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MicroRNAs in Brain Tumors

A New Diagnostic and Therapeutic Perspective?

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Abstract MicroRNAs (miRNAs, miRs) are small, non-coding RNA molecules that regulate gene expression posttranscriptionally. Although discovered only recently in the early 1990s, this relatively new group of molecules has already been proven to play an essential role in the regulation of many physiological and, most importantly, pathological processes such as cancer. A large number of miRNAs has been found to be involved in the pathogenesis of various human malignancies, and expression of miRNAs has been demonstrated to correlate with clinic and outcome. In tumors of the brain, however, the investigations on the role of miRNAs are still in its infancy, and most publications refer to the most common primary brain tumor, the glioma (mostly glioblastoma). But despite the fact that there is only limited data available so far, these first results are very promising and implicate that miRNAs might open a new perspective for diagnostics and treatment of this disease. With this review article, we aim to provide a short overview of miRNA biogenesis, function and regulation in general. Thereafter, the clinical relevant data about miRNAs in the two most common primary malignant brain tumors in adults (glioblastomas) and children (medulloblastomas) will be summarized, and their potential impact on diagnostics and treatment will be highlighted.

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Introduction

The expression "brain tumor" describes a fairly inhomogeneous collection of various tumors of the brain (either malignant or benign), which originate either primarily in the central nervous system or represent metastases from other tumors. In contrast to most other malignancies, brain tumors can also occur at a very young age, in fact, they represent one of the most common tumor types in children. The worldwide overall incidence (age-standardized incidence rates [ASR]) of primary malignant brain and central nervous system tumors in 2002 was 2.6 (female) and 3.7 (male) per 100,000 persons [1, 2]. The 5-year survival rate of patients affected with brain tumors varies widely between the different subtypes. For example, glioblastomas (the most frequent primary malignant brain tumor in adults which belongs to the family of the gliomas), present an overall 5-year survival of only <10% [3], whereas medulloblastomas (the most frequent primary malignant tumor type in children) have a far better prognosis with an overall 5-year survival between 55% and 80% [4]. Treatment of brain tumors includes surgery, radiotherapy and chemotherapy in various settings depending on the tumor type, but outcome of these treatments differs markedly between subtypes.

Only recently, a new class of molecules, the microRNAs, attracted increasing attention as potential diagnostic or even therapeutic tools in brain tumors. MicroRNAs (miRNAs, miRs) are a class of naturally occurring, endogenous, small (19–25 nucleotides) RNA molecules. They had first been

described back in 1993 by two research groups around Lee et al. [5] and Wightman et al. [6]. They discovered these small RNAs in *C. elegans* and found that these molecules obviously do not encode proteins but negatively regulate the expression of the *lin-14* gene on a posttranscriptional level via an antisense (micro)RNA-(messenger)RNA interaction [5, 6]. Nowadays, these molecules are considered as an essential part of the so called epigenetic machinery which participates in regulation of global gene expression, and miRNAs are now known to be involved in many physiological and pathological processes such as cancer. The latest release of miRBase (Release 16; September 2010), a database with information about sequence and putative targets of miRNAs, listed a total of 15,172 miRNAs in different species such as animals, plants and viruses. In *Homo sapiens* alone, there are 1,048 miRNAs described (http://microrna.sanger.ac.uk/) [7].

Regarding the role of miRNAs in brain tumors, there are so far only very limited data available, and the majority of publications refer to the most common primary tumor, the glioma. Gliomas are a very heterogeneous group of tumors that can be classified according to the WHO into four categories: WHO grade I (e.g., pilocytic astrocytoma, subependymoma or myxopapillary ependymoma), WHO grade II (e.g., oligodendroglioma or ependymoma), WHO grade III (e.g., anaplastic astrocytoma or anaplastic oligodendroglioma) and WHO grade IV (e.g., glioblastoma). In this context, malignant gliomas (=astrocytomas WHO grade III and IV) represent the most frequent primary brain tumors in adults. However, first reviews published only recently suggest that miRNAs might indeed play a crucial role in development and progression of brain tumors, as known from other malignancies [8–13]. With this article, we aim to focus on the "clinical aspect" of using miRNAs as potential diagnostic and therapeutic tools in the two most common malignant brain tumors in adults (glioblastoma) and in children (medulloblastoma). The first part of the article provides a general overview about miRNAs, their biogenesis and their function. The second part refers to the aforementioned tumor types highlighting some clinically relevant and very interesting findings.

Background

MiRNA Biogenesis and Regulation

The first step of the biogenesis of miRNAs is the transcription of a long primary transcript called pri-miRNA from the encoding gene in the nucleus by the RNA polymerase II (Pol II). The pri-miRNA is then processed into the pre-miRNA, a shorter precursor miRNA, by a complex consisting of Drosha and DGCR8, and is finally exported from the nucleus by Exportin-5. Outside the nucleus, Dicer and TRBP cleave the pre-miRNA into a mature miRNA duplex containing the single-stranded mature miRNA molecule. The final step presents the incorporation of the mature miRNA into an effector complex, called RNA-induced silencing complex (RISC), which binds to messenger RNA by (im-)perfect base pairing of the guiding miRNA to the 3′ untranslated region of a messenger RNA, and causes either block of translation or, less frequently, mRNA degradation [14–17].

It makes sense that miRNAs, as part of the very complex network of gene expression regulators, are regulated themselves at multiple steps. Until today, these complex regulatory mechanisms are only poorly understood, and there is much more work to be done. But from what is known so far, regulation of miRNAs in general can occur at the transcriptional or at the posttranscriptional level, and the latter includes the multiple steps of biogenesis and maturation [17].

