Number of studies reporting the same regulation pattern for each miRNA (see Table 1). †In these studies, an up-regulation of selected miRNAs was observed. NA, Functional studies are not available in literature

Some of the several mRNA targets controlled by miR-21 are directly associated with the tumor invasiveness. For example, Gabriely et al. [97] reported that miR-21 regulates MMPs and glioma cell invasiveness by directly controlling the MMP inhibitors RECK and TIMP3. Authors showed that the forced inhibition of miR-21 decreases MMP activities both *in vitro* and *in vivo*, leading to reduction of glioma cell motility and invasion [97].

Furthermore, miR-21 could control GBM cell growth by maintaining active the telomerase activity, which is fundamental to maintain the DNA integrity of the replicating tumor cells, establishing cell immortality and survival. As suggested by Wang and colleagues [113], miR-21 regulates hTERT (human telomerase reverse transcriptase) expression in a STAT3- (signal transducer and activator of transcription 3) dependent manner and guarantee GBM cell growth.

In a study by Zhou et al. [20] it was demonstrated that the tumor suppressor PTEN could be a direct target of miR-21. A decreased expression of EGFR and Akt activation was also reported in response to an inhibition of miR-21, concurrently with increased apoptosis through decreased levels of the anti-apoptotic Bcl-2. This suggested that the control of miR-21 on cell growth and apoptosis pathways could be mediated by other targets in parallel with PTEN [20].

In the present series, seven studies reported an up-regulation of miR-10b in GBM tissues [15,33,34,44,47,48,50] (Tables 1,2 and Supplementary Table 1). In 2009, Sasayama et al. showed that the up-regulation of miR-10b was associated with glioma grading and malignancy, according to data obtained on 43 glioma samples (seventeen GBM, six AA, ten low-grade astrocytoma, six oligodendroglioma and four ependymoma) and six glioma cell lines [44]. Furthermore, they showed that miR-10b inhibits the expression of the transcription factor HOXD10 [44]. It was demonstrated that HOXD10 is able to regulate several genes promoting invasion, migration, extracellular matrix remodeling and tumor progression, including α 3-integrin, β -integrin, MMP-14 and two molecules, uPAR (urokinase receptor) and RhoC (Ras homolog gene family member C), which expression is correlated with expression levels of miR-10b [44].

Gabriely et al. [114] demonstrated in several glioma cell lines that miR-10b inhibition could block cell cycle progression and in some cases lead to the accumulation of senescent glioma cells. MiR-10b acts as cell cycle regulator modulating in an indirect manner the

cyclins B1 and D1 expression levels, whereas the inhibition of miR-10b expression is significantly associated to lower levels of this two factors [114]. Moreover, miR-10b inhibition is strictly associated to an increase activation of caspase-3 and -7 suggesting a direct role of miR-10b on apoptotic pathway [114].

In the same study, Bim, TFAP2C, p16, and p21 were validated as direct targets of miR-10b, confirming its role in cell cycle and apoptosis control. Intriguingly, in this study the regulation of miR-10b reported on HOXD10 by Sasayama et al. [44] was not confirmed [114].

Lin et al. [99] reported that miR-10b directly regulates inhibition of several tumor suppressor genes (*FOXO3*, *CYLD*, *HOXD10*, *TP53*, *PAX6*, *PTCH1* and *NOTCH1*) affecting apoptosis, cell invasion and angiogenesis.

Tumor suppressor miRNAs in GBM

MiRNAs found consistently down-regulated in at least 3 studies performed on fresh or FFPE tissues are reported in tables 1 and 3 and in supplementary table 1. Among miRNAs found consistently down-regulated in GBM (Table 3) [31,33,47,72,80-83,101,115-130], miR-128 and miR-218 have been deeply investigated.

MiR-218 was found deregulated in 5 out of the 19 miRNA profiling studies performed on FFPE or fresh tissues here considered. In all cases, a down-regulation of miR-218 was observed in GBM [28,33,47,48,50] (Tables 1,3 and Supplementary Table 1).

The lack of miR-218 expression leads to an increase of GBM cell invasiveness. In fact, the down-regulation of miR-218 causes an over-expression of IKK- β which promotes the activation of NF- κ B/MMP9 signaling pathway [125]. Also the oncogenic transcription factor LEF-1 regulates the expression of MMP9 and Liu et al. [122] demonstrated that LEF-1 is a direct target of miR-218 supporting the thesis that the deregulation of this miRNA is directly correlated to the GBM cell migration and invasion. In another functional study, Xia and colleagues [129] showed that the over-expression of miR-218 in glioma cells induced apoptosis and inhibited cell viability, proliferation and tumorigenicity. ECOP (Epidermal growth factor receptor-coamplified and over-expressed protein), a proteins that belongs to NF- κ B signaling pathway associated with apoptotic response, was identified as a direct target of miR-218 [129].

Both miR-128 [37,43,48] and miR-128a [15,47,50,65,66] were

reported as down-regulated in eight out of 19 profiling studies performed on FFPE or fresh tissues. The exogenous expression of miR-128 significantly reduced *in vitro* and *in vivo* glioma cell proliferation. One of validated miR-128 target is Bmi1, a molecule that regulates the epigenetic gene silencing by chromatin modifications and stem cell renewal [31,131]. MiR-128 promotes GBM cell proliferation by controlling the expression of ARP5 (angiopoietin-related growth factor protein 5) which normally regulates cell regeneration, proliferation and promotes neovascularization; expression of E2F-3a, a transcription factor which plays a crucial role in the control of cell-cycle progression [131], is also controlled by miR-128. The WEE1 tyrosine kinase, that phosphorylates CDK1 playing a role in cell-cycle control, is a direct target of miR-128, as reported by Wutchy et al. [128].

Papagiannakopoulos and colleagues [132] suggested that miR-128 could also control the mitogenic tyrosine kinase signaling targeting EGFR and PDGFR α oncogenes. Finally, Shi et al. [133] reported p70S6K1 as another direct target of miR-128, which is involved in glioma angiogenesis and tumorigenesis.

MiRNA Expression in other Glial tumors

MiRNA expression in Astrocytoma

Diffuse astrocytoma (WHO grade II) is characterized by a high degree of cellular differentiation and slow growth.

