Cancer and Metastasis Reviews (2019) 38:813–828
https://doi.org/10.1007/s10555-019-09820-6

Aberrantly expressed microRNAs and their implications in childhood central nervous system tumors

Julia Alejandra Pezuk[1] · Karina Bezerra Salomão[2] · Mirella Baroni[2] · Carolina Alves Pereira[2] · Lenisa Geron[2] · María Sol Brassesco[3]

Published online: 3 December 2019

Abstract
Even though the treatment of childhood cancer has evolved significantly in recent decades, aggressive central nervous system (CNS) tumors are still a leading cause of morbidity and mortality in this population. Consequently, the identification of molecular targets that can be incorporated into diagnostic practice, effectively predict prognosis, follow treatment response, and materialize into potential targeted therapeutic approaches are still warranted. Since the first evidence of the participation of miRNAs in cancer development and progression 20 years ago, notable progress has been made in the basic understanding of the contribution of their dysregulation as epigenetic driver of tumorigenesis. Nevertheless, among the plethora of articles in the literature, microRNA profiling of pediatric tumors are scarce. This article gives an overview of the recent advances in the diagnostic/prognostic potential of miRNAs in a selection of pediatric CNS tumors: medulloblastoma, ependymoma, pilocytic astrocytoma, glioblastoma, diffuse intrinsic pontine glioma, atypical teratoid/rhabdoid tumors, and choroid plexus tumors.

Keywords miRNA · Cancer · Children · Central nervous system · Review

1 MicroRNA biogenesis, function, and in cancer

MicroRNAs (miRNAs) are small non-coding RNAs that play important roles in regulating gene expression. MiRNAs were first described in 1993 in *Caenorhabditis elegans* as a temporal control that regulated protein levels and allowed the development of the nematode [1]. Soon after that, miRNAs were observed in vertebrates and invertebrates as conserved molecules approximately 22 nucleotides long and characterized as important post-transcriptional gene regulators that inhibit protein translation [2]. These molecules are involved in all critical biological processes with unique expression patterns defined for each cell type. Alterations of their expression is commonly associated with disease, and thus miRNAs can be used as biomarkers [3].

MiRNAs are encoded in intergenic or intronic regions, and their expression is usually regulated as any other gene [4]. Most of them are transcribed by RNA polymerase II in the nucleus, originating a primary transcript (named pri-miRNA). Then, pri-miRNAs are processed by a microprocessor complex containing a RNase III enzyme, called DROSHA, that leads to the formation of a miRNA precursors (pré-miRNA). Then, pré-miRNA are exported to the cytoplasm by Exportin-5, where another RNAse III enzyme called Dicer catalysis the ends of the molecule bringing forth miRNA duplexes. Next, the miRNA duplexes are associated to a protein complex named RISC (RNA-induced silencing complex) where only one strand will be selected to play its function [3, 5, 6]. Alterations on each step of the process can be associated with gene expression dysregulation causing many types of pathological conditions [7].

Numerous studies have shown that miRNA are important players for cancer initiation and progression [8, 9]. Although specific miRNAs are fundamental for each cell and tumor type, each miRNA can act as oncomiR or tumor suppressor,

✉ María Sol Brassesco
solbrassesco@usp.br

[1] Anhanguera University of São Paulo, UNIAN/SP, Av. Dr. Rudge Ramos, 1501 - Jardim Abc, São Bernardo do Campo, SP 09639-000, Brazil

[2] Ribeirão Preto School of Medicine, University of São Paulo, Av. Dr. Rudge Ramos, 1501 - Jardim Abc, São Bernardo do Campo, SP 09639-000, Brazil

[3] Departamento de Biologia, FFCLRP-USP, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, University of São Paulo, Avenida Bandeirantes, 3900 - Vila Monte Alegre, Ribeirão Preto, SP 14040-900, Brazil

according to the target mRNAs present in those cells. Moreover, being short sequences, each miRNA can have different mRNA targets and alternatively each miRNA can have opposite roles according to the tumor or cell type. Hence, it is important to understand the cell expression patterns to interpret the role of a specific miRNA dysregulation and potentially explored them as diagnostics, monitors, and the potentiality of using them therapeutically [10]. This review compiles up-to-date evidence about the role of miRNAs in the biology of leading central CNS tumors in the pediatric setting, coupled with a discussion of their diagnostic/prognostic potential.

2 Medulloblastoma

Medulloblastoma (MB), an invasive embryonic tumor of the cerebellum [11], is a highly malignant CNS tumor that occurs mainly in children. The procedures adopted for treatment are surgical tumor resection, chemotherapy, and radiotherapy [12]. Nonetheless, despite recent treatment advances, approximately 30% of affected children will die from their disease [13], and those who survive generally suffer from enduring neurological deficits and cognitive dysfunction due to the intensive therapies administered to the developing brain [14]. Thus, the current challenge is to identify and project effective molecular-targeted strategies to improve prognosis and reduce mortality and morbidity [15].

Traditionally, MB are classified by the World Health Organization (WHO) according to their histology, though over the last decade, extensive transcriptional profiling revealed the existence of at least four distinct molecular subgroups: (i) Wingless (WNT), (ii) Sonic Hedgehog (SHH), (iii) Group 3, and (iv) Group 4 [16, 17]. WNT tumors are characterized by activated Wingless pathway and connote the most favorable prognosis. SHH tumors possess activation of Hedgehog signaling and present an intermediate prognosis under current treatment regimens. Conversely, Group 3 is characterized by high MYC levels, and Group 4 tumors, with less well-characterized molecular profiles, are usually associated with poor prognosis [18]. More recently, a final classification emerged and divided MB in 12 distinct subgroups [19].

In addition to these features, in recent years, many studies have been conducted to evaluate miRNAs dysregulation in MB. Initial expression analysis showed miR-124 as significantly downregulated in tumor samples and cell lines. Restoration of its function *in vitro* inhibited cell proliferation through the cyclin dependent kinase 6 (*CDK6*), a member of a family of serine-threonine kinases involved in the control of cell cycle progression [20–22]. Moreover, miR-124 was found to directly act on Nur77, a gene that is commonly upregulated in MB cell lines and whose inhibition decreases cell proliferation and viability [23].