The regulation of the transcription for example depends on the function of RNA polymerase II and RNA polymerase II associated transcription factors, such as p53, Myc and muscle-specific myogenin. An altered transcription can lead to "miRNA production" of the encoded miRNAs only under specific conditions. In addition, several miRNAs are embedded within the introns of known coding genes and might be regulated by the promoter of their host gene [18, 19]. Furthermore, miRNAs are considered as a part of the "epigenetic machinery" which includes besides miRNAs as well DNA cytosine methylation or the post-translational modification of histones. All these compounds interact with each other, and miRNAs are regulated by the other parts of this network. One crucial aspect in this context seems to be the interaction of miRNAs with DNA methylation, which has been described to control and "fine tune" various miRNAs by epigenetic inactivation due to aberrant hypermethylation [20–27]. Most interestingly, DNA methylation has been proven to play an important role in drug resistance, and there is effort to assess "DNA methylation-targeted" therapy with drugs such as DNA methyltransferase inhibitors (e.g., decitabine) as potential weapon against cancer [28–31]. Single nucleotide polymorphisms which can occur in miRNA-coding genes or in miRNA-binding sites in mRNAs present another regulatory mechanism that could strengthen or weaken the function of the respective miRNA. A polymorphism in pre-miR-146a sequence for example had been demonstrated to decrease the generation of mature miR-146a and weaken the inhibition of its target genes [22].

On a posttranscriptional level, the processing of the pri-miRNA into the pre-miRNA by Drosha seems to be an important key point of miRNA regulation. Several proteins including RNA binding protein Lin-28, p53 or SMAD

(activated by TGFβ) had been shown to interact with Drosha and to either promote or inhibit cleavage of specific pri-miRNAs [18, 19, 32]. Other posttranscriptional mechanisms include the miRNA editing (an alteration of adenines to inosines, mediated by adenine deaminases [ADARs], that might represent a fine tuning process), the modulation of miRNA expression in circadian rhythm or the not yet thoroughly studied regulation of the nuclear export step or the miRNA turnover [8, 22, 32].

MiRNA Function in Cancer

As outlined above, miRNAs regulate gene expression at the post-transcriptional level via a negative regulation of messenger RNA by an antisense complementarity to a mRNA molecule. Given the fact that miRNAs are very short RNA molecules it is postulated that one miRNA can target and regulate up to 100 different mRNAs. More than 10,000 mRNAs are believed to be directly regulated by this mechanism [33–36]. Thus, it is not surprising that there is strong evidence that miRNAs are involved in the regulation of various (physiological and pathological) cellular functions and pathways involved in organ morphology, bilateral asymmetry, stress response, metabolism, cell proliferation and apoptosis amongst others [37]. Regarding the role of miRNAs in cancer, there is no doubt that miRNAs play a key role in the initiation and progression of cancer. Calin et al. [38] found that more than 50% of miRNA genes are located in cancer associated genomic regions or in fragile sites. Furthermore, variations in miRNA expression levels were shown to be associated with dysplasia and cancer, and there is a clear link between the expression of certain miRNAs and cancer development [14, 17, 39–41]. Specific miRNAs had been demonstrated to modulate known oncogenes or tumor suppressor genes or acting themselves as so called onco-miRs or tumor suppressor-miRs by directly targeting other genes involved in cell differentiation, proliferation, angiogenesis, apoptosis or invasion in various cancer types [14, 17, 33, 39, 41–45]. Finally, miRNA expression profiles have been found to correlate with clinicopathological features, disease progression, prognosis and outcome in various human cancers [14, 17, 45–47].

MiRNAs in Brain Tumors: Clinical Perspectives

Overall, there are so far only very limited data available about miRNAs and their role in brain tumors, most of which refer to the impact of miRNAs on development and progression of this disease. But there are as well first very promising data about more clinical relevant aspects of miRNAs in these tumors which highlight miRNAs as potential diagnostic tools (e.g., different expression profiles between malignant and benign tissue, or correlations between expression profiles and outcome) and/or therapeutic tools (effects of miRNAs on tumor cell proliferation and growth, or on chemotherapeutic treatment). At this point, it is important to mention that non-tumorous controls in different studies were taken from different sources: the majority of studies used commercially available RNA from normal human brain tissue or control samples from large tissue banks. In the context of glioblastoma, several studies used normal brain samples from patients undergoing surgery for other diagnoses (e.g., epilepsy, severe traumatic brain injury or "autopsy brain"). However, a number of studies did not provide detailed information about their "non-neoplastic brain control tissue samples", and very few authors used normal non-tumorous tissue adjacent to the malignant tumor of the same patients (e.g., [48]).

