Role of micrornas in medulloblastoma biology essay

Science, Biology

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ABSTRACT

Medulloblastomas are the most frequent brain tumors in children and remained a major therapeutic challenge. Clinical and histopathological features are used for disease classification and patient prognostication. Currently, several molecular studies using transcriptomic and genomic approaches suggested the existence of four molecular subtypes, increasing the complexity and knowledge of medulloblastoma biology. Despite these significant advances, the molecular basis of medulloblastomas is not fully

understood. MicroRNAs (miRNAs) are a group of small non-protein coding RNA molecules that target genes by inducing mRNA degradation or translational repression. They represent an evolutionary conserved mechanism that controls fundamental cellular processes, such as development, differentiation, metabolism, proliferation and apoptosis. Aberrant expression of miRNAs correlates with various cancers. This altered expression can arise from mutation, methylation, deletion, and gain of miRNA-encoding regions. We here review the knowledge of miRNAs in medulloblastomas. The expression patterns of miRNAs in medulloblastomas were comprehensively evaluated and their diagnostic, prognostic and therapeutic biomarker role assessed. miRNAs are important players in medulloblastoma tumorigenesis and their therapeutic exploitation can constitute an alternative approach to this devastating disease. Keywords: miRNAs, medulloblastoma, children, diagnostic, prognostic.

INTRODUCTION

Childhood cancer is relatively uncommon, however approximately 15, 000 children (<19 years old) are diagnosed with cancer each year and[American Cancer Society, 2009 #6] it remains the leading cause of disease-related mortality in children [1]. Central Nervous System (CNS) tumors, primarily occurring in the brain, are the second most frequent malignancy (first is leukemia) and is the most common solid tumor of the childhood, representing at least 30% of all cancers in children and adolescents in the United States [2]. In Brazil, it is the third most common tumor in children [3]. Among childhood CNS tumors, medulloblastoma (MB) is the most frequent

representing ~20% of all brain tumors in children [4]. Medulloblastoma is a highly aggressive tumor that arises from altered (mutated) remaining primitive neuroectoderm cells in the ventricle and grows in the cerebellar vermis, often invading through the ependyma and brainstem [5]. Around 30% of children present evidence of disseminated disease at diagnosis [6]. The classical neurological symptoms in children are associated with an increasing in intracranial pressure and include nausea, headache, vomit, irritability and ataxia [5]. According to the WHO (World Health Organization) classification, medulloblastomas are grade IV malignancies and can be divided into five subtypes: classical, anaplastic, large cell, nodular desmoplastic and medulloblastoma with extensive nodularity [7]. The choice of treatment in medulloblastoma is based on the risk stratification (high risk or standard risk) of the disease, and the current treatment modalities range from surgery, craniospinal radiotherapy and standard chemotherapy treatment [8]. The risk stratification is established according to the possibility of tumor resection, presence of metastasis and histology. The overall five-year survival stands around 75%, ranging from 55% to 80% in high risk and standard risk patients, respectively [9]. Despite the diversity of therapeutic options, the major concern about the treatment is that it lacks specificity, often resulting in long late-effects that include neurocognitive impairment, neuropathy, endocrinal alterations, impaired bone growth and impaired motor function, hearing loss as well as secondary tumors [8]. The majority of medulloblastomas is not associated with a predisposing genetic cause, however its occurrence may be associated with inherited disorders such as Li-Fraumeni syndrome (TP53 mutations), Turcot syndrome (APC