Later, miRNA expression analysis in primary MB samples demonstrated downregulation of miR-let-7, miR-103, miR-124a, miR-128a, miR-128b, miR-134, miR-138, miR-149, and miR-181b, while miR-21 was upregulated. When the expression of each miRNA was analyzed in relation to the histological classification, miR-let-7, miR-106b, miR-191, and miR-19a were upregulated in the more aggressive anaplastic histotype regarding classic and/or desmoplastic tumors; miR-let7 and miR-106b were differentially expressed between desmoplastic and classic tumors; and miR-19a resulted upregulated in anaplastic *versus* classic MBs. Also, when clinical data was considered lower expression of miR-153 and miR-31 was observed in high risk versus average risk patients, suggesting their loss as an indicator of poor prognosis [24]. SNP-array on MB cell lines, on the other hand, identified an amplified region (8q24.22-q24.23) harboring miR-30b and miR-30d that present increased expression in this tumor [25]. Large-scale analysis also revealed overexpression of miR-216 and miR-25 and downregulation of miR-92b [26]. Alternatively, downregulation of miR-34a is a common feature in MB. *In vitro*, its forced expression significantly inhibited cell proliferation, induced cell death and promoted the expression of its targets (*SIRT1* and *MYCN*), while, in mouse models, its absence accelerated tumor formation [27].

Another study showed decreased expression 167 miRNAs in MB compared with normal cerebellum samples, though the downregulation of miR-128a was the most prominent. Re-expression of this miRNA *in vitro* showed a decrease in cell growth and inhibited colony formation, indicating a possible role as a tumor suppressor [28]. Interestingly, miR-128a targets *Bmi-1*, a protein required for the normal development of the cerebellum that is found overexpressed in MB [29]. Additionally, other analyses suggested that the growth inhibition exerted by miR-128a is due to the signaling pathway triggered by senescence as a result of increased reactive oxygen species (ROS) [28]. Other miRNAs with low levels of expression in MB are represented by miR-9, miR-22, miR-31, and miR-449a. Forced expression of these miRNAs reduced cell viability and proliferation, while increased apoptosis *in vitro* and *in vivo* [30–32]. MiR-192, miR-221, and miR-4521 also exhibit low expression in MB samples and cell lines and when their expression is restored, there is a decrease in proliferation, cell cycle disruption, and increase apoptosis [33–35].

Likewise, miR-495 also has reduced expression in MB samples as compared with non-neoplastic cerebellum being directly correlated with survival [36]. In addition, a positive association between overall (OS) and event free survival (EFS) and miR-100, miR-126, and miR-219 expression were found in samples from pediatric MB patients [37]. Conversely, miR-328, miR-133b, miR-181b, miR-154, miR-433, miR-488, miR-584, miR-329, miR-299-5p, miR-330, miR-770-5b, miR-656, and miR-642 were found to have

low expression in MB samples even though their roles in MB remain unclear [38].

More recently, efforts to determine miRNA profiles that characterize each of the four distinct molecular subgroups have been made (Fig. 1). In a first attempt, grouping was defined according to miRNA profiling in 19 MB samples divided into clusters "A," "B," "C", and "D". The subtype "A," found in 6 tumors, was characterized by overexpressing several miRNAs involved in the WNT pathway, such as miR-224, miR-193a, and miR-23b. Subgroup "B" showed overexpressed genes from the SHH pathway, with miR-23b as the most prominent miRNA. "C" and "D" groups did not include any of those two pathways [39]. A recent study showed 19 miRNAs that can be used to improve MB classification, specifically to distinguish MB group 4 [40]. From those, miR-362-3p, miR-376a-2-5p, miR-135b-5p, miR-181c-5p, miR-501-3p, miR-504-5p, miR-660-5p, miR-187-3p, miR-892a, miR-888-5p, miR-4705, and miR-891a-5p were upregulated; while miR-17-5p, miR-18a-3p, miR-20a-5p, miR-181a-2-3p, miR-92a-3p, miR-224-5p, and miR-4417 were downregulated. In another study with a bigger cohort ($n = 103$), WNT tumors showed overexpression of miR-193a-3p, miR-224, miR-148a, miR-23b, miR-365, and miR-10b, compared with other groups. MiR-182 was found overexpressed in all WNT-MB samples. Moreover, miR-592 was overexpressed in tumors of group 4, miR-10b was found with high levels of expression in the WNT subtype, followed by group 3 MB, and miR-376a showed a higher expression in the group 4 MB compared with group 3 MB. On the other hand, miR-182 along with miR-135b and miR-204 were at low levels of expression in SHH variants. MiR-135b also appeared downregulated in tumors classified as groups 3 and 4 [41]. In addition, samples classified as WNT showed increased expression of miR-449a in relation to the other subgroups, and there may be a difference in DNA methylation patterns according to the molecular subgroup of MB [42].

Another high-throughput screening demonstrated low levels of miR-125b, miR-324-5p, and miR-326 in association with the regulation of the activator components of the SHH pathway. The re-expression these miRNAs in MB cells showed a significant reduction in mRNA levels of *Smo*, *Gli1*, and *Pitch*, endogenous SHH target genes. Furthermore, significant inhibition of cell proliferation and reduced clonogenicity was observed [43]. MiR-326, is related to the development of tumor stem cells derived from MB-SHH subgroup, promotes the inhibition of the Hh/Gli signaling pathway, causing decreased proliferation and cellular auto-renovation. This mechanism occurs due to miR-326 regulation of two important genes of this pathway, *Smo* and *Gli2* [44]. Additionally, miR-466f downregulation seems to be important to maintain the mesenchymal phenotype in SHH by allowing the overexpression of Vegfa and its receptor Nrp2 [45]. Comparatively, miR-218 is significantly downregulated in SHH and Group 3 subgroups. When overexpressed, decreases cell proliferation, colony formation, invasiveness and cell motility [46, 47]. MiR-182 and miR-183 also present decreased expression levels in SHH-MB in comparison with non-SHH-MB [48].