Glioblastoma

MiRNAs as Diagnostic Tools

There is increasing evidence that various miRNAs are deregulated in glioblastoma in vitro and in vivo [49]. Ciafrè et al. [50] found nine (miR-10b, miR-130a, miR-221, miR-125b-1, miR-125b-2, miR-9-2, miR-21, miR-25, miR-123) and four miRNAs (miR-128a, miR-181c, miR-181a, miR-181b), respectively, out of 245 miRNAs to be up-/downregulated in human glioblastoma samples, and nine (miR-221, miR-23a, miR-24-2, miR-24-1, miR-23b, miR-21, miR-222-prec, miR-191, miR-220) and seven miRNAs (miR-181a, miR-181b, miR-128b, miR-197, miR-181c, miR-125b-2, miR-125b-1), respectively, to be up-/downregulated in human glioblastoma cell lines [50]. Chan et al. [51] demonstrated five (miR-21, miR-138, miR-347, miR-291-5′, miR-135) and three miRNAs (miR-198, miR-188, miR-202), respectively, out of 180 miRNAs to be up- and downregulated in glioblastoma samples. Sasayama et al. [52] found miR-10b, miR-21, miR-183, miR-92b and miR-106b to be up-, and miR- 302c*, miR-379, miR-329, miR-134 and miR-369-3p to be downregulated in human glioblastoma samples. Other studies reported several miRNAs to be significantly deregulated in glioma samples of Chinese patients (including miR-34a, miR-15b, miR-200a and miR-146b) [53], or miR-29b, miR-125a and miR-149 to be downregulated in glioblastomas [54]. In an array study with 192 miRNAs, 13 miRNAs (miR-101, miR-128a, miR-132, miR-133a, miR-133b, miR-149, miR-153, miR-154*, miR-185, miR-29b, miR-323, miR-328, miR-330) were found to be downregulated and three miRNAs to be upregulated (miR-21, miR-155, miR-210) in glioblastoma multiforme [55]. Another microarray study identified 55 miRNAs out of 756 miRNAs to be upregulated and 29

miRNAs to be downregulated in malignant astrocytomas (primary and secondary glioblastoma and anaplastic astrocytoma, respectively) compared to controls [56].

Regarding astrocytoma grades, several authors described an association between miRNA expression and tumor grade. MiR-10b was demonstrated to be highly overexpressed in glioblastomas, and expression was correlated with tumor grade and malignancy in astrocytic tumors. Most interestingly, this study provided first evidence that miR-10b expression is correlated with multifocal lesions of malignant glioma [52]. MiR-21 and miR-221 were found to be overexpressed in glioma. MiR-21 was homogeneously overexpressed in low and high-grade tumors, but miRNA-221 overexpression was more evident in high-grade tumors [57]. Investigation on members of the miR-17-92 cluster in astrocytoma grade II, anaplastic astrocytoma grade III, secondary glioblastoma and primary glioblastoma, and normal brain showed that expression levels of miR-17-3p respectively of miR-17-3p, miR-17-5p, miR-92a-1 and miR-106b were significantly higher in primary and secondary glioblastomas relative to non-neoplastic brain. Mir-19a and mir-19b were downregulated in both, primary and secondary glioblastomas. Most interestingly, miR-17-5p, miR-19a and miR-106b were significantly higher expressed in secondary glioblastomas compared to astrocytomas grade II [58]. MiR-128 was demonstrated to be downregulated in low and high grade gliomas when compared to controls, but expression was lower in high grade versus low grade tumors (no statistical analysis provided) [59]. MiR-181a was as well shown to correlate with tumor grade in human glioma samples [60]. Rao et al. [56] identified 67 differentially expressed miRNAs between (primary and secondary) glioblastomas and anaplastic astrocytomas, and a cluster of only 23 miRNAs (miR-126, miR-21, miR-146b-5p, miR-155, miR-16, miR-193a-3p, miR-199a-3p/miR-199b-3p, miR-22, miR-335, miR-143, miR-381, miR-24, miR-552, miR-886-5p, miR-142-5p, miR-34a, miR-128, miR-513a-5p, miR-509-3-5p, miR-376c, miR-886-3p, miR-219-2-3p, miR-451) was found to be sufficient to discriminate glioblastomas from anaplastic astrocytomas with an overall diagnostic accuracy to 94.8% [56]. Another very interesting study investigated miRNA expression of patients who presented initially primary WHO grade II gliomas (three diffuse astrocytomas and one astrocytoma-predominant oligoastrocytoma), and then developed recurrences that were histologically classified as glioblastoma (WHO grade IV). They found a set of 14 out of 157 miRNAs (miR-184 and miR-328 downregulated; miR-9, miR-15a, miR-16, miR-17, miR-19a, miR-20a, miR-21, miR-25, miR-28, miR-130b, miR-140 and miR-210 upregulated) to be deregulated in the secondary glioblastoma compared to the initial WHO grade II tumors. PCR validation on a selection of these miRNAs (miR-16, miR-17, miR-19a, miR-20a, miR-140 and miR-184) in the same patients and in three additional patients with gliomas that progressed from WHO grade II to WHO grade III widely confirmed these findings. However, in an independent validation cohort of 14 diffuse astrocytomas versus 13 secondary glioblastomas, miR-19 and miR-19a failed to reach significance [61].

We found only three studies describing correlations between miRNA expression or polymorphisms and clinico-pathologic features of glioblastoma patients (see Table 1). Dou et al. [62] investigated associations between miR-196a genotype and glioma disease risk in a Chinese population. They included 643 glioma patients (astrocytoma, glioblastoma, oligodendroglioma, ependymoma, medulloblastoma, gliomatosis cerebri, or mixed glioma) and 656 controls, and analyzed genotype distributions of miR-196a T/C polymorphism. MiR-196a rs11614913 (CC) polymorphism was associated with a decreased risk of glioma, and significant associations were observed between the miR-196a CC genotype and glioma risk in the subgroups of adult glioma, male glioma and patients with glioblastoma. However, the restriction of this study to a Chinese population, the population size of different subtypes and the missing expression analysis of miR-196a or its potential targets limit the applicability of these data to