Diffuse astrocytoma inherently tends to locally recur and spontaneously progress to AA (WHO grade III) and eventually to secondary GBM (WHO grade IV). Progression from primary low-grade glioma to secondary GBM was associated with up-regulation of 12 miRNAs (miR-9, miR-15a, miR-17, miR-19a, miR-20a, miR-21, miR-25, miR-28, miR-130b, miR-140, miR-210) and with the down-regulation of 2 miRNAs (miR-184 and miR-328) [134] (Table 4).

The miR-21 and miR-221 were also observed up-regulated in astrocytoma [25,43,53], a diffuse infiltrating malignant astrocytoma (WHO grade III) characterized by nuclear atypia, increased cellularity and significant mitotic activity. In contrast, miR-106a, miR-124, miR-125 and miR-181b were reported as down-regulated in this type of tumors [25,53].

As regards some of the above reported deregulated miRNAs, miR-181b, miR-106a and miR-21 were seen significantly associated with poor survival in patients with astrocytoma. These data could lead to

hypothesize that miR-21, miR-106a or miR-181b may have a strong potential to serve as novel prognostic markers of astrocytoma [53].

The miRNAs analysis in the serum of malignant astrocytoma patients, compared to normal controls, revealed several miRNAs (miR-15b*, miR-23a, miR-133a, miR-150*, miR-197, miR-497 and miR-548b-5p) whose concentrations were significantly lower in serum of the patients. Moreover, the concentrations of miR-23a, miR-150*, miR-197 and miR-548b-5p were markedly decreased in malignant cases if compared to the astrogliosis group [52].

Considering miRNAs found deregulated in at least 2 studies, miR-21 results to be up-regulated in astrocytoma while miR-181b is down-regulated.

MiRNA expression in oligodendroglial tumors

Oligodendrogliomas (WHO grade II) are diffusely infiltrating, well-differentiated gliomas, composed of neoplastic cells morphologically resembling oligodendroglia [11] (Table 5).

A comparative study between ten oligodendrogliomas and non-neoplastic samples identified several miRNAs up- (let-7a, let-7f, miR-17, miR-21, miR-155, miR-17, miR-16, miR-26b, miR-374a, let-7d, miR-20a, miR-15b, miR-7b, miR-9) or down-regulated (miR-132, miR-134, miR-7, miR-330-3p, miR-127-3p) [37]. Moreover, in the same study, a set of seven markers (miR-21, miR-128, miR-132, miR-134, miR-155, miR-210 and miR-409-5p) was proposed to help in distinguishing GBM from oligodendroglial tumors [37].

Nelson et al. demonstrated that miR-9 was highly expressed in oligodendrogloma and fetal brain, but had a low expression in adult brain, suggesting that miR-9 up-regulation may be important in the development of oligodendrogliomas [62]. The same authors observed reduced level of miR-124 in oligodendrogloma compared to normal brain [62].

A comparison between newly diagnosed anaplastic oligodendrogloma and recurrent tumors showed that 7 miRNAs (miR-124, miR-128, miR-139-5p, miR-153, miR-210, miR-582-5p and miR-96) were highly increased in the patients with recurrent anaplastic oligodendrogliomas. In particular, 5 miRNAs (miR-124, miR-128, miR-139-5p, miR-210 and miR-582-5p) exhibited more than 10-fold increased expression [35].

In contrast, 21 miRNAs (miR-1, miR-1180, miR-133b, miR-135b,

Table 4: MiRNAs deregulated in astrocytomas

miRNA up-regulated	miRNA down-regulated	Samples Setting	Methods	References
-21	None Found	Astrocytoma vs Normal Brain	Microarray	[43]
-21, -221	-181b	AA vs Normal Brain	qRT-PCR	[25]
-21, -24	-181b, -124, -125, -106a	AA vs Normal Adjacent Tissue	qRT-PCR	[53]
-21	-106a, -181b	Correlated with poor survival		[53]
-9, -15a, -17, -19a, -20a, -21, -25, -28, -130b, -140, -210	-184, -328	Astrocytoma Correlated to progression to GBM	qRT-PCR	[134]
	-15b*, -23a, -133a, -150*, -197, -497, -548b-5p	Serum vs Normal Controls	NGS and qRT-PCR	[52]
	-23a, -150*, -197, -548b-5p	Serum vs Astrogliosis		[52]

AA: Anaplastic Astrocytomas, GBM: Glioblastoma, qRT-PCR: quantitative Real-Time Polymerase Chain Reaction, NGS: Next Generation Sequencing.

Table 5: MiRNAs deregulated in oligodendroglial tumors

Tumor Type	miRNA up-regulated	miRNA down-regulated	Samples Setting	Methods	References
Oligodendrogloma	-124, -128, -139-5p, -153, -210, -582-5p, -96	-1, -1180, -133b, -135b, -1539, -193a-5p, -196a, -196b, -200b, -21*, -221*, -224, -24-1*, -31, -31*, -32*, -34a*, -34c-5p, -455-5p, -503, -631	Recurrent vs De Novo Oligodendrogloma	Microarray	[35]
	-9	-124	Oligodendrogloma vs Normal Brain	Microarray and ISH	[62]
	let-7a, let-7f, -21, -155, -17, -16, 26b, -374a, let-7d, -20a, -15b, -7b, -9	-132, -134, -7, 330-3p, -127-3p	Oligodendrogloma vs Non-Neoplastic tissues	Microarray and qRT-PCR	[37]
	-21, -155	-132, -134	Oligodendrogliomas vs GBM		[37]
Oligoastrocytoma		-137	Oligoastrocytomas vs Normal Brain	qRT-PCR	[38]

GBM: Glioblastoma, ISH: In Situ Hybridization, qRT-PCR: quantitative Real-Time Polymerase Chain Reaction

miR-1539, miR-193a-5p, miR-196a, miR-196b, miR-200b, miR-21*, miR-221*, miR-224, miR-24-1*, miR-31, miR-31*, miR-32*, miR-34a*, miR-34c-5p, miR-455-5p, miR-503 and miR-631) showed a huge (0.01-fold) decreased expression in patient with recurrent anaplastic oligodendroglioma, if compared with those with newly diagnosed tumors [35].

Moreover, in oligodendroglioma also miR-137 was found down-regulated, as already reported for oligoastrocytoma [38].

MiR-9 is the only miRNA found deregulated (up-regulated) in oligodendroglioma in at least 2 papers [37,62].