mutations) (Hottinger and Khakoo, 2009) or Gorlin syndrome (PTCH1, PTCH2 and SUFU mutations) [11, 12]. Recently, a collaborative effort involving groups that performed strategies combining high throughput screening (genomics and transcriptomics) has resulted in a consensus that medulloblastomas can be grouped into four distinct categories (WNT/β-catenin, Sonic Hedgehog (SHH), group 3 and group 4) according to unique molecular characteristics [13-17]. In this way, it is possible to establish risk stratification for patients with medulloblastoma based on the molecular features of the tumor. Despite the great progress made in the understanding of medulloblastoma biology, it is still a heterogeneous disease with a different molecular behavior. Therefore, more efforts needed to be driven to define the most relevant prognostic stratification factors and also to identify biological alterations that could be targets for molecular specific therapies contributing to the decreasing of deleterious and long-term effects of the current treatment strategies. Emerging in this scenario is the increasing number of small RNA molecules, particularly microRNAs (miRNAs) and the established evidence regarding the key roles of these molecules in human diseases, mainly cancer. Despite the fact that miRNAs are involved in the tumorigenesis of a range of different tumors [18], the knowledge about the prognostic, diagnostic and/or therapeutic target potential of these molecules in brain cancer, especially medulloblastomas, is still in its beginning.

microRNAs

Biogenesis and Function

miRNAs are short (19 to 25 nucleotides) and evolutionary conserved RNA structures that can bind to the messenger RNA (mRNA) of protein coding genes [19]. Usually, miRNAs bind within the 3’ untranslated region (UTR) of the genes and they are responsible for gene expression silencing by repressing translation or directing the sequence-specific degradation of target mRNAs [20]. These small RNAs are encoded within the genome and are initially transcribed as primary transcripts that can be several kilobases (kb) in length. The primary transcripts are initially cleaved by a RNase III enzyme called Drosha in the nucleus resulting in a precursor miRNAs (pre-miRNAs) with ~70 nucleotides (nt). The pre-miRNAs are quickly transported to the cytoplasm through the action of exportin-5 (Exp5), a nuclear exportation protein that makes use of Ran-GTP as a cofactor. Once in the cytoplasm the pre-miRNA is cleaved again by another RNase III enzyme called Dicer, that produces a mature miRNAs with 22 nt. Finally, the mature miRNAs are incorporated to the RNA-induced silencing complex (RISC) and can regulate the gene expression post-transcriptionally by pairing with their target mRNA, leading to the inhibition of translation or degradation of target mRNA (Figure 1) [21]. In general, to exert its function the perfect biding of miRNA is only necessary at the seed region (2-8 nt in the 5’ region of miRNA) often found in the 3’ UTR of the target mRNA. Thus, their small size and their combined capacity to the imperfect matching recognition site allow miRNAs to regulate an excessive number of mRNAs. It is estimated that a single miRNA might be involved in the regulation of more than 200 different mRNA.

Even though, they represent less than 2% of human genes, miRNAs are responsible for the regulation of the expression of ~ 30% of the human genes [22]. Although, we are at the beginning of understanding the biology of miRNAs and their action mechanism, a growing number of studies have revealed the importance of these small RNAs in diverse biological processes. Furthermore, through the global regulation of gene expression of cells associated with different functions, it is reasonable that miRNAs can be involved in the progression of several diseases, including cancer [18, 23, 24].

miRNAs in cancer

Recently, miRNAs have been proposed to be involved in the pathogenesis of cancer and altered miRNA expression profiles are associated with prognosis in several human cancers [24, 25]. The expression of miRNAs is deregulated in human cancer and some of them are consistently up or downregulated in more than one type of malignancies. miRNA expression profiles in human solid and hematologic malignancies have shown their potential value as tumor markers in cancer patient management [26]. The miRNAs may have a dual role in cancer and can act as oncogenes (oncomRis -upregulated) or tumor suppressor genes (downregulated) [27]. The first known reporting of deregulated miRNAs in cancer showed a deletion of miR-15a and miR-16-1 in chronic lymphocytic leukemia (CLL), which suggested their role as tumor suppressor genes in CLL [28]. Members of the let-7 miRNA family also act as tumor suppressor genes and are frequently downregulated in human cancer, leading to the upregulation of several proto-oncogenes [29]. In addition, increased expression of miRNAs may also act as oncogenes (oncomiRs), as