Moreover, miRNAs expression profiling revealed miR-17~92 cluster as overexpressed in MB with an aberrantly activated SHH/PTCH. Interestingly, mouse models with mutations in important genes of the SHH pathway, developed MBs

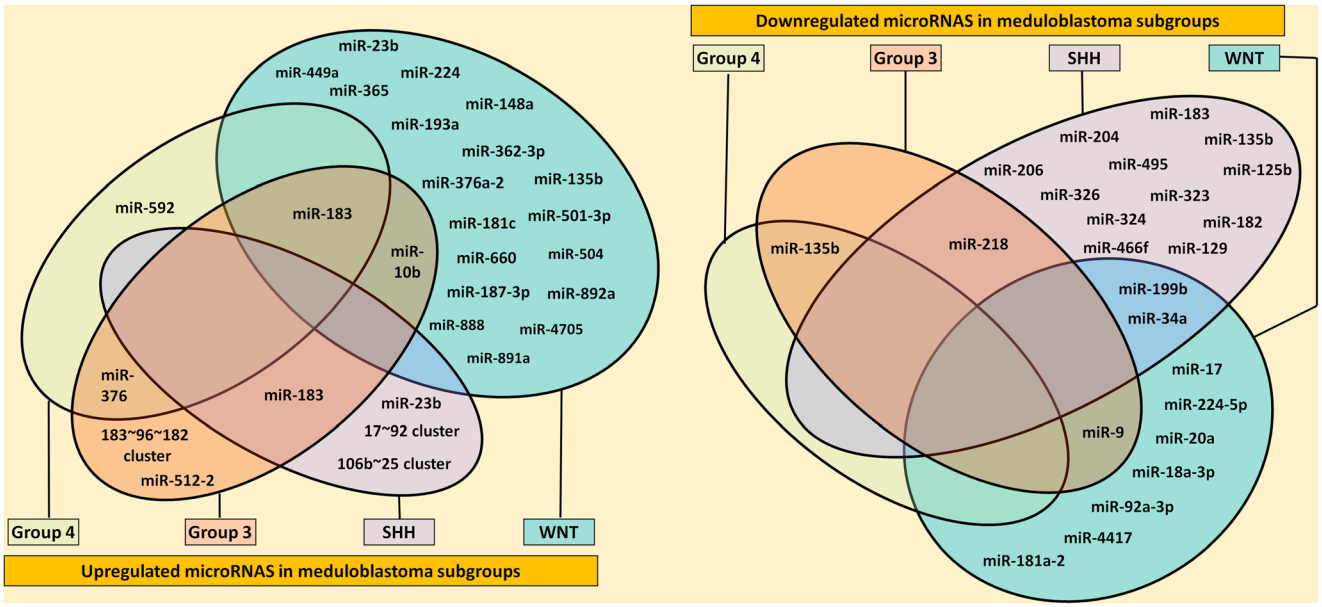

Fig. 1 MiRNA expression profiles characterize each of the four MB distinct molecular subgroups: WNT, SHH, group 3 and group 4 (see separate figure attached)

with complete penetrance when this cluster was overexpressed in neuronal progenitor cells of the cerebellum [49]. In addition, miR-17~92 overexpression was related with increased expression of MYC genes [50]. Moreover, when miR-17 and miR-19a are silenced, reduced proliferation and tumor growth are observed [49]. Likewise, the overexpression of miR-106b (a member of the paralog cluster miR-106b~25) in precursor cells promoted increased expression of targets of the SHH pathway, including *Gli1*, *Gli2*, and *Ptch1* [51]. Silencing miR-106b in MB cell lines inhibited cell proliferation, colony formation, and migration with the consequent cell cycle arrest and increased apoptosis. High expression of *p21* and *PARP* and decreased expression of *cyclin D1* were also observable. Furthermore, reduced miR-106b increased *PTEN* expression a target commonly associated with MB tumorigenesis [52].

Microarray analyses of desmoplastic MB samples probably belonging to SHH subgroup, found 64 downregulated and 20 upregulated miRNAs when compared with normal cerebellum. Thirty-two of those downregulated belong to a cluster located on human chromosome 14q32. Among the putative signaling pathways regulated by this cluster, *TGF-β*, oxidative phosphorylation and ubiquitin-mediated proteolysis were acknowledged. To confirm these data miR-206, miR-129-5p, miR-323-3p, and miR-495 were chosen and used for functional studies. Re-expression of miR-206 and miR-323-3p showed no differences in cell proliferation, though overexpression of miR-129-5p reduced cell growth [53].

Furthermore, the relationship between miRNAs dysregulation and *MYC* levels have also been addressed. *MYCC* presents over 30 putative miRNA target regions. This gene was found upregulated in 11 out of 37 MB samples analyzed; however, only the correlation between its overexpression and deletion of miR-512-2 was observed. Knockdown of this miRNA caused increased expression of *MYCC* in tumor cells, and consequently improved cellular proliferation [54]. Another cluster found overexpressed in the group of MB with *MYC* amplification is the miR-183~96~182. This cluster regulates the PI3K/AKT/mTOR signaling axis and its silencing decreases cell viability and proliferation, reducing cell invasion and metastasis [55]. Furthermore, low miR-494 expression has also been linked to high c-myc in MB cells, and that its increased expression lead to decreased cell proliferation, migration, and invasion, and increased apoptosis by inactivating the p38 MAPK pathway [56].

Also, in an attempt to identify miRNAs potentially targeting *HES1*, the principal Notch-responsive gene that normally prevents migration of neural progenitor cells out of the ventricular zone, dysregulation of miR-199-5p was described [57, 58]. The expression of this miRNA in non-metastatic cases is significantly higher than in metastatic cases and is associated with improved OS. Also, besides the inhibition of *HES1*, overexpression of miR-199b-5p impairs proliferation and the chlorogenic potential of MB cell lines while reducing tumor formation in mice models. *CD15* was also found to be a target of miR-199b-5p [59].