OligoAstrocytomas. Oligoastrocytomas are diffuse infiltrating glioma composed of a mixture of two distinct neoplastic cell types morphologically resembling the tumor cells in oligodendroglioma and diffuse astrocytoma (WHO Grade II) [11].

In a study of 16 oligoastrocytomas, miR-137 was down-regulated in the vast majority of analyzed samples [38]. According to data obtained in transfected cell lines, miR-137 could play an important role in the suppression of invasion of glioma cells. A putative target of miR-137 in oligoastrocytomas could be CSE1L (Chromosome Segregation 1-Like), a protein involved in tumor cell invasion and metastasis in cancer progression [38].

MiRNA expression in ependymal tumors

Ependymoma (WHO grade II) is a common pediatric central nervous system (CNS) tumor that is believed to originate from

ependymal cells located in the lining of ventricular surfaces in the brain [11] (Table 6).

Costa and colleagues analyzed the expression of 365 miRNAs in 34 ependymoma samples compared with normal brain tissue of the same patients [26]. The authors identified 28 miRNAs that were differentially expressed in ependymoma samples. Specifically, 23 miRNAs (miR-10a, miR-17-5p, miR-19a, miR-19b, miR-20a, miR-21, miR-32, miR-34a, miR-106b, miR-130a, miR-135a, miR-142-3p, miR-193a, miR-210, miR-301, miR-449b, miR-502, miR-518b, miR-551b, miR-565, miR-591, miR-594 and miR-601) were up-regulated and 5 miRNAs (miR-139, miR-323, miR-383, miR-433 and miR-485-5p) were down-regulated in ependymoma specimens [26]. Moreover, miR-203 was proposed as a biomarker for management of disease progression in ependymomas and as a predictor for the likelihood of recurrence. In fact, while higher expression of miR-432, miR-411, miR-376a, miR-381 and miR-487b was associated with a lower relapse free probability, a lower expression of miR-203 was correlated to a trend to develop recurrences [26].

MiRNA Expression in Medulloblastoma

Medulloblastoma (MB, WHO grade IV), the most frequent brain neoplasia in children, is an aggressive infratentorial primitive neuroectodermal tumor that originates from aberrant development of cerebellar progenitor neurons [11,135,136]. Nevertheless, the division into molecular subgroups is a useful tool for the risk stratification of the patients and has generated several hypotheses for new treatment [137]; further molecular characterization will allow

Table 6: MiRNAs deregulated in ependymoma

miRNA up-regulated	miRNA down-regulated	Samples Setting	Method	Reference
-10a, -17-5p, -19a, -19b, -20a, -21, -32, -34a, -106b, -130a, -135a, -142-3p, -193a, -210, -301, -449b, -502, -518b, -551b, -565, -591, -594, -601 -432, -411, -376a, -381, -487b	-139, -323, -383, -433, -485-5p -203	Ependymoma vs Normal Brain Correlated with lower relapse Correlated with high risk of recurrence	qRT-PCR	[26]

qRT-PCR: quantitative Real-Time Polymerase Chain Reaction

Table 7: MiRNAs profiling studies in medulloblastoma

Number of Human miRNAs analyzed	Samples setting	Methods	Up-regulated miRNAs	Down-regulated miRNAs	References
248	34 MBs vs 14 adult and fetal NBs	qRT-PCR	-17-5p, -214, -199b	-22, let-7a, let-7b, let-7d, let-7e, let-7f, let-7g, let-7i, -26a, -26b, -29c, -30b, -30c, -31, -98, -103, -107, -124a, -125a, -127, -128a, -128b, -129, -132, -133b, -134, -138, -143, -145, -149, -150, -153, -154, -181b, -181c, -190, -191, -192, -194, -212, -219, -320, -323, -324-3p, -324-5p, -326, -328, -330, -331, -346, -370, -381, -382, -383, -425	[29]
762	10 fresh MB vs CD133+ NSCs	qRT-PCR	-127-3p, -539, -495, -409-3p, -494, -376c, 379, -376a, -323-3p, -203, -193a-5p, -144*, -21*, -338-3p, -143, -145, -146a, -126, -126*, -223, -361-3p	-483-5p, -10a, -373, -935, -92b*, -302b*, -302d*, 219-1-3p, 219-5p, -219-2-3p, -106a*, -504	[30]
NS	6 fresh MB vs ANBs	Microarray	-17, -99a, -100, -106b, -204	-218, -29a, -29c, -128a, -379, -127-3p, -9, -214	[39]
NS	90 MBs vs 10 NBs	qRT-PCR	-216a, -106a, -182, -17, -93, 20a, -20b, -106a, -18a, -21, -106b, -25, -183, -96*, -19b, -135a, -130a, -19b, -130b, -217, -19a, -345, -33a, -183*, -375, -32, -92a, -148a, 582-3p, -210, -135b, -137	-329, -124, -409-3p, -1, -129-5p, -34b, -134, -7-2*, -380, -496, -124, -432, -329, -379, -376b, -129-5p, -218, -130a*, -338-5p, -133a, -433, -410, -382, -299-5p, -376c, -376a, -495, -133a, -218, -138, -128a/b, -323-3p, -219-2-3p, -129*, -539, -128a/b, -129-3p, -219-5p, -138	[42]
250	17 MB-Gli ^{High} vs 14 MB-Gli ^{Low}	qRT-PCR	-199b, -214	-7, let-7a, let-7e, let-7f, -9, -25, -30b, -100, -103, -124a, -125b, -132, -135a, -135b, -142-5p, -143, -150, -153, -181c, -190, -191, -203, -324-3p, -324-5p, -326, -331, -338, -425	[56]
NS	Mouse model: MBs, P6 GNPs vs 1-month-old rodent cerebellum	NGS	-17, -18, -20a, -19b, -216b, -9*, -449, -130a, -217, -92, -93, -25, -19a, -216a, -34c, -335, -106b, -503, -130b, -217*, -672, -17*, -191, -20a*, -423, -32	-204, -127, -29b, -129, -128a, -138, -338, -206, -29a, 382, -124a, -218, -143, -127*, -128b, -125a, -434, -487b, -381, -150, -433, -378, -300, -136	[19]