the example of the miR-17~92 cluster that is overexpressed in several tumors [30]. The deregulation of miRNAs expression is the main mechanism of loss or gain of function of these molecules in cancer cells. In fact, the activation of oncogenic transcription factors, such as MYC, represents an important mechanism for altered miRNA expression [31]. These data strongly suggest that miRNAs play an important role in human cancer. The mechanisms underlying miRNA gene deregulation in cancer are not well understood. However, the expression of miRNAs can be modulated in response to various stimuli as oncogene activation or DNA damage [32]. Considering that more than one-half of the miRNAs have been aligned to genomic fragile sites or chromosomal regions associated with cancers [33], genome copy number aberrations (CNA) is considered one of the major causes of miRNA deregulation in tumors [34].

microRNAs IN MEDULLOBLASTOMAS

Despite the importance of miRNAs in tumorigenesis [35] just recently these molecules have been involved in the medulloblastoma pathogenesis (Table I). In the next section we will address the knowledge of clinical and functional impact of miRNAs deregulation in medulloblastomas.

Diagnostic and prognostic biomarkers

The first report describing the involvement of a miRNA in medulloblastomas showed that miR-124, an enriched miRNA in normal brain, presented decreased expression in MB samples. miR-124 modulates cell cycle regulation by targeting CDK6, a well known adverse prognostic marker in MB [36]. The low expression of miR-124 in MB was corroborated in a subsequent

report and it was suggested as a regulator of cell glycolysis (bioenergetics hallmark of cancer) by targeting SCL16A1 (solute carrier family) [37]. In a series of 61 MB samples, miR-199b-5p was described as upregulated in non-metastatic cases and its high expression showed an association with a better overall survival [38]. A well known miRNA associated with metastasis in several tumors, miR-21 is upregulated in MB samples. It was observed that miR-21 regulates the expression of the metastasis suppressor PDCD4 and lack of this protein is essential for metastatic dissemination in MB [39]. In a high throughput approach, MB of the SHH subgroup (increased GLI1 expression) was associated with a notably downregulation of 28 miRNAs, suggesting a tumor suppressor function acting through the regulation of the SHH pathway. In fact, miR-125b and miR-326 were identified as regulators of SMO (smoothened) and miR-324-5p of GLI1, that are key activator components of the SHH pathway. In the same work, miR-214 was demonstrated with an increased expression in SHH subgroup tumors and its oncogenic function could be associated with the regulation of SUFU, a SHH pathway inhibitor [40]. Mutations or loss of function of SUFU are associated with medulloblastoma development [12]. In a second approach, the same authors reported the expression of 248 miRNAs in a representative collection of MB histopathological variants with different clinical features and molecular characteristics. Consequently, the authors focused in 86 miRNAs already reported as expressed in neuronal tissues and/or associated with tumor (oncomiRs). They observed that the majority of the miRNAs were downregulated in MB samples [41]. The expression of only four miRNAs (miR-let7g, miR-19a, miR-106b and miR-191) allowed the distinction of MB

histotypes (classic, anaplastic and desmoplastic). Also, the differential expression of a group of six miRNAs (miR-10b, miR-135a, miR-135b, miR-125b, miR-153, miR-199b) was associated with tumors overexpressing ERBB2 and a group of three (miR-181b, miR-128a, miR-128b) with tumors overexpressing MYC. Furthermore, miR-31 and miR-153 were differentially expressed among high risk and standard risk MBs and they were suggested as poor prognostic markers of the disease [41]. A recent report demonstrated the increased expression of miR-182 and miR-183 in association with metastatic non-SHH MB subgroups [42]. A strategy using deep sequencing of MB mouse models revealed the increased expression of 26 miRNAs and decreased expression of 24 miRNAs in MB tissue compared to normal brain [43]. In the group of downregulated miRNAs were included miR-124a and miR-128, previously identified by others [36, 37, 41]. Interestingly, among the upregulated miRNAs were nine members of the miR-17~92 cluster, a group of miRNAs implicated as oncogenes in several tumors [44]. The expression of three of these miRNAs (miR-19a, miR-20 and miR-92) was associated with the SHH subgroup in MB human samples [43]. These results were also corroborated in another report using microarray that identified miR-18a, miR-19b, miR-20a and the paralogous miR-106a (miR-106a/363 cluster) as upregulated in activated SHH pathway MB samples. In this work, it was shown that the overexpression of these miRNAs occurred as a result of an amplification on chromosome 13q31. 3 (miR-17~92 cluster regions) in a subset of tumors. Moreover, the high expression of these miRNAs was associated with NMYC amplification in these samples [45]. Novel recurrent chromosome 8q24. 22–q24. 23 amplification was also described in

