Abstract

**Purpose:** To provide a critical assessment of the clinical translational applications of microRNA (miRNA) genes in medulloblastomas.

**Methods:** Data were obtained from MEDLINE using Boolean-formatted keyword queries. Top articles were selected for critical analyses - depending on the novelty of findings, qualitative assessment of the citation index and relevance to the diagnosis, prognosis and therapeutic targeting of medulloblastomas.

**Results:** MiRNAs, non-protein-coding RNA molecules, negatively regulate gene expression in a sequence-specific manner during biological processes. In the past few years, miRNA genes have emerged as key regulators of not only molecular events involved in normal brain development and function but also in the molecular pathogenesis of medulloblastomas. In this manner, microRNA genes are identified with functional roles as oncogenes and tumor suppressor genes. At least four miRNAs have proven useful in improving the molecular classification of medulloblastomas, and eight others have shown potential in predicting patients’ overall prognosis. Moreover, more than 10 miRNA genes can be potentially utilized in therapies against medulloblastomas, using nine recent methods of targeting miRNAs.

**Conclusion:** The quest to identify miRNA genes that are of biological significance in medulloblastomas is on an ongoing venture. Most importantly, these miRNAs have been shown to be of clinical importance for improving the accuracy of diagnosis and prognosis and even developing therapies that can significantly improve patients’ overall survival from this deadly disease.

According to the World Health Organization, medulloblastomas are the most frequent and aggressive infratentorial primitive neuroectodermal tumors. These tumors are genetically and epigenetically heterogeneous, with ongoing world-wide research aimed at defining precisely the inherent cellular and molecular mechanisms regulating tumor growth and recurrence. Unfortunately, despite current treatment modalities (combined surgery, radiation and chemotherapy) outcomes are poor, which justifies the need to better understand the biology of these tumors and to explore alternative therapeutic strategies. These tumors are most common among children, representing up to 30% of diagnosed primary brain tumor pediatric cases. In adults with primary brain tumors, less than 2% account for medulloblastomas and the incidence tends to be higher in males in comparison with fe-
Approximately 40% of cases are diagnosed in children younger than five years of age and these cases tend to be therapeutically challenging and correlate with the poorest prognosis. In addition, about 30% of cases are diagnosed between the ages of five and nine, while the majority of remaining cases are diagnosed between 10 and 19 years of age. The majority of neurological symptoms among the children are associated with an increase of intracranial pressure in the fourth ventricle, and with progressive worsening symptoms when the increase in tumor size begins to affect other major cranial nerve functions.

Medulloblastomas originate from mutations in cells of the cerebellar neural precursors as an invasive embryonal tumor. They arise from the remnants of the primitive neuroectoderm in the roof of the fourth ventricle and grow in the cerebellar vermis, filling this ventricle and often invading through the ependyma to enter the brainstem. The World Health Organization has established four histologic variants of medulloblastomas: classic medulloblastomas, desmoplastic medulloblastomas, medulloblastomas with extensive nodularity and large cell medulloblastomas. Patients with desmoplastic medulloblastomas have a better survival, following current treatment modalities. Medulloblastomas can also be classified in two groups according to risk-adapted treatments: high-risk and average risk groups. Karyotypic abnormality and the dysregulation of several prominent signaling pathways contribute to the cause of these medulloblastomas, which may also be caused by mutations in miRNA genes. Consequently, high prevalence of iso-chromosome 17q and the loss of heterozygosity on chromosome 9q are evident in ~50% of medulloblastomas. In addition, evidence suggests that aberrances in Sonic Hedgehog (SHH) signaling, which play an essential role in cerebellar development, can promote medulloblastoma tumorigenesis. The oncogenic expression of SHH and tumor suppressive role of the SHH receptor, namely Patched (PTC), can synergistically promote medulloblastomas. Furthermore, mutations in SUFU, which encodes a negative regulator of Sonic Hedgehog and Wnt signaling, can synergistically induce the growth of medulloblastomas.

Aberrant Growth Factor signaling also plays essential roles in medulloblastoma tumorigenesis. Mutations or aberrant activities of growth factor receptors such as ERBB-2, PDGFA and IGFR1, and their downstream effectors such as Ras/mitogen-activated protein kinase, and c-Myc contribute to medulloblastoma tumorigenesis. Among these effectors, amplifications of c-Myc on chromosome 6q, are associated with very poor prognosis. Aberrancies in pRb and p53 signaling pathways, which regulate the cell cycle, can likewise induce the growth of medulloblastomas.