MB: Medulloblastoma, NB: Normal Brain, ANB: Adjacent Normal Brain, NSC: Neural Stem Cells, GNP: Granule Neuron Progenitors, NS: Not Specified, NGS: Next Generation Sequencing, qRT-PCR: quantitative Real-Time Polymerase Chain Reaction. Only miRNAs which showed a fold change greater than or equal to twofold, were considered.

the identification of novel targeted therapies and will improve the reliability of risk stratification. To develop novel therapies and to identify new biomarkers useful to classify histological variants or to predict patient's overall survival, in the last years many groups have investigated the miRNAs layout of this neoplasia [138]. In table 7, six miRNA profiling studies are reported: miRNAs expression in MB samples has been evaluated, for example, in fresh tumors compared with non-neoplastic samples [29,39,42] or with CD133+ Neuronal Stem Cells (NSCs) [30] or, as in the study of Uziel et al., a rodent model of MB was studied [19]. In the work of Ferretti et al. [56] they compared miRNA expression patterns between MB with high or normal expression of Gli1, a transcription factor of Sony Hedgehog (SHH) signaling pathway which is often hyperactive in MBs [56,135]. They obtained 31 significantly deregulated miRNAs, most of them were down-regulated in MB-Gli1^{High}, suggesting that the deregulation of SHH pathway could be due to the loss of specific miRNAs [56] (Table 7).

According to miRNAs found consistently deregulated in at least three studies, a putative miRNA profile for MB could be the following: miR-17, miR-21, miR-106b, miR-199b and miR-214 are up-regulated in MB; miR-9, miR-29c, miR-30b, miR-103, miR-124a, miR-128a, miR-129, miR-132, let-7a, let-7e, let-7f are down-regulated in MB.

MiRNA control of Hedgehog signaling in MB

The SHH signaling pathway is a major mitogenic regulator of granule progenitor cells (GPCs) and its deregulation has been implicated in MBs [135]. Figure 3 presented a schematic view of how several functional studied miRNAs could control this oncogenic pathway in MB. Among characterized miRNAs in MB pathogenesis, the over-expression of the miR-17-92 cluster, also known as Oncomir-1, has been deeply investigated. The oncogenic expression of miR-17, miR-19a, miR-20 and miR-92, all of them included in the Oncomir-1 cluster, induces elevated SHH pathway which lead to tumorigenic effects in MB development, among which the c-Myc activation [19,42,135]. Northcott et al. [42] showed a 13q31.3 locus amplification, in which the miR-17/92 cluster mapped. An over-expression of this cluster and its related paralogous (miR-106a/363 and miR-106b/25) was demonstrated in MB if compared to normal human cerebellum [42]. In particular, this over-expression was associated with activated SHH signaling and elevated c-Myc and n-Myc expression levels, supporting the hypothesis that the up-regulation of this miRNA cluster promotes the growth of MB in a SHH-mediated manner [42]. In particular, it is demonstrated that Myc and n-Myc transcriptional regulate miR-17/92 by binding to the promoter of primary miR-17/92 cluster [42,139]. Uziel and colleagues [19] obtained similar results, analyzing miRNAs profiles in rodent models of MB (Ink4c^{-/-}; Ptch ^{+/-}; Ink4c^{-/-}; p53^{-/-} genotypes) compared to mature rodent cerebellum and developing cerebellum (P6 GPCs): among the 26 miRNAs that they obtained over-expressed, nine miRNAs belonging to the miR-17-92 cluster. Furthermore, the authors demonstrated that three miRNAs of this cluster, miR-19a, miR-92 and miR-20, were significantly over-expressed in human SHH-active MBs when compared to normal control cerebellum [19].

Ferretti et al. [56] identified a specific signature in tumors with activated SHH (MB-Gli1^{High}): miR-125b, miR-326 and miR-324-5p were down-regulated and the forced over-expression of these three miRNAs inhibited SHH-signaling pathway (Figure 3). In particular, miR-324-5p targets both Smo (the transmembrane protein Smoothed, a SHH-pathway activator) and Gli1 (a downstream transcriptional activator that acts as a SHH-pathway effector) and cooperates with miR-326 to further reinforce the inhibitory activity [56]. The deregulation of these three miRNAs was involved in cell proliferation stimulus through a hedgehog-dependent mechanism [56].

Finally, miR-214, found up-regulated in human MB by two profiling studies (Table 7) [29,56], could regulate SHH signaling targeting SUFU (Suppressor of fused, a Hh antagonist, which inhibits Gli1 and Gli2 from entering the nucleus) as already demonstrated by Flynt et al. in zebrafish [140].

MiRNA Expression in other Brain Tumors

Tumors of the hematopoietic system

Lymphoma CNS: Primary central nervous system lymphoma (PCNSL) is a rare subtype of extranodal non-Hodgkin's lymphoma that is confined to the CNS in the absence of systemic disease. In patients with PCNSL, definitive diagnosis often is difficult only on the basis of radiographic features and responsiveness to corticosteroids, which both do not specifically distinguish between lymphoma and inflammatory CNS disease [141]. Identification of PCNSL specific miRNAs could provide a potentially useful tool as novel noninvasive biomarker for the diagnosis of this neoplasia (Table 8).

MiR-21, miR-19b, miR-92a were found up-regulated in a study of 23 patients with PCNSLs and were proposed as marker for PCNSL [21]. These deregulated miRNAs were evaluated also as circulating miRNAs in serum of 14 PCNSL patients. However, no significant differences in the expression levels of miR-21, miR-19b, and miR-92 were observed in serum of PCNSL patients in comparison with that of the control group [21].

MiR-21 and miR-19b were the most abundant miRNAs in 9 CNS diffuse large B-cell lymphomas also in a study by Robertus et al. performed using a qRT-PCR technique [64]. Other than up-regulation of miR-21 and miR-19b, the authors observed also low expression levels of miR-16 and miR-127 [64].

The expression of 13 miRNAs (miR-9, miR-20b, miR-155, miR-340, miR-17-5p, miR-148a, miR-30b, miR-27b, miR-26b, miR-146b, miR-20a, miR-30c and let-7g) was significantly higher in primary CNS lymphomas compared with nodal diffuse large B-cell lymphomas, while five miRNAs (miR-199a, miR-214, miR-432, miR-193b and miR-145) showed a reduced expression in PCNSL if compared with nodal diffuse large B-cell lymphoma. Of the 13 up-regulated miRNAs, the most expressed were miR-9, miR-20b, miR-155, miR-340, miR-17-5p and miR-148a [61]. According to these data, the down-regulation of specific miRNAs, leading to an activation of the MYC (V-Myc Avian Myelocytomatosis Viral Oncogene Homolog) pathway or to a blockade of germinal center exit in B cells, might play a key role in PCNSL pathogenesis [61].