MB and it was independent of MYC amplification (8q24. 1). In this chromosome region, spanning 3 megabases (Mb), are mapped two miRNAs (miR-30b and miR-30d) that presented higher expression in 54% and 12% of the MB samples compared to normal cerebellum, respectively. The expression of these miRNAs was strictly related to the presence of the chromosome amplification [46]. A miRNA profiling that evaluated 365 miRNAs was capable to identify the four distinct molecular subgroups of MBs. Additionally, the authors identified a specific and robust miRNA signature (16 miRNAs) associated with WNT subgroup and mutations of CTNNB1. Overexpression of miR-23b, miR-148a, miR-182, miR-193a, miR-224 and miR-365 was validated in WNT subgroup of MBs. The authors suggested that the expression level of these potential tumor/metastasis suppressive miRNAs is likely to determine the response to treatment and would be an important biomarker for risk stratification in a WNT subgroup [47]. A similar analysis using microarrays revealed a miRNA signature based on the differential expression of 9 miRNAs (4 upregulated and 5 downregulated) that distinguish MB samples from normal cerebellum specimens. Interestingly, four of these miRNAs (miR-17, miR-100, miR-106b and miR-218) were not reported as differentially expressed in previous studies regarding MBs [48]. Since the development of high throughput techniques, the first reports regarding integrative genomic analysis in medulloblastomas have begun to be published with very promising results. Recently, the expression of 663 miRNAs was evaluated in MB primary samples, MB cell lines, neural stem cells (CD133+) and neural progenitor cells (CD133-). This analysis revealed 33 differentially expressed (21 upregulated and 12 downregulated) miRNAs

in MB primary samples compared to CD133+ cells. An integrative and functional analysis of negatively-correlated predicted targets of both up and downregulated miRNAs revealed 106 altered significant pathways in MB. Enrichment was observed in pathways regulating neuronal migration, nervous system development and cell proliferation [49]. Based on gene expression, other report proposed that MB presents six molecular subgroups. The authors identified specific miRNA profiles that were correlated with the mRNA signatures. Integrating mRNA expression, miRNA expression and gene copy number analysis they showed that a specific group of MB characterized genetically by MYC copy number gains and transcriptionally by the enrichment of photoreceptor pathways and increased expression of miR-183~96~182 cluster is associated with a poor prognosis [13]. miRNA profiling has also been used to evaluate differences among all pediatric CNS tumors. Actually, an effort was driven in the analysis of 470 miRNAs in a variety of pediatric CNS malignancies including atypical teratoid/rhabdoid tumor (AT/RT), glioblastoma (GBM), MBs, ependymoma (EPN) and pilocytic astrocytoma (PA). Three miRNAs (miR-25, miR-129 and miR-142-5p) were found to be differentially expressed in every tumor type when compared to the normal controls. The analysis also revealed significant differences in miRNA expression between the cancer groups and allowed the assignment of specific miRNA signatures to each CNS tumor type. Performing unsupervised hierarchical clustering analysis using miRNA expression of the 20% most variable miRNAs demonstrated that pediatric tumor samples formed tighter diagnostic groups, with the exception of GBMs that presented a widespread clustering [50]. The same clustering pattern was observed in a subsequent