MiR-34a is also associated with the Notch signaling pathway, regulating *DLL1*, *Jagged1*, *Notch1*, and *Notch2*. Transfection of vectors expressing miR-34a in MB cell lines negatively regulates *DLL1* activity, while no significant inhibition was observed for the other studied genes [60]. MiR-34a has also been associated with sensibility to chemotherapeutic agents, including mitomycin C and cisplatin. This sensibility is facilitated by the direct targeting of the *MAGE-A* genes [61]. Comparatively, miR-584 has also been associated to chemotherapeutic response in MB. In fact, miR-584 over expression is correlated with increased levels of HDAC1/eIF4E3 and can potentiate vincristine and radiation response by regulating cell cycle arrest, DNA damage, and microtubule dynamics and may be useful as a therapeutic adjuvanted for MB [62].

3 Ependymoma

Ependymoma (EP) is a pediatric tumor of the CNS that originates from ependymal cells located in the lining ventricular surfaces in the brain. It is the third most common pediatric brain tumor with over 50% occurring in children younger than 5 years of age [63, 64]. This tumor appears in different places along the entire craniospinal axis, including the cerebral hemispheres, the region around the brainstem, posterior fossa and the spinal cord [65–67]. However, while the majority of tumors in adult patients are located in the spine (SP; 46%), the brain is the predominant location in children and adolescents, with 90% of tumors located intracranially, a third of which are supratentorial (ST), and two-thirds are located in the posterior fossa (PF) [68]. EP treatment consists of surgical resection followed by radiation therapy [69, 70]; however, 5-year EFS and OS rates are only 60% and 30%, respectively [63, 69]. Moreover, as is the case with MB, most survivors have long-term side effects such as physical and cognitive disabilities [63, 71].

EP have traditionally been classified by the WHO Classification of Tumors of the Nervous System as grades I, II, and III, based on the presence and degree of anaplasia. From a genetic point of view, this tumor is characterized by some instability with loss of chromosomes 6q, 17p and 22q and gains of 1q and 9q. A recent analysis through CGH-array revealed subsets of EP with distinct patterns of gene expression and aneuploidies that correlated with the anatomic location, but not with clinical parameters [67]. Nonetheless, about 40% of EP in children have a balanced genomic profile, with few detectable chromosomal abnormalities [72]. Consequently, there is still a need for better biomarkers for diagnosis, prognosis and management of disease progression [73].

On this regard, recent methodological advances have allowed, extensive epigenetic and transcriptional analysis leading to the elucidation of nine distinct molecular subgroups of ependymoma, across all compartments of the CNS [65, 74, 75]. When comparing to other brain tumors, EP showed low expression of miR-10a and over-expression of miR-10b and miR-29a [76]. Accepting that most relevant analyses in tumors are connected with epigenetic and regulatory studies, initial miRNA profiling showed overexpression of miR-34b, miR-34c, miR-200a, miR200b, and miR-483. Moreover, miR-124a, miR-137, miR-138, miR-193b, and miR-181d appeared underexpressed in these samples [26]. Other miRNAs that showed differences in expression compared with non-neoplastic brain tissues were miR-10a and miR-10b [77]. MiR-34a, miR-135a, and the mir-17~92 cluster also showed to be overexpressed in tumor samples, whereas miR-485-5p was downregulated [73]. The later targets genes from the TGF-β family, important for the biology of several tumors, including EP [78, 79].

Other studies showed that the expression of miRNAs could be used as biomarkers to differentiate histological and molecular subgroups (Table 1), like the study of Lourdusamy et al. that showed a differential miRNA expression in pediatric spinal EP. Those miRNAs included miR-10a and -b, both of which were found upregulated and target chromatin modification genes and miR-124, which was downregulated in and normally represses genes involved in cell communication and metabolism [80]. Alternatively, miR-106-b-5p and miR-19a-3p are mostly expressed in samples from the posterior fossa compared to tumors from other compartments [83] and the expression of these two miRNAs also differentiates between grades II and III [82, 83]. Recently, it was shown that miR-135a-3p, miR-137, miR-181d, and let-7d-5p are upregulated in EP, and that higher miR-203a (previously named miR-203) expression can be used as a prognostic or diagnostic marker once that it is found in grade III EP [85]. Another study found some differentially expressed miRNAs in posterior fossa samples that are CD44 positive. Among these, miR-495-3p and miR-299 were validated by qRT-PCR and showed to activate the PI3K-Akt pathway, which is related to tumor progression and worse prognosis [87]. Also, Zakrzewska et al. found that lower miR-17-5p expression correlates with better prognosis and lower expression of *EZH2* [82], which was suggested as a marker of poor prognosis in children with posterior fossa tumors [88].

Other miRNAs that correlate with EP prognosis are miR-124-3p [84], miR-203 [73, 86], miR-15a, and miR-24-1 [81]. High expression of miR-124-3p is associated with increased metastatic rates and higher histological grade [84]. In addition, despite the use of chemotherapy in EP remains controversial [65, 89], some studies associate this treatment response to the activation of different molecular pathways. Lower expression

Table 1 Dysregulated MiRNAs in Ependymoma associated with prognosis and differentiate histological and molecular subgroups

MiRNA	Expression	Prognostic value	Target	Reference
miR-10a	Upregulated	Pediatric S-EP	Chromatin modifier genes	[80]
miR-10b	Upregulated	Pediatric S-EP	RhoC, chromatin modifier genes	[77, 80]
miR-15a	Upregulated	Relapsed and deceased		[81]
miR-17-5p	Downregulated	Better prognosis	*EZH2*	[82]
miR-19a-3p	Upregulated	PF-EP, differentiates between grades II and III		[82, 83]
miR-24-1	Upregulated	Relapsed and deceased		[81]
miR-34a	Upregulated	Overexpressed in ST-EP		[73]
miR-106-b-5p	Upregulated	PF-EP, differentiates between grades II and III		[82, 83]
miR-124-3p	Upregulated	Metastasis rate and high histological grade	*TP53INP1*	[84]
miR-135a	Upregulated	Associated with less responsive phenotype		[73, 76]
miR-146b	Upregulated	Associated with less responsive phenotype		[76]
miR-192-5p	Upregulated	Tumor recurrence		[83]
miR-203	Upregulated	Worse prognosis		[73, 85, 86]
miR-221-3p	Upregulated	Tumor recurrence		[83]
miR-222-3p	Upregulated	Tumor recurrence		[83]
miR-299	Upregulated	PF-EP – CD44 positive	PI3K-Akt pathway	[87]
miR-326	Upregulated	Tumor recurrence		[83]
miR-371-5p	Upregulated	Tumor recurrence		[83]
miR-495-3p	Upregulated	PF – CD44 positive	PI3K-Akt pathway	[87]
miR-520-3p	Upregulated	Tumor recurrence		[83]

S-EP, spinal ependymoma; *PF-EP*, posterior fossa ependymoma; *ST-EP*, supratentorial ependymoma

of miR-135a and miR-146b, for instance is related to a less responsive phenotype leading to remission and recurrence of the tumor [76]. Other miRNAs associated with higher rates of tumor recurrence are miR-192-5p, miR-221-3p, miR-222-3p, miR-326, miR -371a-5p, and miR-520g-3p [83].