Considering miRNAs found deregulated in at least two papers, diffuse large B-cell lymphomas are characterized by up-regulation of miR-19b and miR-21.

Meningeal tumors:

Meningioma: Meningiomas are frequent - accounting for 15% to 20% of all CNS tumors - intracranial neoplasia of adulthood. Most meningiomas are benign (WHO grade I), but certain histological subtypes are associated with less favorable outcomes and correspond to atypical (WHO grade II) and anaplastic (WHO grade III) meningiomas [142].

Five different miRNAs (let-7a, let-7b, let-7c, let-7d and miR-145) were investigated in a series of primary meningioma tissues compared with non-neoplastic arachnoid specimens [36]. A significant down-regulation of miR-145 and let-7d expression in the tumor tissues was observed when compared with non-neoplastic controls. Moreover, lower expression of miR-145 was detected in the group of atypical (WHO grade II) and anaplastic (WHO grade III) meningiomas if compared with benign ones (WHO grade I) [36]. The malignant cell lines transfected with pre-miR-145 were demonstrated to be less invasive and with reduced motility (Table 8) [36].

In a study on 14 human sporadic benign meningiomas compared with arachnoidal tissue controls, 43 miRNAs were found deregulated in most tumor samples. Of the over-expressed miRNAs, miR-335, miR-98, and miR-181a were the most up-regulated (from 25- to 30-fold). Additional miRNAs (miR-19b, let7d, let7g, miR-100, miR-125a, miR-103, miR-370, miR-106b, miR-106a, miR-125b, let7b, miR-26a, miR-23b, and miR-29a) were confirmed to be up-regulated (4- to 10-fold) using qRT-PCR. The three down-regulated miRNAs (miR-200a, miR-373* and miR-575) showed marked decreases [45].

The over-expression of miR-200a in transfected cells showed inhibitory effect on meningioma cell growth, while down-regulation of miR-200a significantly increased, *in vitro*, the growth of meningioma cells [45].

A TaqMan-based real-time stem-loop RT-PCR analysis in 30 human meningioma specimens compared with normal brain arachnoid tissue, revealed elevated expression levels of miR-335, especially in WHO grade III samples [46]. Moreover, it was demonstrated that miR-335 inhibition lead a statistically significant level of inhibition of cell proliferation compared with that of the control group [46].

The analysis of expression levels of 200 miRNAs from 110 meningioma samples compared to the miRNA expression profiles of 35 normal adjacent tissues revealed that 14 were miRNAs differentially expressed in tumor specimens. Twelve miRNAs (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 up-regulated whereas two miRNAs (miR-29c-3p

and miR-219-5p), were significantly down-regulated [54]. Based on their respective miRNA expression profiles, an unsupervised cluster analysis revealed a clear separation of the meningioma samples from the normal adjacent tissues. Moreover, authors observed that miR-96-5p and miR-190a were up-regulated in patients with tumor recurrence if compared to those without recurrence. In contrast, miR-29c-3p and miR-219-5p were up-regulated in patients without recurrence compared to those with tumor recurrence. Finally, the univariate analysis indicated that WHO tumor grade and expression levels of miR-190a, miR-29c-3p and miR-219-5p were significantly correlated with recurrence [54].

Intriguingly, considering all papers about miRNA expression in meningeal tumors, no miRNAs have been found consistently deregulated in at least two papers.

Tumors of the sellar region

Pituitary adenoma: Pituitary adenomas account for at least 10% of intracranial tumors with a prevalence of 1 in 1,000 people in the general population [143].