MicroRNAs (miRNAs) are groups of gene regulators consisting of ~22 nucleotides of non-coding single stranded RNA molecules. miRNAs bind to their target mRNA and repress RNA translation or facilitates RNA degradation. Since their discovery in C. elegans in 1993, miRNAs have captured the attention of the world-wide research community with >700 miRNA genes identified in humans, and with each molecule having the potential to regulate the expression of >200 genes. Unfortunately, much research is still required to understand the precise roles in conjunction with the diagnostic and therapeutic applications of these genes. Despite this, in recent years miRNAs have been emerging as pivotal regulators of biological processes in both normal and neoplastic tissues. In fact, oncogenic and tumor suppressive roles of miRNA genes are identified in medulloblastomas, leading to the possibility that these genes can be exploited to develop novel therapies to combat this disease. Furthermore, direct therapeutic targeting can be enhanced or even used in predicting patients’ survival following conventional treatments.

In this article, we report current findings on key miRNA genes identified in medulloblastomas, with a focus on those that are of importance for use as informative functional biomarkers, as potential novel tumor pathological/classification biomarkers, and for use in potentially predicting patients’ overall survival. We also discuss the potential for some of these miRNAs
for therapeutic targeting to induce tumor regression and with novel strategies of how these miRNAs can be delivered or targeted in neoplastic cells for prospective treatments.

Methods

Data were archived from MEDLINE, using Boolean-formatted queries on the keywords including: medulloblastoma, miRNA, microRNA, prognosis, classification, tumor regression, gene therapy, miRNA therapy, gene delivery, patient survival, cancer, tumor suppressor, oncogene. Only the top articles were selected for critical analyses depending on the qualitative assessment of the citation index, novelty of the findings, and relevance to functional and translational applications of miRNAs in medulloblastomas.

Results

MicroRNA genes and relevance to cancers

miRNA biogenesis and processing are summarized in Figure 1. miRNAs are known to regulate numerous cellular processes including proliferation, differentiation and stress responses in normal cells, and to play oncogenic and tumor suppressive roles in the regula-
tion of cell cycle, proliferation, apoptosis, cell migration and even angiogenesis in cancerous cells. Aberrancies in miRNA expression, therefore, represent a key mechanistic hallmark of cancer. The mechanisms that contribute to abnormal miRNA expression are yet to be defined precisely, but there are a number of possibilities. Firstly, since miRNA genes are located in clusters in the genome, genomic alterations such as chromosomal abnormalities (deletions, duplications, point mutations, etc.) and epigenetic alterations in these regions, are anticipated to induce oncogenic activations or tumor suppressive inactivations in functionally relevant miRNA genes, leading to tumorigenesis. Secondly, perturbations in biological processes that effect the correct processing of miRNAs such as those caused by point mutations or by a loss of the RNase III Drosha, can cause altered miRNA expression leading to predisposed cellular transformation. Thirdly, alterations in the cellular microenvironment can induce changes in miRNA expression leading to transformation. For instance, the miR-21 gene undergoes altered expression while adapting to hypoxia in order to promote the survival of cancerous cells. Collectively, in support of aberrant miRNA gene expression in tumorigenesis, numerous studies have thus far identified both oncogenic and tumor suppressive expression patterns of miRNA genes in a variety of human cancers including: colon, kidney, prostate, bladder, lung, breast and brain. Aberrant miRNA expressions may also play pivotal roles in the proliferation, self-renewal and differentiation of both normal stem cells and cancer stem cells, with such cell types currently speculated as being implicated in the origin and recurrence of tumors. In this manner, miRNAs may regulate the division and self-renewal of normal stem cells by targeting cell cycle regulators. In addition, recent data suggests that miRNAs play a role in driving both processes of normal cell differentiation in non-neoplastic tissues and in the transformation of normal stem cells into cancer-stem cells. Overall, these miRNA expression pattern signatures not only distinguish cancerous tissues/cells from normal tissues/cells but can also serve as biomarkers in understanding and predicting the multistep pathological stages implicated in cancer initiation and progression. In addition, they can be useful as diagnostic biological markers, and even for designing novel therapies. Such clinical applications, however, will require a better understanding of the biology of miRNAs in tumorigenesis from a variety of cancers.

Applications of microRNA genes in medulloblastomas

Recent studies have uncovered several dysregulated miRNAs that contribute to tumorigenesis in medulloblastomas. Listed in Table 1 and illustrated in Figure 2 are generalized functional interactions of some of these prominent miRNA genes in medulloblastomas. Table 1 outlines miRNAs which have been shown to be useful in defining the pathological hallmarks (tumor classification) and even in the prognostic outcome of the patients. Mentioned below are the biological and translational applications of miRNA biomarkers in medulloblastomas.