4 Pilocytic astrocytoma

Pilocytic astrocytomas (PAs) are the most common brain tumors in childhood between 5 and 14 years of age. These tumors are classified as WHO grade I and exhibit low rates of growth. PAs originate in the cerebellum and chiasmatic hypothalamic region, being disseminated just in 3–5% of cases. Patients with this tumor show a 10-year OS greater than 90%. Development of PAs is associated with neurofibromatosis (NF1). In most cases, PA treatment is surgical, and patient prognosis depends on resection, however radiotherapy and chemotherapy can be applied as adjunctive treatment [90–92].

Alterations in molecular pathways such as Ras/ERK, and KIAA1549-BRAF fusions are common in PAs [93]. Meanwhile, genome wide studies have demonstrated the importance of miRNAs as biomarkers and targets in this tumor. Microarray analysis, for instance, allowed the identification of a subset of differentially expressed miRNAs in pediatric PAs when compared to normal brain tissue. Main fold-changes were described for miR-124, miR-124*, miR-129, miR-129*, and miR-218, which were underexpressed; and for miR-21, the Xq26.3 cluster (miR-542-5p, miR-542-3p, miR-503, mir-450a and miR-450b-5p); miR-224, miR146a, miR34a, and the miR-106a~miR-363 cluster, that were upregulated [94, 95]. Mir-124 and miR-129-5p target multiple components of receptor tyrosine kinase/MAPK/ERK signaling pathways. MiR-34a targets MAP2K1 (MEK1), while miR-503 targets MAPK1 (ERK1). MiR-146a, on the other hand, is known to modulate senescence associated with inflammatory mediators [95]. In another set of PA samples ($n = 44$), miR-15 and miR-24-1 were found downregulated. These miRNAs showed four common predicted pathways: PI3K signaling pathway, fatty acid metabolism, IL3-mediated signaling, and focal adhesion [81].

Comparing among PA groups, miR-650 and miR-1276 were overexpressed, and miR-744* and miR-187* were downregulated between NF1-associated tumors and BRAF alteration [94]. PA showed downregulation of miR-129 in relation to other CNS pediatric tumors (Atypical teratoid, Ependymoma, Medulloblastoma and Glioblastoma); in this context of comparison, it was observed the downregulation of miR-93, miR-135a, miR-135b, and miR-106b, and overexpression of miR-432, miR-29a, miR-138, miR-299-5p, and miR-34a [26].

A comparison between PA and white matter showed over 80 differentially expressed miRNAs, those underexpressed regulate classical tumorigenic pathways, while the most overexpressed miRNAs were related to pathways such as focal adhesion, P53 signaling pathway and [96]. More recently, in a nanostring platform, that quantified the expression of 800 miRNAs, PA clustered together with subependymal giant cell astrocytomas, and rosette forming glioneural tumors, and farthest from non-neoplastic brain, and showed upregulation of miR-487b, miR-129-3p, miR-219-5p, miR-338-3p, miR-478b, miR-21, and miR-34a, and downregulation of miR129-5p [97].

5 Glioblastoma multiforme

Glioblastoma multiforme (GBM) is the most frequent and aggressive neoplasm of the CNS. It is considered a grade IV tumor by the WHO [98], and highly lethal with survival around 12–18 months after diagnosis. It can occur at any age or gender, though is more common in adults (average 97% of cases) [99]. In children, they only represent approximately 8–12% of all CNS tumors [100]. Current treatment consists of surgery, followed by radiation and chemotherapy [99]. However, these procedures are still inefficient, with survival rates stagnated in a few months [101]. Thus, in order to elucidate potential markers for the diagnosis and prognosis in GBM, and to contribute to the development of new therapies, intense research is in progress, focusing on the role of the altered expression of miRNAs in key molecular pathways [98].

Currently, the role of many miRNAs in the development and progression in adult GBM has been unveiled. However, in children this kind of data is still somewhat scarce, mainly due to the rarity of samples [102] (Table 2). One oncomiR quite studied is miR-21, which is overexpressed in this tumor, and is associated with defective apoptotic pathways [98]. Moreover, miR-21 regulates several targets that function as tumor suppressors (including the network of p53, TGF-β, and mitochondrial apoptosis) whose dysregulation has several biological effects leading to increased cellular proliferation, and invasion/migration [103, 110, 111].

Another miRNA that has been extensively studied is miR-221/222, which is also found overexpressed in GBM. The expression of both miRNAs is co-regulated and they share target specificity, such as p27Kip1 and p57 (CDK inhibitors) and the pro-apoptotic gene PUMA [98, 104–106]. Moreover, Tomaselli and colleagues [112] reported that ADAR2, an enzyme essential for brain development and function is impaired in pediatric GBM, and is correlated with increasing tumor grade. This enzyme is capable of modulating the expression of miRNAs involved in tumorigenesis, among which are miR-21 and miR-221/222. This inhibition leads to reduced cell proliferation, tumor growth and migration in GBM, through the modulation of the CDC14B/Skp2/p21/p27 pathway. In

Table 2 MiRNAs with clinical and biological significance in pediatric glioblastoma

MiRNA	Clinical and biological significance	Reference
miR-21	Defective apoptotic pathway, targets p53 and TGF-β	[98, 103]
miR-221/222	Targets CDK inhibitors and pro-apoptotic PUMA	[98, 104–106]
miR-124-3p, miR-128-1, miR-221-3p	Differentiation of glioma stem-cells	[107]
miR17/92, miR-106b/25, and 14q32 cluster	Upregulated in patients is associated with shorter overall survival	[108]
miR-15a, miR-424, miR-30e, miR-378c	High in H3F3A mutants	[108]
miR-487b	Overexpression leading to a decrease in a colony formation	[97]
miR-3666	Downregulated with a role in GBM progression	[109]

addition, downregulation of miR-129, miR-124-3p, miR-128-1, and miR-221-3p and upregulation of miR-142-5p, and miR-25, was reported in pediatric GBM [26, 107]. MiR-124-3p, miR-128-1, and miR-221-3p are important for the differentiation of glioma stem-cells and are also expressed in adults gliomas [107].