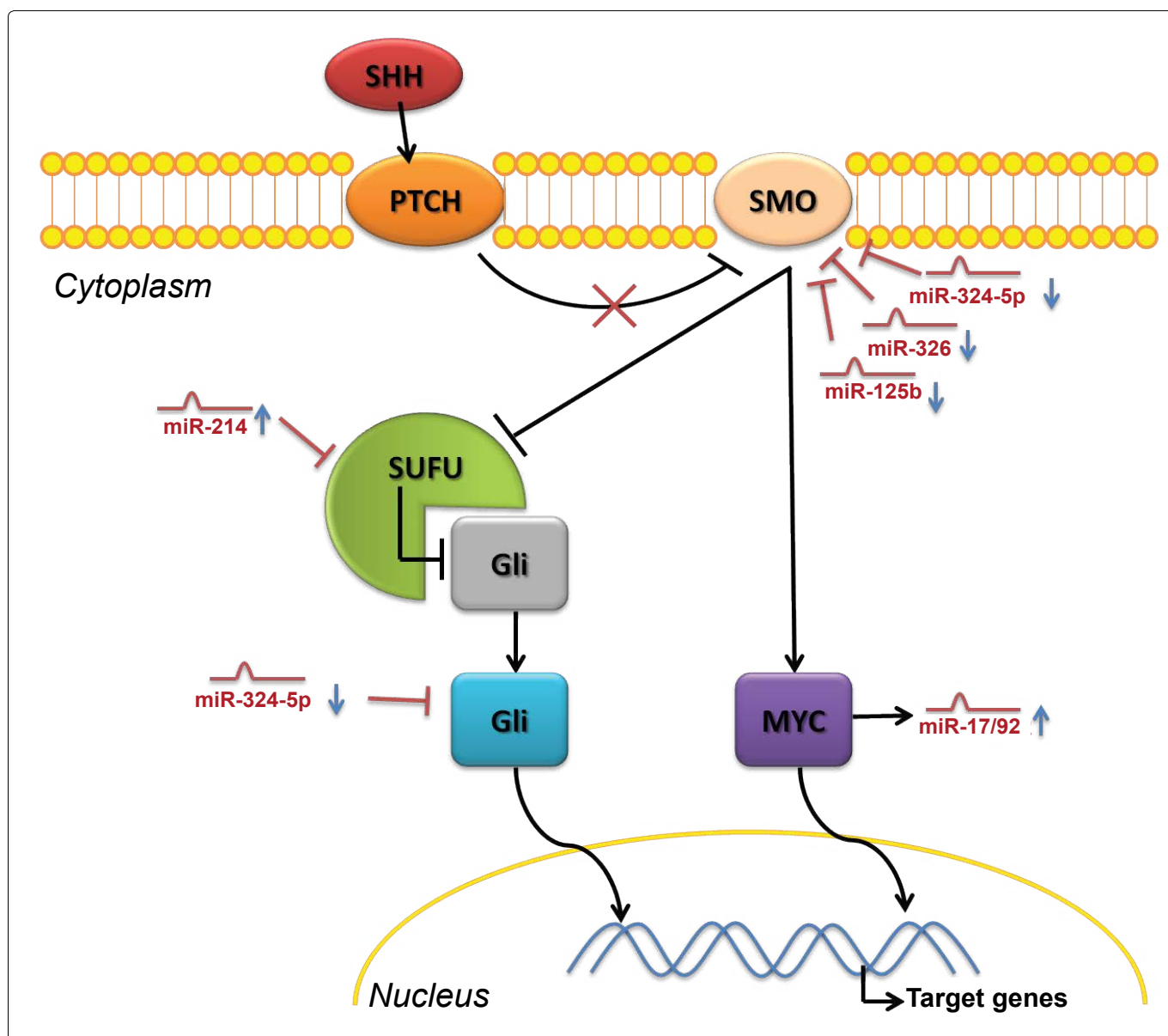


Figure 3: MiRNAs control of Sony Hedgehog pathway in medulloblastoma

When SHH binds to its receptor PTCH, it releases its repression on a transmembrane protein Smoothed (Smo) which becomes able to inhibit the repression of SUFU on Gli proteins, which could translocate to the nucleus. A second mechanism induced by activated Smo, by which SHH signaling exerts a proliferative effect, is through the up-regulation of N-Myc. As described in the text, in medulloblastoma the deregulation of several miRNAs support the activate status of this oncogenic pathway: the lack of miR-324-5p, 326, 125b leads to an over-expression of Smo and Gli factors. In parallel the up-regulation of miR-214 could repress the inhibition of SUFU on Gli proteins. The up-regulation of N-Myc correlates with a deregulation of the Oncomir-1, which is the oncogenic miR-17/92 cluster.

SHH: Sony Hedgehog, PTCH: Patched Gene SHH Receptor, SMO: Transmembrane Protein Smoothened, SUFU: Suppressor of Fused. Blue arrows indicate the expression pattern of miRNAs in medulloblastoma (up-arrow means up-regulated, down-arrow means down-regulated).

Table 8: MiRNAs deregulated in “other” brain tumors

Tumor Type	miRNA up-regulated	miRNA down-regulated	Samples Setting	Methods	References
Lymphoma CNS	-21, -19b, -92a		CNS DLBCL vs Inflammatory CNS disorders	qRT-PCR	[21]
	-21, -19b	-16, -127	CNS DLBCL vs extranodal DLBCL	qRT-PCR and ISH	[64]
	-9, -20b, -155, -340, -17-5p, -148a	-199a, -214, -432, -193b, -145	CNS DLBCL vs LN DLBCL	qRT-PCR	[61]
Meningioma	-335, -98, -181a, -19b, let7d, let7g, -100, -125a, -103, -370, -106b, -106a, -125b, let7b, -26a, -23b, -29a	-200a, -373*, -575	Meningioma vs Normal Brain	Microarray and qRT-PCR	[45]
		-145, let-7d -145	Meningioma vs Normal Brain G2-G3 vs G1	qRT-PCR	[36] [36]
	-335		Meningioma vs Normal Brain	qRT-PCR	[46]
	-17-5p, -22-3p, -24-3p, -26b-5p, -27a-3p, -27b-3p, -96-5p, -146a-5p, -155-5p, -186-5p, -190a, -199a	-29c-3p, -219-5p	Meningioma vs Normal Brain	qRT-PCR	[54]
	-96-5p, -190a		Patients with Recurrence vs Without		[54]
	-29c-3p, -219-5p	Patients Without Recurrence vs With Recurrence		[54]	
Pituitary Adenomas	-26a, -149	-21, -141, -144	Pituitary Adenomas vs normal	Microarray	[22]
	-320	-34b, -326, -374b, -432, -548c-3p, -570, -603, -33	GH-adenomas vs normal	Microarray	[27]
	-183, -193a-5p, -222, -516b, -524-5p, -601, -629, -99b	-124, -32, -574-5p, -744, -96	Macro- vs Micro-adenomas	Microarray	[41]
	-135a, -140-5p, -582-3p, -582-5p, -938, -124*, -515-5p, -872	-198, -299-5p, -497*, -548c-3p, -622	Pituitary Adenomas vs normal	qRT-PCR	[23]
	-378, -516-3p, -151-3p, -224, -618, -455-3p, -29b	-509-3-5p, -508-5p, -452, -330-5p, -200a, -503, -424, -449a, -199-5p	Pituitary Adenomas vs normal	qRT-PCR	[51]
	-217, -216a, -215, -502, -338, -10b, -96, -202, -501, -18a, -450a, -329		Non-functioning pituitary adenoma		[51]
	-1, -760, -196b, -188-5p, -146b-3p		GH-adenoma		[51]
-205, -132*, -523		PRL-adenoma		[51]	

DLBCL: Diffuse Large B-Cell Lymphoma; GBM: Glioblastoma, CNS: Central Nervous System, G: Histological Grade, LN: Lymph Node, GH: Growth Hormone, PRL: Prolactin, qRT-PCR: quantitative Real-Time Polymerase Chain Reaction, ISH: In Situ Hybridization.

In a study of eight pituitary adenomas compared with four normal pituitary samples, three miRNAs (miR-124*, miR-515-5p and miR-872) were expressed only in adenoma tissues while five miRNAs (miR-198, miR-299-5p, miR-497*, miR-548c-3p and miR-622) only in normal pituitary tissues. Of the 457 miRNAs expressed in both normal and tumor tissues, 92 were significantly down-regulated and 70 significantly up-regulated in pituitary adenomas [23]. Five miRNAs (miR-135a, miR-140-5p, miR-582-3p, miR-582-5p and miR-938) were predicted to target Smad3 (Small mother against decapentaplegic 3) and all these miRNAs were significantly over-expressed in pituitary adenoma samples. These data support the possibility that the TGFβ signaling is repressed and modulated by miRNAs in pituitary adenomas via Smad3 whose expression can be directly regulated by several up-regulated miRNAs, including the experimentally validated miR-140 (Table 8) [23].