Table 1 Overview about miRNAs that are correlated to clinico-pathologic features in glioma/glioblastoma and medulloblastoma patients

miRNA	Details	Impact on clinic	Reference
Glioma/Glioblastoma			
miR-26a	miR-26a located within an amplicon often containing two oncogenes	miR-26a amplicon present → median survival ↓	Kim et al. 2010
miR-196a	Polymorphism in the miR-196a region	Polymorphism evident → disease risk ↓	Dou et al. 2010
miR-196 (a/b)	–	miR-196a + miR-196b ↓ → overall survival ↑ miR-196 ↑ → independent predictor of short survival	Guan et al. 2010
Medullablastoma			
miR-31/miR-153	–	Inverse correlation: miRNAs – disease risk	Ferretti et al. 2009
miR-199b-5p	–	miR-199b-5p ↑ → non-metastatic disease miR-199b-5p ↑ → trend towards better survival	Garzia et al. 2009

the general "glioma population", and mandates a very careful discussion of these findings [62]. Kim et al. [63] performed a genome analysis and found that miR-26a was located within an amplicon at 12q13.3–14.1 which often contained as well CDK4 and CENTG1, two oncogenes. The median survival for glioblastoma patients with tumors harboring the miR-26a amplicon was significantly lower than that of patients lacking this amplicon. The authors concluded that miR-26a, CDK4 and CENTG1 compromise a functionally integrated oncomir/oncogene DNA cluster which impacts on survival of glioblastoma patients [63]. Guan et al. [64] demonstrated a significantly different expression of 16 miRNAs (miR-196a, miR-15b, miR-105, miR-367, miR-184, miR-196b, miR-363, miR-504, miR-302b, miR-128b, miR-601, miR-21, miR-517c, miR-302d, miR-383, miR-135b) in glioblastoma versus anaplastic astrocytoma. Classification of malignant gliomas via these 16 miRNAs showed significant correlations with the WHO grade of the tumor. Most notably, two miRNAs (miR-196a, miR-196b), were highly significantly associated with overall survival of 46 malignant glioma patients (anaplastic astrocytomas and glioblastomas), and glioblastoma patients with lower expression of both miRNAs (lower than average) showed a better overall survival than patients with high (higher than average) expression of either miRNA. A multivariate analysis demonstrated that a high level of miR-196 was an independent and significant predictor of short overall survival in glioblastoma patients [64].

In summary, these (predominately human in vivo) data demonstrate an increasing body of evidence that miRNAs might be useful biomarkers to discriminate glioblastomas from normal brain tissue in clinical settings, and to identify different astrocytoma grades. Furthermore, first promising results implicate that miRNAs even correlate with clinical outcome of glioblastoma patients highlighting their possible impact on staging investigations and clinical decision making.

MiRNAs as Therapeutic Tools in Cell Lines

Glioblastomas are highly resistant to all chemotherapy options to the moment. Drug resistance is believed to be a multifactorial phenomenon that includes various aspects such as reduced intracellular accumulation of drugs, altered cellular response and microenvironment affection. A number of miRNAs had previously been shown to impact on cellular functions or pathways that might play a role in drug resistance such as drug metabolizers or efflux pumps (Fig. 1), or tumor suppressor genes (Fig. 2 [17, 65]). And in fact, first in vitro studies described an impact of miRNA modulation on response to various anticancer drugs in glioblastoma cell lines [49]. As expected, most studies examined a possible impact of miRNA modulation on TMZ (temozolomide, an alkylating agent), a drug that methylates DNA purine residues and is commonly used in the therapy of malignant gliomas. O^6-Methylguanine methyltransferase (MGMT) methylation status is highly correlated with the tumor responsiveness to TMZ. MGMT encodes a DNA repair protein, and patients with high levels of MGMT present a worse response to TMZ what might be explained by the neutralization of the effects of the alkylating drug via DNA repair. In the development of acquired TMZ resistance, deficiency of mismatch repair (MMR) seems to play a crucial role. A loss of for example MSH2 or MSH6 is