Likewise, genome-wide microarray analysis showed that 266 miRNAs were differentially expressed in pediatric GBM when compared with controls, being 55 upregulated, and 71 downregulated. All miRNAs from the miR17/92 and miR106b/25 clusters were found upregulated; while the 14q32 cluster was upregulated in patients that have a shorter OS. Moreover, differentially expressed miRNAs were found when considering mutations in histone H3.3, in chromatin remodeling genes or in *TP53*. Expression of miR-15a, miR-424, miR30e, and miR-378c was found to be higher in H3F3A mutants as compared with wildtype counterparts. Also, the comparison between pediatric and adult GBM samples showed that 227 miRNA probes were differentially expressed, where 69 were upregulated, and 108 were downregulated [108].

More recently, miR-487b was found downregulated in gliomas using a nanostring platform and functionally validated in a cell line established from a 16-year-old patient with GBM. The overexpression of miR-487b decreased the expression of PROMI and Nestin, leading to a decrease in colony formation, but did not affect cell proliferation, sensitivity to temozolomide, migration, or invasion [97]. Additionally, miR-3666 was found downregulated in GBM cells when compared with normal cells, while its overexpression shows to inhibit proliferation, migration and invasion, and to cause GBM cells cycle arrest by regulating KDM2A expression [109].

6 Diffuse intrinsic pontine glioma

Diffuse intrinsic pontine gliomas (DIPG) are the most common malignant gliomas of the brainstem in childhood, affecting typically children between 3 and 12 years old [113]. Their median survival is approximately 10 months and despite decades of clinical research, DIPG prognosis remains disappointing [114, 115]. Most affected children die within 2 years after diagnosis, and currently there is no effective treatment [113, 116].

Children with this neoplasia have classical symptoms, which include a triad of neurological signs, such as cerebellar involvement with ataxia and/or incoordination, cranial neuropathies, and involvement of a long tract (extremity weakness and hyper-reflexia) [117, 118]. Due to the DIPG localization, the surgical intervention is not an option and biopsies are rarely performed. Thus, the current diagnosis is based on radiological features and clinical findings [119]. Radiotherapy is the predominant way of treatment, though it is mainly palliative [113, 114, 116, 120].

Historically, the study of the underlying biology of DIPG has been limited by the shortage of tumor tissue accessible for research and the lack of experimental models [117, 121]. Because biopsy is still not very feasible or reliable for the evaluation of molecular characteristics, the use of other body fluids such as blood and cerebrospinal fluid, has become quite attractive to investigate [116]. However, a few recent studies based on DIPG biopsies have been conducted safely and have opened opportunities for future clinical trials, in an attempt to understand the molecular characteristics of this tumor, and define target therapeutic approaches [114, 117].

Thus, over the past few years, some genetic characteristics present in patients' subgroups have been described. *TP53* is one of the most common mutated genes (40–77% of cases). High-frequency heterozygous mutations of H3.3 histone at K27 residue (approximately 70% of cases) are also frequently observed [114, 118]. Nowadays, with the recent increase in utilization of experimental models, better preclinical studies and therapeutic tests may be performed. Studies involving miRNAs would then be inserted in this context, assisting in the of drug selection and in understanding of resistance mechanisms of this tumor [113].

A pioneer miRNA profiling analysis revealed 2 groups of miRNAs as significantly altered in DIPG. Of the 25 overexpressed miRNAs, most are known oncomiRs (miR-183~96~182 and mir-17~92 clusters). Conversely, many of the 19 downregulated miRNAs were previously reported to act as tumor suppressors (i.e., miR-29b, miR-124, and miR-

145). Remarkably, 11 of the down-regulated miRNAs are located on chromosome 14, which is frequently lost in this tumor [122]. Later, Yadavilli and colleagues [123] described the downregulation and hypermethylation of miR-129-2 in DIPG samples, what results in increased expression of NG2, a transmembrane protein that contributes to the neoplastic transformation of glioma cells.

More recently, an *in silico* analysis, with the aim to explore interactions between miRNAs and transcription factors (TF), demonstrated 27 altered miRNAs associated with DIPG, and showed 141 relationships between miRNA and target-genes, from those, miR-26b which interacts with the TF AP-2 Gamma (TFAP2C) featured a higher degree in the TF-miRNA-target gene network suggesting important roles in the development and progression mechanisms of DIPG [124]. Therewith, it is necessary to continue the search for specific biomarkers to explore the unique molecular characteristics of DIPG, which would reflect at new therapeutic strategies [113, 116, 117].

7 Atypical teratoid/rhabdoid tumors

Atypical teratoid/rhabdoid tumor (AT/RT) are highly aggressive primitive neuroectodermal tumors that preferentially affect the cervical region, and represent the most frequent malignant CNS tumor in infants [125]. The current treatment approach consists of multimodal therapy, including chemotherapy, radiotherapy, and surgery [126]. Nonetheless, despite the aggressive treatment-free survival rates are low, usually less than 3 years after diagnosis, special due to chemo- and radioresistance [127, 128].

Genomic alterations of AT/RT tumors are not completely understood. However, inactivation of the tumor suppressor SMARCB1, or in rare cases of SMARCB4, gene has already been shown and used to diagnose this disease [129, 130]. Thus, proposals to divide AT/RT into subtypes still conveys on treatments response and clinical outcomes [130].