A microarray assay performed on 12 human human growth hormone-secreting (GH) pituitary adenomas (GH-adenomas) compared normal pituitary gland showed 18 miRNAs down-regulated with at least a 2-fold change in the neoplastic tissues. Conversely, only one miRNA (miR-320) was over-expressed (with a 13.3-fold change) in GH-adenomas [27]. Eight miRNAs (miR-34b, miR-326, miR-374b, miR-432, miR-548c-3p, miR-570, miR-603, and miR-633) were confirmed as down-regulated by qRT-PCR while miR-320 was confirmed as up-regulated [27].

Using bioinformatics tools, three miRNAs (miR-326, miR-432, and miR-570) potentially target the *HMG2* (High Mobility Group AT-Hook 2) gene, while two miRNAs (miR-34b and miR-548c-3p) have both the *HMG1* (High Mobility Group AT-Hook 1) and *HMG2* genes as predicted targets. Finally, miR-326 and miR-603 were predicted to regulate *E2F1* (E2F Transcription Factor 1) [27].

A miRNA microarray statistical analysis on 32 pituitary adenomas generated a list of 30 miRNAs differentially expressed between pituitary adenomas and normal pituitary gland [22]. According

to expression profile of 29 miRNAs it was possible to predict the different pituitary adenoma histotypes: ACTH- (adrenocorticotrophic hormone), GH-, PRL- (prolactin) secreting adenomas and non-functioning adenoma. MiR-26a and miR-149 were found to be up-regulated in pituitary adenomas compared with normal pituitary samples, while miR-21, miR-141 and miR-144 were down-regulated [22].

In an analysis on 21 GH-secreting pituitary adenoma samples (three micro-adenomas and 18 macro-adenomas), 23 miRNAs were found to be up- and 29 down-regulated in GH-adenomas [41]. Moreover, nine miRNAs were differentially expressed between macro- and micro-adenomas: miR-183, miR-193a-5p, miR-222, miR-516b, miR-524-5p, miR-601, miR-629, miR-99b were up-regulated; miR-124, miR-32, miR-574-5p, miR-744 and miR-96 were found to be down-regulated [41].

Trivellini et al. [51] found nine miRNAs (miR-509-3-5p, miR-508-5p, miR-452, miR-330-5p, miR-200a, miR-503, miR-424, miR-449a and miR-199-5p) expressed in normal pituitary gland but not in pituitary adenomas. Different miRNA expressions were observed in different adenoma histotypes. Seven miRNAs (miR-378, miR-516-3p, miR-151-3p, miR-224, miR-618, miR-455-3p and miR-29b) were expressed in all types of adenomas but not in normal pituitary gland. Twelve miRNAs (miR-217, miR-216a, miR-215, miR-502, miR-338, miR-10b, miR-96, miR-202, miR-501, miR-18a, miR-450a and miR-329) were detected only in the non-functioning pituitary adenomas. Five miRNAs (miR-1, miR-760, miR-196b, miR-188-5p and miR-146b-3p) were detected only in the GH-adenoma samples. Three miRNAs (miR-205, miR-132* and miR-523) were detected only in the PRL- adenomas group [51].

Considering miRNAs found deregulated in at least two papers, pituitary adenoma are characterized by up-regulation of miR-516 and down-regulation of miR-548c.

Conclusion

In this review, we have focused on the expression profile of up- or down-regulated miRNAs in brain tumors. MiRNAs have emerged to play a key role in several cellular processes in brain: they are involved in cancer development and progression by regulating gene expression *via* post-transcriptional regulation, but reliable miRNA profiles of brain tumors are far to be defined. The data reported in the present paper highlight the importance to standardize the tissue source, the analysis methods and the choice of control tissue. In fact, changes in one of these factors could contribute to differences in expression of investigated miRNAs. Identification of tumor-specific miRNAs and of their target genes will improve the knowledge in brain neoplasm and supply additional molecular biomarkers for diagnosis of brain tumors and prospective target therapies. Moreover, the available data provide a basis for *in vivo* studies to determine the effect of miRNA modulation in chemotherapy. Finally, circulating cancer-related miRNAs could provide an important tool for early non-invasive tumor diagnosis.

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Supplementary Table 1: MiRNAs profiling studies in glioblastoma