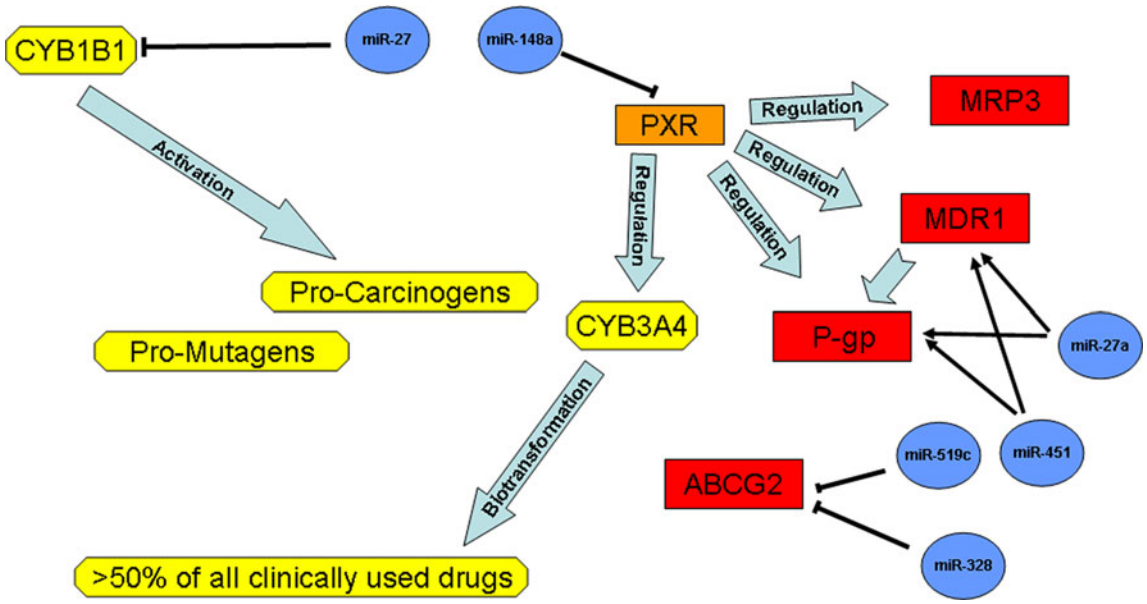

Fig. 1 MiRNAs regulate drug metabolizers and drug efflux pumps

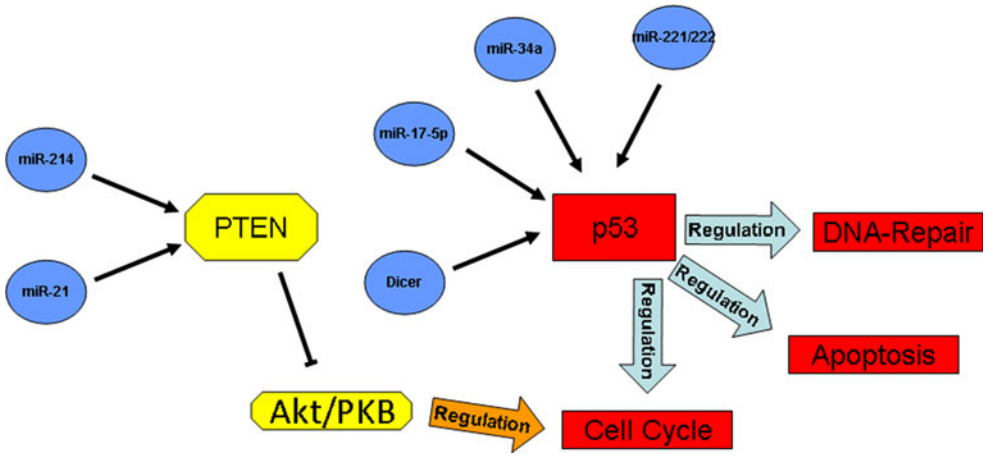

Fig. 2 MiRNAs regulate tumor suppressor genes

associated with increased resistance to TMZ, and MMR deficiency might present an alternative way to develop resistance in tumors with low levels of MGMT [88–91]. However, a number of studies worked with other drugs such as teniposide (VM-26, a topoisomerase II inhibitor) or taxol (paclitaxel, an antimicrotubule agent), which are known be less effective treatment options. But given the drug-resistant character of glioblastomas, it is important to assess new therapeutic approaches (that aim to increase the success of well-know anticancer therapies) as well in the context of drugs that present a lack of efficiency in the first place. If these experimental in vitro studies show that response to various chemotherapeutic drugs can be affected by up- or downregulation of specific resistance-related miRNAs, and glioblastoma can be sensitized to these drugs, a basis for further studies could be provided that might finally increase treatment options in this highly fatal disease.

Feng et al. [66] used small interfering RNAs (siRNA), a synthetic form of miRNAs made of short double stranded RNA, against bFGF (basic fibroblast growth factor), and treated U251 and A172 glioma cells with both, siRNA and chemotherapeutics (BCNU (Carmustine, an alkylating agent) or VM-26). They found a significantly improved effect of the combined treatment compared to treatment with either siRNAs or chemotherapeutics alone. The authors highlighted that high levels of bFGF were previously found to be associated with malignant grades of glioma, and that bFGF has been shown to be overexpressed in neoplastic astrocytes and affect cell proliferation in vitro and in vivo [66]. Li et al. [67] transfected glioblastoma cell lines with miR-21 inhibitors and treated them subsequently with VM-26. They could show that the knock-down of miR-21 led to a dose-dependent reduction in cell survival after VM-26 therapy and found LRRFIP1 (which can inhibit NF-κB activation) to be a direct target of miR-21 [67]. Another group demonstrated miR-21 inhibitors to cause a decrease in cell viability and cell invasion, as well as an increase in apoptosis in PTEN-mutant (U251) and PTEN-wild-type (LN229) human glioblastoma cell lines, and to further induce G1 and S phase arrest. More importantly, inhibition of miR-21 increased the cells' sensitivity to taxol and improved the effect of this treatment. Treatment with either miR-21 inhibitor or taxol combination therapy led to reduced expression of phosphorylated Akt (p-Akt), and combination therapy resulted in a marked reduction of EGFR protein as well as downregulation of STAT3 and p-STAT3. The authors concluded that inhibition of miR-21 might interrupt activity of EGFR/STAT3 signaling pathway, and this effect might be independent from the PTEN pathway (PTEN is a direct target of miR-21) [68]. Furthermore, co-delivery of miR-21 inhibitors and 5-FU significantly improved the cytotoxicity of 5-FU and led to dramatic increase of apoptosis and diminished migration in U251 cells [69]. Another study showed that overexpression of miR-21 prior to chemotherapeutic treatment in glioblastoma cells (U87MG) led to a decrease of apoptosis and to an increase of cell survival after treatment with TMZ. As possible mechanism, the authors found miR-21 overexpression to be associated with a significant decrease in the Bax/Bcl-2 ratio and caspase 3 activity implicating that overexpression of miR-21 protected cells from TMZ induced apoptosis [70]. MiR-451 was demonstrated to show a considerable neurosphere inhibition and to negatively impact on cell viability and cell growth. But more importantly, miR-451 was reported to generate a synergistic effect in inhibiting neurosphere formation in glioblastoma cells (A172) when applied in combination with Imatinib mesylate [71].