report using a different technical approach, which was interesting regarding the heterogeneity of brain tumors. These results could be explained by the greater degree of heterogeneity of GBMs, the most aggressive and non-responsive pediatric brain tumor [51]. Recently, in a different approach, miRNAs sequences were assayed for base pair deletions, amplifications and mutations. The authors evaluated nine specific miRNA (miR-33b, miR-135a-1, miR-135a-2, miR-135b, miR-186, miR-200b, miR-512-2, miR-548d-1 and miR-548d-2) that were selected on the basis of the presence of potential target sequences within the 3′ UTR of the MYC mRNA. At least 73% (35/48) of the analyzed MB samples presented an alteration in one of the nine miRNAs, indicating an alternative mechanism of MYC overexpression in MB [52]. The data presented so far, regarding pediatric medulloblastoma, indicate that miRNA profiling could be an important tool to support risk stratification and molecular classification of MBs. Meanwhile, due to the high heterogeneity of this specific group of tumors, new efforts with greater cohorts and/or the use of new technologies (especially deep sequencing), which allow a deeper coverage of miRNAs, will be crucial to address this issue in the future.

Potential therapeutic targets: miRNA functional role in medulloblastomas

Current evidence demonstrates the involvement of miRNAs in the pathogenesis of MB. Under this circumstances, the modulation of this RNA molecule points to the possibility of the use of new therapeutic strategies for MB treatment. Indeed, several groups around the world evaluated the ectopic modulation of miRNAs in MB. Experimentally up or downregulated miRNAs and its effects in MB are shown in Table II. The first evidence was

demonstrated for the re-expression of miR-124 in MB cell lines. Transient expression of a synthetic miRNA that mimics miR-124 function resulted in a reduction in MB cell proliferation [36, 37], not mediated by cell apoptosis [36]. The re-expression of miR-124 was associated with downregulation of CDK6 [36] and SLC16A1 [37], which could explain the cell growth impairment. Ectopic expression of miR-129 was proposed to regulate CDK6 resulting in cell growth arrest by the same mechanism [53]. The re-expression of miR-199b-5p resulted in diminished MB tumor growth in vitro and in vivo. miR-199b-5p restoration also impairs the engrafting potential of MB cell lines by decreasing the stem-cell-like (CD133+) subpopulation of MB cells, as a consequence of downregulation of HES1, a key effector of Notch signaling pathway [38]. The decrease in cell proliferation was also achieved when miR-125b, miR-324-5p and miR-326 were ectopically expressed in vitro, by the targeting of SHH signaling pathway. The same molecular regulatory circuitry could be observed in the development of cerebellar neural progenitors, in which the miRNAs expression increases through differentiation steps thereby allowing cell maturation and growth inhibition [40]. In another report, the same authors demonstrated that the rescue of expression of miR-9 and miR-125 in MB cell lines, using retinoic acid treatment, was associated with cell growth arrest and apoptosis and with a decrease in cell proliferation while targeting the truncated isoform of the neurotropin receptor TrkC (t-TrkC) [41], a deregulated mechanism already described for neuroblastoma [54]. Enforced expression of the entire miR-17~92 cluster in a MB mouse model led to an early tumor development in the animals. miR-19a and miR-92 were expressed at elevated levels in the