So far, miRNA expression patterns in AT/RT has been scarcely explored (Table 3). A study performed by Sredni et al. that analyzed a panel of 365 miRNA was the first to associate miR-221 and miR-222 dysregulation with AT/RT progression [136]. Both microRNAs present increased expression in this tumor and have shown to regulate SUN2, a tumor suppressor that participates in the nuclear envelope protein complex LINC (linker of nucleoskeleton and cytoskeleton) pointed as a key regulator of cell proliferation and tumor malignancy [137]. Another early study showed that AT/RT samples have also increased levels of miR-520b, miR629, miR-221, miR-498, and miR-373* and decreased levels of miR-140, let-7b, miR-139, miR-153 and miR-376b compared to normal tissue controls [26].

Later, in the search for diagnosis and new therapeutic choice the copy number of let-7a3 and let-7b was analyzed. Both were confirmed to be decreased in AT/RT with the concomitant overexpression of their target HMGA2 (high-mobility groups AT-hook 2) [138]. Furthermore, it was shown that miR-142-3p was lower in stem-like AT/RT cells, and that its downregulation increases the cancerogenic characteristics of AT/RT cells, by promoting mesenchymal transitional, radioresistant, tumor initiation, and stem-like capacities [139]. Moreover, miR-155 secreted in exosomes by tumor-associated mesenchymal stem cells mediate AT/RT tumor migration in a SMARCA4-dependent pathway [134]. Even so, profiling of AT/RT-specific miRNA signatures needs to be better scrutinized to assist the establishment of biomarkers with diagnostic or prognostic utility.

8 Choroid plexus tumors

Choroid plexus tumors (CPTs) are rare pediatric brain neoplasms characterized by papillary and intraventricular growth as a consequence of the uncontrolled replication of the choroid plexus epithelium. CPT can be classified according to histopathological features into three grades: choroid plexus papilloma (CPP) (grade I), atypical choroid plexus papilloma (aCPP) (grade II), and choroid plexus carcinoma (grade III) [140, 141]. Clinical manifestations on patients are consequences of increased intracranial pressure such as headache, diplopia, and ataxia, and the main therapeutic approach consist of surgical removal [142]. Nevertheless, CPT are challenging in pediatric patients mainly due to the potential for massive blood loss [143].

The molecular biology of CPT is not completely understood, and most information available relays on indirect evidence (Table 4). A primary study performed by Redshaw

Table 3 Important miRNAs for Atypical teratoid/rhabdoid tumors

MiRNA	Clinical and biological significance	Reference
miR-449	Cancer initiation and progression	[131, 132]
miR-146a and miR-155	Cell fate Circulating in extracellular vesicles	[133, 134]
miR-34a	Hypermethylation is associated with decreased in overall survival	[135]

et al., for instance, identified miR-449 is an important regulator of choroid plexus development and function, suggesting that its dysregulation could be associated to cancer initiation a progression [131]. This miRNA has indeed a role in cell fate by regulating cell death, cell cycle arrest, and/or cell differentiation [132], but still, its contribution to tumor development needs to be better explored. Another customized miRNA array was developed by Wang et al. for the expression profiling of cerebrospinal fluid that might be useful to identify and monitor diseases of the choroid plexus [144]. In addition, a study that explored extracellular vesicles secreted by the choroid plexus epithelium showed that the number of vesicles were increased and cargo more miR-146a and miR-155 in the presence of systemic inflammation, pointing to the possibility of using miRNA profiles to identify pathological conditions related to the choroid plexus [133]. Even so, a recent genome-wide DNA methylation analysis in CPA samples from patients with Li-Fraumeni syndrome showed that the hypermethylation miR-34a was associated with cancer susceptibility [135].

9 Circulating miRNAs as cancer biomarkers for childhood CNS tumors

Many studies have shown that specific cancer characteristics, both genetic and epigenetic, are detectable in the plasma and serum of cancer patients and may be useful as tools for early detection, patient follow-up and even increase the precision with which a disease can be classified into subtypes [145]. Therefore, the characterization of circulating miRNAs (c-miRNAs) in cancer has received more attention in identifying promising diagnostic and/or prognostic biomarkers. These non-coding RNAs are ideal candidates for biomarker development due to their stability, ease of isolation and detection, and the unique expression patterns associated with disease stages [146].

C-miRNAs can be detected in diverse biological fluids including blood, urine, serum, plasma, cerebrospinal fluid, or saliva. Their extracellular presence may result from their passive release from injured cells, chronic inflammation, apoptosis or necrosis, active secretion by a protein-miRNA complex (e.g., high-density lipoprotein-HDL or Ago2 protein) and/or via exosomes [147], playing important roles in intracellular communication [148] and may promote or inhibit tumor progression. C-miRNAs have shown to be highly resistant to degradation after temperature oscillations, pH changes and/or fragmentation by chemicals or enzymes [149], making ideal tumor biomarkers for translation into clinical practice even for CNS tumors [150]. Indeed, several studies have focused on their potential use for pediatric cancer [134].

Table 4 MiRNAs with clinical and biological significance in choroid plexus tumors

MiRNA	Expression	Target	Reference
miR-221	Upregulated	SUN2	[137]
miR-222	Upregulated	SUN2	[137]
Let-7a3	Downregulated	HMGA2	[138]
Let-7b	Downregulated	HMGA2	[138]
MiR-142-3p	Downregulated	Sox9 and AC9	[139]

One example is the microRNA profiling of cerebrospinal fluid (CSF) that enables the detection of GBM, the discrimination between primary and metastatic brain tumors, and reflects disease activity. The levels of miR-10b and miR-21, for example, were found significantly increased in the CSF of patients with GBM and brain metastasis of breast and lung cancer, compared with tumors in remission and a variety of nonneoplastic conditions. Likewise, members of the miR-200 family are highly elevated in the CSF of patients with brain metastases but not with any other pathologic conditions, allowing discrimination between glioblastoma and metastatic brain tumors [151].

In pediatric gliomas some miRNAs were found to be important biomarkers for screening and tracking the tumor. Higher levels of miR-21, miR-15b, miR-23a, and miR-146 were found in the serum of juvenile pilocytic astrocytoma patients compared with control [152]. Differential levels of miR-130, miR-145, and miR-335 in serum of PA patients have also been described demonstrating the potential use of c-miRNAs as non-invasive biomarkers [153]. Other eight miRNAs were significantly elevated in the serum of juvenile PA patients as compared to non-oncologic controls: miR-21, miR-15a, miR-15b, mir-17 miR-23a, miR-23b and miR-106b, and miR-146b, previously identified in the tissues. Moreover, mir-21, miR-23a, miR-146b, miR-106b, miR-15a, and miR-15b were correlated with tumor nodule size [152].