So far, we found only one article presenting data about miRNA expression in resistant variants of glioblastoma cell lines. Ujifuku et al. [72] established TMZ-resistant variants of glioblastoma cell lines (U251MG, U87MG, M059K and M059J) by applying TMZ continuously to the cells. The authors could demonstrate via microarray that 12 (hsa-miR-455-3p, hsa-miR-195, hsa-miR-10a*, hsa-miR-502-3p, hsa-

miR-193b*, hsa-miR-584, hcmv-miR-US25-2-5p, hsa-miR-500*, hsa-miR-193a-5p, hsa-miR-452, hsa-miR-132, hsa-miR-503) and two miRNAs (hsa-miR-106b*, hsa-miR-210), respectively, were up-/downregulated in TMZ-resistant U251MG cells. PCR validation for miR-455-3p, miR-195 and miR-10a* in all resistant variants confirmed these findings widely, only miR-195 was not significantly downregulated in resistant M059J cells. Furthermore, while inhibition of miR-455-3p and miR-10a* did not affect cell survival, miR-195 knockdown led to a moderate growth inhibition in TMZ-resistant U251MG cells. More importantly, a combined treatment with TMZ and miR-455-3p or miR-10a* inhibitors showed a modest negative effect on cell survival, the combination of miR-195 inhibition with TMZ on the other hand enhanced cell death strongly in these cells. The same experiments in TMZ-resistant M059J cells (which did not present miR-195 downregulation) basically confirmed these findings, but downregulation of miR-195 alone strongly induced cell death, and combination with TMZ did not make significant difference. The authors provided a number of promising targets for the described miRNAs by in silico identification and cDNA microarray analysis [72] (for an overview about the effects of miRNA modulation on sensitivity to chemotherapy in glioblastoma cell lines, see Table 2 and Fig. 3).

Taken together, these (so far only) in vitro data imply that a number of miRNAs impact on the response of glioblastoma to a variety of chemotherapeutic drugs such as TMZ, VM-26, taxol, 5-FU and others, and TMZ-resistant tumors seem to present distinct miRNA expression signatures. These findings might represent a first important step on the way to understand, diagnose and fight resistance to conventional anticancer strategies in highly drug-resistant glioblastoma on a molecular level.

Medulloblastoma

There is increasing evidence that various miRNAs are deregulated in medulloblastoma in vitro and in vivo [49].

MiRNAs as Diagnostic Tools

Ferretti et al. [73] demonstrated that 30 (including let-7a, let-7e, let-7f, miR-7, miR-9, miR-25, miR-30b, miR-100, miR-103, miR-124a, miR-125b, miR-132, miR-135a, miR-135b, miR-142-5p, miR-143, miR-150, miR-153, miR-181c, miR-190, miR-191, miR-203, miR-324-3p, miR-324-5p, miR-326, miR-331, miR-338, miR-425) out of 250 miRNAs were downregulated in a subset of human primary medulloblastomas with high Hedgehog (Hh) signaling strength. The Hedgehog signaling pathway is believed to play an important role in the malignant transformation into medulloblastoma [73]. High-throughput profiling in a large sample set of human medulloblastomas showed that 78 miRNAs out of selected 86 miRNAs (which had been reported to be expressed in neuronal tissues or to be associated with different tumors [onco-miRs]) were differentially expressed between tumors and controls. Interest-

Table 2 Effects of miRNA modulation on sensitivity to chemotherapy in glioblastoma cell lines

miRNA	(Potential direct or indirect targets)	Up-/down-regulation	Effect on Sensitivity to CTX	CTX agent	Ref.
miR-10a*	CCL2, SULT1A3, **EPHX1**, CDK5, GRTP1, **BRD7**, PDIA5, IDS, PSPC1, RWDD2B, COL6A1, GIYD2, HSPA1B, GRM6, MRPL19[a]	↓	↑	TMZ (temozolomide)	Ujifuku et al. 2010
miR-21	LRRFIP1	↓	↑	VM-26 (teniposide)	Li et al. 2009
miR-21	STAT3	↓	↑	Taxol (paclitaxel)	Ren, Zhou et al. 2010
miR-21	–	↓	↑	5-fluorouracil	Ren, Kang et al. 2010
miR-21	Bax/Bcl-2/caspase-3	↑	↓	TMZ (temozolomide)	Shi et al. 2010
miR-195	PPP2R1A, AP2A1, **SIAH1**, HAS2, ALS2CR2, CCNE1, SESN1, **WEE1**, **RANBP3**, VAT1 [a]	↓	↑↑	TMZ (temozolomide)	Ujifuku et al. 2010
mir-451	-	↑	↑	Imatinib mesylate	Gal et al. 2008
miR-455-3p	LHX2, ALS2CR8, DIP2A, DYNLL2, C6orf145, FBXL15, **LTBR**, ASB1, PNPLA6, RTN4, RUSC1, **EI24**, HSF1, **SMAD2**, GNL1[a]	↓	↑	TMZ (temozolomide)	Ujifuku et al. 2010

Data in bold potential TMZ resistance-related candidates of target genes identified by literature search. *CTX* chemotherapy

[a] Predicted target genes of the respective miRNA that were downregulated (<0.8-fold) in a cDNA microarray