tumors and expression analysis revealed the loss of expression of the wild type Ptch1 allele, a bona fide tumor suppressor gene. Also, the tumors presented increased levels of GLI1 indicating the constitutive activation of SHH signaling pathway. Tumors expressing this miRNA cluster were sensitive to cyclopamine, which decreased cell proliferation in addition to the downregulation of GLI1 [43]. Other work demonstrated that the higher expression of miR-17~92 cluster was driven by NMYC overexpression, which acts in synergy for the increased proliferation in a SHH subgroup of MB tumor cells. This way, the authors suggested that miR-17~92 can provide a selective growth advantage to MB cells [45]. Exogenous overexpression of miR-193 and miR-224, the most upregulated miRNAs in WNT subgroups, was found to inhibit cell proliferation, anchorage-independent growth and increases radiation sensitivity in MB cell lines. These characteristics refer to the better response to treatment and excellent prognosis in this subgroup of MB tumors [47]. Reintroduction of miR-128a in MB cell lines was associated with decreased cell growth through the downregulation of Bmi-1 and induced cell senescence by the upregulation of reactive oxygen species (ROS) levels [55]. miR-34a impairs cell proliferation, invasion and survival by targeting c-MET (MB and gliomas) and Notch-1, Notch-2, CDK6 (in glioma) [56, 57]. Recently, miR-34a expression was shown to induce apoptosis, senescence and chemotherapy sensitivity of MB cell lines thought a positive feedback mechanism promoting MAGE-A downregulation with concomitant increasing of the expression of p53 and its targets (p21/WAF1/CIP1) [58]. Also, miR-34a expression was related to cell proliferation inhibition, induction of apoptosis and differentiation in MB cell lines by the regulation of Dll1, an important

effector protein of the Notch signaling pathway [59]. Because most of the studied miRNAs are downregulated in medulloblastomas, the vast majority of the reports evaluated the re-expression of these miRNAs in MB cells. However, there are few examples in which inhibition of miRNA resulted in changes of biological processes in MB cells. So, inhibition of miR-21 impaired the cell ability of migration and invasion in MB cell lines due to the induction of the expression of PDCD4, which negatively regulated µPAR and integrin proteins (essential for cell invasion) and positively regulated E-cadherin and TIMP-2 proteins (cell migration control) [39]. Knockdown of miR-182 and miR-183 was related with decreased cell migration in MB cell lines. In xenograft experiments, MB cells overexpressing miR-182 were able to invade normal tissue and capable to metastasize to the leptomeninges, this way representing a very aggressive MB phenotype [42]. So far, several efforts reported the importance and impact of miRNA modulation in a series of cellular biological processes. These studies are of great value to clarify the biology of medulloblastomas and more important to define novel targets for therapeutic development in future approaches.

CONCLUSION

The development of high throughput methodologies and analysis of all biological molecules is leading to a wider and deeper understanding of their involvement and cross-talk in several human diseases. We here highlighted the advances and evidences showing miRNAs as a key molecular effector in medulloblastoma pathogenesis and the potential use of these molecules as biomarkers of diagnosis, prognosis and therapeutics. Despite the new insight

and enthusiasm in medulloblastoma regarding miRNAs, the regulation of these molecules as a therapeutic approach is still in its infancy and caution most be taken during their design. Even more, once the majority of these findings were observed in in vitro studies, more research is needed in vivo in MB models and in extensive pre clinical approaches to clarify the key role of miRNAs in the pathogenesis of MB and to establish its safety and real importance as a therapeutic target for future interventions. Moreover, miRNAs present the characteristic to regulate hundreds of mRNAs, thereby the identification of the targets involved in the deregulated miRNA-mRNA network will be of pivotal importance to elucidate novel molecular pathways that could also be used for new therapeutic approaches in MB. A great challenge relies on the ability of the researchers in bringing the findings observed in large scale studies to routine diagnostic. It is responsibility of these professionals to identify a concise and smaller panel of relevant miRNAs that could be evaluated by routine techniques for a faster assessment of the related MB subtype resulting in a better management of the treatment for the patients. Concluding, miRNAs are major regulators of mRNA expression levels and are frequently deregulated in medulloblastomas. Its characterization, biological and clinical impact in medulloblastomas is far from complete, but their unraveling is crucial in order to identify novel clinical biomarkers and development of new and more effective future therapeutic options bringing hope for the patients affected by this dismal disease.

Declaration of interest statement

The authors have no conflict of interest to disclose. The authors alone are responsible for the content and writing of the paper.