Number of Human miRNAs analyzed	Samples setting	Method	Up-regulated miRNAs	Down-regulated miRNAs	References
180	3 fresh GBMs, versus 8 NBs	Microarray	-21, -138, -347, -291-5', -135	-198, -188, -202	[24]
245	9 fresh GBMs versus 9 ANBs 10 GBM cell lines versus 4NBs	Microarray	-10b, -130a, -221, -125b-1, -125b-2, -9-2, -21, -25, -123 -221, -23a, 23b, -222-prec, -21, -24-1, -24-2, -191, -220	-128a, -181c, -181a, -181b -125b-1, -197, -181b, -181a, -125b-2, -181c,	[15]
756	26 fresh GBMs versus 13 AAs	Microarray	-16, -21, -22, -24, -34a, -126, -142-5p, -143, -146b-5p, -155, -193a-3p, -199a/b-3p, -335, 376c, -381, -451, -509-3-5p, -513a-5p, -552, -886-3p/5p	-128, -219-2-3p	[43]
248	Unspecified # fresh mouse GBMs versus ANBs	Microarray	-383, -519d, -21, -516-3p, -26a, -10b, -486, -451	-124a, -137, -323, -139, -218, -128-2, -483, -128-1, -299, -511-1, -190	[31]
435	5 GBM cell lines, 1 AA cell line, and 1 commercial RNA	Microarray	-23b, -23a, -222, -221, -106, -15b, -21	-451, -124, -495, -223, -329, -126, -219, -1, -330, -342, -323, -127, -128, -132, -95, -137	[20]
8	10 fresh GBMs, 10 AAs, 8 LGAs, versus 4 NBs	qRT-PCR	-21, -221	-181b	[25]
192	4 fresh GBMs, 4 AAs vs 4 NBs	qRT-PCR	-10b, -21, -155, -210	-7, -31, -107, -124, -124b, -129, -137, -138, -139, -187, -203, -218, -101, -128a, -132, -133a, -133b, -149, -153, -154*, -185, -29b, -323, -328, -330	[47]
723	CD133+ (stem) vs CD133- (non-stem) cells from 3 GBM tissues	Microarray	-451, -486, -425, -16, -107, -185	None found	[16]
365	8 fresh GBMs vs 4 AAs	qRT-PCR	-15b, -21, -135b, -196a, -196b, -363	-105, -128b, -184, -302b, -302d, -367, -383, -504, -517c, -601	[32]
127	3 fresh GBMs versus ANB	Microarray	-10b, -21, -183, -92b, -106b	-302c, -379, -329, -134, -369-3p	[44]
157	4 fresh secondary GBMs versus LGGs	qRT-PCR	-9, -15a, -16, -17, -19a, -20a, -21, -25, -28, -130b, -140, -210	-184, -328	[40]
1145	82 fresh GBMs versus 5 NBs	Microarray	-518b, -566	-181d, -524-5p, -1227	[55] †
whole-genome small RNA sequencing	3 GBM stem cell lines vs 3 normal neural stem cell lines	Microarray AND NGS	-10a, -10b, -140-5p, -204, -424, -34a, -193a-5p, -455-5p	-124, -874	[17]
875	3 fresh GBMs vs 3 NBs	NGS	Most 10 up-regulated: -10b, -96, -10b*, -182, -135a*, -21*, -21, -542-3p, -148a, -92b	Most 10 down-regulated: -433, -7-1*, -129*, -628-5p, -935, -218, -31, -876-3p, -1258, -132	[33]
8	22 FFPE-dissected GBMs vs 6 NBs	qRT-PCR	-21, -125b	-128a, -222, -221, -181b, -181c, -181a	[65]
282	12 fresh GBMs, 12 fresh O versus 4 fresh NBs	Microarray AND qRT-PCR	-21, -155, let-7f, let-7a, -16, -15b, -9, -210	-132, -128, -7, -330-3p, -139-5p, -124	[37]
8	38 FFPE GBMs vs 6 NBs and 4 commercial RNAs	qRT-PCR	-196a, -196b, -21	-128a, -221, -195, -222, -181c	[66]
866	7 GBM cell lines: migrating cells vs non migrating cells	Microarray	-99a*, -767-3p, -202*, -556-3p, -655, -451, -495, -579, -223, -381, -329, -769-3p, -524-3p, -93*, -220a, -491-5p, -200c*, -133b, -19b-1*, -520h, -92b, -657, -891a, -326, -541*	-16, -30c, -15b, let-7f, let-7a, -23b, -103, -107, -24, -93, -15a, -151-5p, -23a, -30b, let-7e, -30a, -29a, -455-3p, let-7b, -25, let-7c, -106b, -92a, let-7d, -125b, -125a-5p, -222, let-7g, -17, -20a, -29c, let-7i, -26a, -574-3p, -181b, -30e, -320, -99a, -424, -181a, -10b, -17*, -140-5p, -197, -151-3p, -30e*	[18]
305	222 GBMs vs 10 NBs (data from TCGA)	Microarray	-20a, -106a, -17-5p, -148a, -146b, -200b, -193a	-221, -222, -31	[49]
whole-genome small RNA sequencing (according to miRBASE v 16.0)	6 fresh GBMs versus 3 NBs	NGS	-10b*, -10b, -891a, -93, -196b, -21*, -320d, -217, -4448, -25, -660, -21, -320c, -296-3p, -92b, -10a, -92a, let-7i*, -148a*	-124, -95, -132, -139-5p, -7, -543, let-7d, -323-3p, -128, -598, -103a, -103b, -139-3p, -487b, -873, -323b-3p, -1381*, -301b, -107, -411-3p, -124*, -342-3p, -379*, -212, let-7g, -153, -181d, -22, -889, -885-5p, -379, -138, let-7e, -218, -221, -136, -24, -4787-3p, -126, -548i, -382, -1270, -495, -2392, -1273d, -767-5p, -770-5p, -504, -490-3p	[48]

>1000	35 FFPE GBMs: short-term vs long-term survivors	Microarray	miR-3163	-539, -1305, -1260, let-7a	[63]
534	240 GBMs vs 10 NBs (data from TGCA)	Microarray	-15a, -15b , -16 , -17-5p , -25 , -92, -93 , -106a , -106b , -320, -502, -532, -590, -660	-487a, -323 , -329 , -218	[28]
NS	261 GBMs vs 10 NBs (data from TGCA)	Microarray	-21 , -10b , -210 , -27a, -25 , -15b , -23a , -92b , -106b , -92, -155 , -93 , -339, -494, -565, -130b, -34a, -16 , -20a , -320, -106a , -19b, -100, -17-5p , -424	-124a, -139 , -134, -7, -218 , -338, -129, 2-19, -128b, -128a , -136, -410, -138 , -29b, -29c, -491, -132 , -379 , -377, -149, -769-5p, -330 , -323	[50]
474	3 fresh GBMs vs 2 NBs	Microarray	-10b , -182, -10a , -183, 21 , -148a , -503, -505, -451 , -144, -542-5p, -542-3p, -26a, -142-3p, -199b, -15b , -550, -25 , 9*, -92b , -142-5p, -27a, -23a , -486 , -362, -532, -93 , -106b , -223	None found	[34]

GBM: Glioblastoma, AA: Anaplastic Astrocytoma, NB: Normal Brain, ANB: Adjacent Normal Brain, LGA: Low Grade Astrocytoma, LGG: Low Grade Glioma, O: Oligodendroglioma, DA: Diffuse Astrocytoma, NS: Not Specified, NGS: Next Generation Sequencing, qRT-PCR: quantitative Real-Time PCR, ISH: in situ hybridization, TGCA: The Cancer Genome Atlas Datasets, FFPE: Formalin-Fixed Paraffin-Embedded. In bold: miRNAs reported up- or down-regulated in at least 3 studies. Only miRNAs showed a fold change greater than or equal to two-fold: were considered. MiRNAs showed a fold change smaller than two-fold are reported in italic font and they were not considered for GBM profiling analysis. † The authors considered a cut-off fold change >1.5.