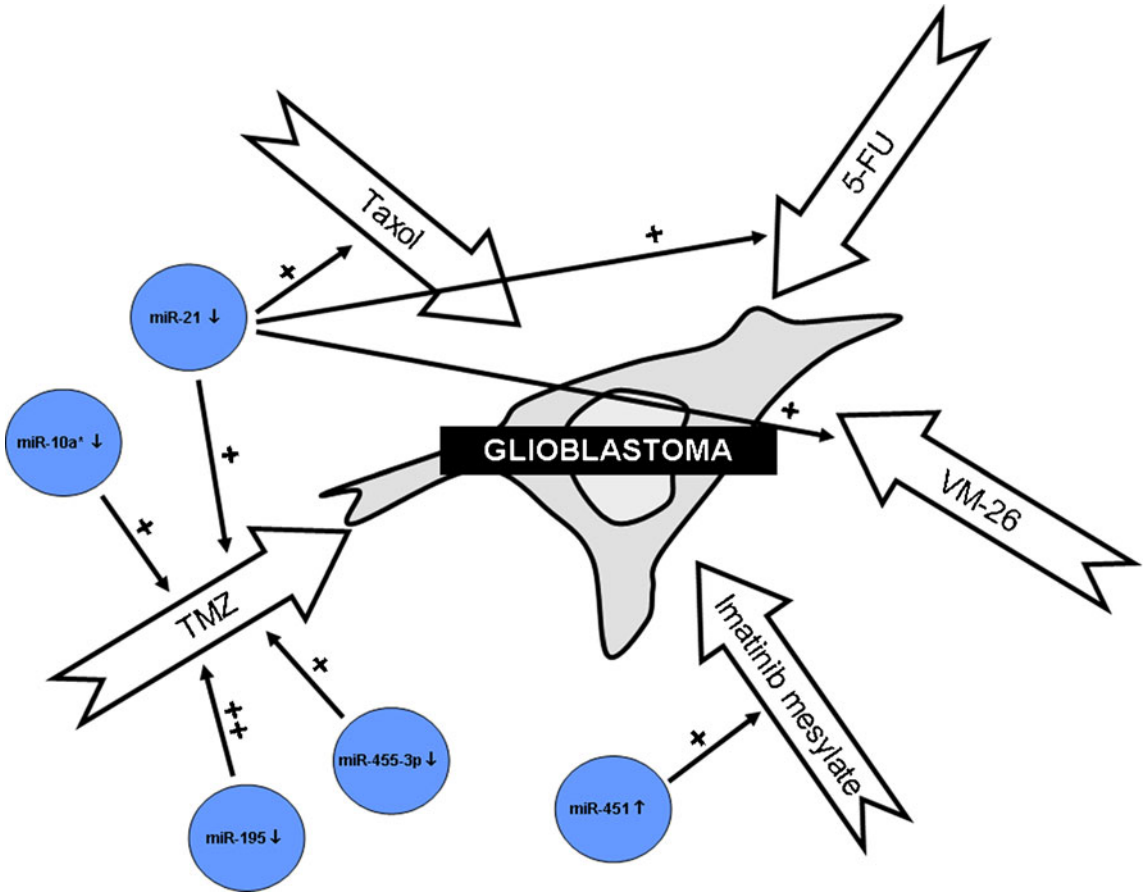

Fig. 3 Effects of miRNA modulation on sensitivity to chemotherapy in glioblastoma cell lines

ingly, most miRNAs were downregulated in medulloblastomas, suggesting a predominantly tumor-suppressive function of miRNAs in this tumor. A subset of only four miRNAs (let-7g, miR-19a, miR-106b and miR-191) allowed the classification of medulloblastoma samples into three different histotypes (anaplastic, classic and desmoplastic), and groups of miRNAs showed different expression between tumors overexpressing, e.g., ErbB2 (miR-10b, miR-135a, miR-135b, miR-125b, miR-153, miR-199b) or c-Myc (miR-181b, miR-128a, miR-128b) and "not-overexpressing" controls [74]. Uziel et al. [75] reported 26 miRNAs (including nine miRNAs that were encoded by the miR-17/92 cluster and its paralogs, and the miR-106b/25 paralog clusters) to be overexpressed in mouse medulloblastoma, and another 24 miRNAs (including miR-124a, miR-128, miR-138, miR-300, miR-381, miR-487b, miR-382, miR-433, miR-127, miR-434 and miR-136) showed decreased expression. Interestingly, investigations on human samples demonstrated that three miR-17/92-cluster-miRNAs (miR-92, miR-19a and miR-20) were overexpressed in medulloblastomas with a constitutively activated Sonic Hedgehog (SHH) signaling pathway [75]. These results were widely confirmed by another microarray study assessing expression of 427 miRNAs: components of miR-17/92 polycistron, including miR-18a, miR-19b and miR-20a, as well as the paralogous miR-106a (miR-106a/363 cluster) were overexpressed in human and murine medulloblastomas. Interestingly, expression of miR-17/92 was highest in the subgroup of medulloblastomas associated with activation of the SHH signaling pathway when compared with other subgroups of medulloblastoma [76]. Liu et al. [77] reported a miRNA expression signature to allow discrimination between medulloblastoma and controls, with four miRNAs (miR-17, miR-99a, miR-100, miR-106b) being overexpressed and five miRNAs (miR-218, miR-29a, miR-29c, miR-128a, miR-127-3p) being underexpressed in tumors. They validated their microarray data for miR-17, miR-100, miR-106b, and miR-218. Notably, most of predicted target genes of these miRNAs had been shown to be involved in medulloblastoma carcinogenesis [77]. Furthermore, 30 miRNA were found to be consistently downregulated in medulloblastoma. Some of these miRNAs were already mentioned in previous studies (miR-124, miR-129, miR-138, miR-150 and miR-323 were mentioned by Ferretti et al. [74] and Northcott et al. [76]), another 12 respectively, of which three were only mentioned in one of these two studies), but ten miRNAs (let-7g, miR-9, miR-124, miR-125, miR-128, miR-139, miR-181, miR-194, miR-324, miR-32) had not

been reported so far. The authors validated some of these new miRNAs and proofed a downregulation for miR-125, miR-128a, miR-139 and let-7g as well as an upregulation for miR-17-5p [78].