Thus, the so-called "liquid biopsies" can allow to detect tumors early and non-invasively survey tumor changes in real time, assist treatment strategy assortment, monitor chemo- or radiotherapy response, and predict outcomes [154–157]. Nonetheless, in some cases low quantity of c-miRNA in liquid samples represent a challenge, requiring sample preparation and pre-amplification to be able to detect variations c-miRNA levels. As a result, new strategies are constantly described to overcome methodological limitations [158–161].

10 Therapeutic potential and future implications of miRNA

Besides patient clinical and genetic features, the optimization of cancer treatment greatly depends on the understanding of the molecular pathways involved in tumor establishment/maintenance. Over the last decade, genomic profiling has

directed the re-classification of different tumors into new molecular and prognostic homogeneous subgroups. The clear epitome of how integrated phenotype/genotype approaches have improved disease stratification is represented by MB, where the amalgamation of cytogenetic aberrations, transcription profiles, and DNA methylation biomarkers along with age at onset and other features of clinical and prognostic relevance has allowed the incorporation of consensus molecular subgroups into the 2016 revised WHO classification [162]. Moreover, the integration of molecular features has fomented the advent of subgroup-directed therapies that are ongoing clinical trials, i.e. the smoothened inhibitors vismodegib [163, 164] or sonidegib [165].

Nonetheless, even with the embracement of this classification, the refinement of molecular approaches has denoted considerable biological heterogeneity within each subgroup that remains to be resolved. On this regard, more recent in-depth studies have further allocated MB into additional novel subtypes [19, 166]. As seen in previous sections, a sizeable body of literature illustrates aberrant expression profiles of miRNAs as a common feature of human malignant cancers. Nonetheless, little efforts have been made to combine all this information and find more precise molecular networks to improve disease subclassification towards personalized therapy.

MiRNA-tailored treatments are supported by several factors. First, the multi-target nature of these molecules allows them to act simultaneously on diverse signaling pathways, and thus, modulate numerous oncogenes and tumor suppressors. Secondly, it has been suggested that targeting a miRNA that coordinately targets and regulates multiple members of a cancer-related pathway would be particularly advantageous [167]. And last but not least, different miRNAs cooperate and exert their inhibitory activity over members of specific cancer-related pathways. As an example, Ferretti et al. [168] demonstrated that miR-324-5p targets Smo and Gli1 in mammalian cells, and together with miR-125b, miR-326 orchestrates the modulation both components of the Hh signaling pathway in MB.

Over the last years, compelling *in vitro* evidence underpins the therapeutic targeting of miRNAs. However, even though is undeniably clinically promising, the transition from bench to bedside is far from reality due to a variety of challenges. The potential of using mimics or antagomiRs to replenish or block specific miRNAs in clinics has confronted restrictions for *in vivo* application as a consequence rapid degradation in extracellular and plasma environment, instability in the cell compartments and stimulation of immunity [169]. Other hurdles with the circulation time, parenteral administration, and cell uptake have not been solved [170]. Furthermore, the miRs tend rapidly be removed from the circulation to the liver and kidneys decreasing plasma levels within minutes after administration [171].

Moreover, different from *in vitro* models, the use of viral vectors faces several drawbacks mainly due to the latent mutagenic effects in the host, what have impulsed the development of new concepts of nanotechnology-based local or systemic delivery to overcome those barriers to materialize into practical therapies. Nanostructured lipid carriers, encapsulation in polymer nanoparticles, for instance, have shown great potential for miRNA delivery in mice models due to low toxicity/immunogenicity, long metabolic cycles, and easy modification during controlled synthesis [172–179]. Other options include cationic polyester nanospheres, micelles and dendrimers [180], and graphene oxide nanocarriers [181].

Currently, there is an increasing myriad of nanosized alternatives that can achieve tumor-targeted delivery been tested for CNS tumors [182], including carriers of polycistronic RNAs [183], copolymers of poly(lactic acid) and hyperbranched polyglycerol [184], transporters of CRISPR/dCas9 systems [185], and polymeric magnetic particles [186]. Nonetheless, each vector system has its own unique properties and may not be suitable for every application. Still, exosomes present themselves as superior bio-shuttles for the delivery of nucleic acids [187]. Among the many advantages are their inherent small size (40–100 nm) and the similarity to cells that allows them to evade phagocytosis/degradation by macrophages [188]. Most importantly, the main asset for these nano-scale and lipid-bound vesicles is their intrinsic ability to readily pass the blood-brain barrier [189, 190] and the feasibility of tailoring them with surface adhesion proteins and/or specific ligands that may provide potential advantages for cell-specific delivery [182].

Nevertheless, irrespective of the delivering strategy, the clinical application of miRNA modulation for cancer treatment remains uncertain. So far, the only approach to enter clinical trials was MRX34 (Mirna Therapeutics, Inc.®), a liposomal miR-34 mimic tested in patients with unresectable primary or metastatic tumors. Initial results showed acceptable safety and antitumor activity in a subset of patients with refractory advanced hepatocellular carcinoma, renal cell carcinoma or melanoma when MRX34 was associated with dexamethasone premedication [191]. However, the trial was terminated prematurely due to immune related serious adverse events (www.clinicaltrials.gov – NCT01829971).

Currently, it is unquestionable how the molecular profiling of tumors has surpassed the histologic classification. Despite the progression on understanding the functionality of miRNAs and how their dysregulation influences tumor development and progression, the study of their expression profiles and prognostic value remains elusive for many tumors of childhood. As seen in previous sections, many miRNAs have been described as dysregulated in CNS tumors, most of which, may aid risk stratification, predict treatment response and survival. Nonetheless, more integrative approaches are still needed to leverage their use as diagnostic biomarkers and outcome predictors. Moreover, better-designed studies are needed to translate miRNA-tailored approaches into actual clinical use.

Funding information The authors would like to thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for the financial support along all these years.

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