Additional researchers investigated the expression of specific miRNAs in medulloblastoma. MiR-124 or miR-129 for example were shown to be consistently downregulated in various medulloblastoma cell lines (e.g., Daoy, D283 Med, D341 Med, D283, D341, D384, D425, D458) and/or in patient tumor samples [79–81]. Lu and colleagues searched for novel recurrent regions of genomic amplification in medulloblastoma cell lines (D341MED and D384MED), and identified one of these regions at 8q24.22–q24.23. When screening this region for critical disease genes by using PCR-based mapping and SNP array methods, they found the encoding genes for miR-30b and miR-30d harbored in this area. Both miRNAs were overexpressed (in 54% and 12%, respectively) in human tumor samples [82].

Only two groups looked at clinically relevant outcomes (see Table 1). Ferretti and colleagues used a disease risk stratification to allocate patients into average risk (patients older than 3 years at diagnosis, non-metastatic and totally or nearly totally resected) or high risk (patients not fulfilling the above-mentioned criteria) groups. With this approach, they could demonstrate that miR-31 and miR-153 were downregulated in all medulloblastoma samples, but, more importantly, both miRNAs were lower expressed in high risk patients compared to average patients. The authors concluded from these data that the loss of these two miRNAs might be a marker of poor prognosis [74]. In addition, Garzia et al. [83] found that patients with non-metastatic medulloblastoma presented a significant higher expression of miR-199b-5p than patients with metastatic disease. Furthermore, there was a (not significant) trend towards a better overall survival in the group of the high-expressing patients [83].

In conclusion, these (mostly human and animal in vivo) studies suggest that miRNAs might as well offer a new diagnostic approach in medulloblastoma as already seen in glioblastoma, by distinguishing for example tumors with activated Sonic Hedgehog signaling pathway from healthy brain tissue. And again, first data reveal a potential connection between miRNA expression and clinically relevant parameters such as disease risk or metastasis, highlighting the possible use of miRNAs in clinical settings.

MiRNAs as Therapeutic Tools in Cell Lines

In the context of medulloblastoma, we could not identify any article describing a similar effect of miRNAs on anticancer treatment in vitro as pointed out for glioblastomas. But as there is strong evidence that miRNAs affect chemotherapy or radiotherapy in a variety of other cancer types including glioblastomas [65], we expect to read about this topic soon. However, several groups assessed the effects of ectopic miRNA modulation on tumor cell proliferation and growth [49]. MiR-9 and miR-125a, for example, were shown to promote growth arrest and apoptosis in medulloblastoma by targeting the truncated isoform of the neurotropin receptor TrkC (t-TrkC) [74]. Transient transfection with miR-34a strongly inhibited cell proliferation, cell cycle progression, cell survival and cell invasion. As miR-34a was shown to inhibit c-Met (in glioma and medulloblastoma), Notch-1, Notch-2, CDK6 (in glioma), and was furthermore downregulated in human glioblastoma versus normal and mutant p53 glioblastoma versus wild-type samples, the authors concluded that miR-34a suppresses brain tumor growth by targeting c-Met and Notch pathways [84, 85]. Ectopic upregulation of miR-124 was shown to inhibit cell proliferation but not to affect apoptosis in various medulloblastoma cell lines. Two possible targets were provided in this context: one group demonstrated that miR-124 targets and regulates CDK6, and another group found transfection with miR-124 to result in downregulation of solute carrier family 16, member 1 (SLC16A1), possibly explaining the impact on cell growth [86, 87]. Furthermore, Ferretti et al. reported that overexpression of miR-125b, miR-324-5p or miR-326 can inhibit medulloblastoma cell growth in vitro by targeting Hedgehog signaling pathway [73]. Regarding miR-128a, Venkataraman et al. [78] showed an inhibition of growth of medulloblastoma cells by targeting the Bmi-1 oncogene. In addition, miR-128a was demonstrated to alter the intracellular redox state of tumor cells and to promote cellular senescence [78]. Finally, Garzia and colleagues [83] could show that miR-199b-5p negatively regulates proliferation and cell growth in medulloblastoma cell lines via regulation of the Notch pathway through targeting of the transcription factor HES1.

To sum up, there is so far no (in vitro or in vivo) evidence that miRNAs affect response to chemotherapy in medulloblastoma as seen in glioblastoma. But there are several reports about distinct effects of miRNA modulation on for example cell proliferation, apoptosis or cell survival in this tumor type. As these characteristics represent important targets of conventional chemotherapy it can be expected that miRNAs will be demonstrated to impact as well on anticancer drug treatment in medulloblastoma in future studies.

Summary

MiRNAs are an astonishing new class of gene regulators, and it had been demonstrated that these molecules play a

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

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