Use for functional biomarkers

With respect to better understanding miRNAs as functional biomarkers for tumor initiation and progression (Table 1, Figure 2), it is now clear that some miRNAs, for instance, the miR-17-92 gene cluster family, act as proto-oncogenes, with roles in enhancing proliferation and angiogenesis, while others like miR-324-5p, act as tumor suppressors when down-regulated, leading to the progressive events in medulloblastomas. The miR-17-92 cluster, Oncomir-1, which is located on human chromosome 13, is the current most studied miRNA gene family in medulloblastomas, and is significantly over-expressed. Most remarkably, the oncogenic expression of members of the miR-17/92 family, including miR-19a, miR-20 and miR-92, induces elevated Sonic Hedgehog signaling leading to c-Myc oncogenic activation and the modulation of E2F1, in SHH dependent medulloblastomas. In some SHH independent medulloblastomas, the onco-
genic roles of other microRNAs, like miR-30b and miR-30d on chromosome 8, can be apparent and independent of c-Myc amplification. Interestingly, the tumor suppressor genes, FASTK and TOPORS, are targets of miR-17 and of another microRNA, miR-106b. The SHH pathway is also regulated by miR-125b, miR-326 and miR-324-5p; miRNAs that target the Smoothened receptor (SMO). Downregulation of miR-324-5p was identified as a consequence of the loss of chromosome 17p, the most frequent chromosome mutation in medulloblastomas. Other miRNAs that target the SHH pathway, such as miR-214, are up-regulated in medulloblastomas that have high levels of Gli1 expression.

Most remarkable, the oncogenic expression of miR-214 leads to the translational silencing of the SHH ligand transcript, in an autocrine regulatory loop of this seminal pathway. miRNAs regulate diverse neoplastic events through target molecules with aberrant expressions leading to tumor progression in medulloblastomas (Figure 2). Indeed, the expressions of miR-10b, miR-135a/b, miR-125b, miR-153 and miR-199 are up-regulated when the EGFR protein family member, ErbB2, is constitutively active or over-expressed. Likewise, the expressions of miR-128a, miR-128b and miR-181b are significantly induced with the expression of c-Myc. Over-expression of miR-9 and miR-125a is associated with promoting growth arrest. In fact, miR-9 targets the repressor element-1 silencing transcription factor complex, REST/NRSF, which initiates tumor formation, whereas miR-125a targets the neurotropin receptor t-Trk-C, which also contributes to the cause of medulloblastoma. Moreover, miR-124, which is regulated by REST during neural differentiation, plays an important role in medulloblastoma pathogenesis by modulating cell-cycle regulation via CDK6, a well defined prognostic biomarker in medulloblastoma.
invasion of tumor cells by targeting pro-oncogenes such as ROS1, EGFR, Bcl-2, β-catenin and MAPK9.5

Uses for tumor pathological classifications and patients’ prognostic applications

The expression profiles of miRNAs can be used to classify the histological types of medulloblastomas; a characteristic which will undoubtedly be of prognostic and diagnostic relevance (Table 1). For example, decreases in the expressions of miR-31 and miR-153 significantly correlate with High-Risk medulloblastoma patients, in comparison with those in the Average-Risk group.3,5 Likewise, the oncogenic expressions of miRNAs, including let-7g, miR-191, miR-19a and miR-106b, and the miR-17/92 targeted gene, MIRHG1, correlate with the aggressive pathological properties of anaplastic medulloblastomas, and inevitably with poor prognosis among patients.3,5,26 In addition, although no oncogenic RAS mutations have been identified among medulloblastomas, the expression of RAS is regulated by the let-7g miRNA.2 Elevated/oncogenic RAS signaling is also associated with the aggressive properties of medulloblastomas and poor prognosis. Let-7g and miR-106b are differentially expressed in desmoplastic medulloblastomas, while miR-19a is up-regulated in anaplastic anaplastomas, in comparison with the classic medulloblastoma histological grade.3 The expressions of other miRNAs like
miR-9 and miR-92 can accurately distinguish between primary and metastatic brain tumors, with the potential to represent novel biomarkers for identifying primary medulloblastomas. Furthermore, activation of the Notch signaling pathway and its downstream effector HES1 significantly associates with poor clinical outcome among medulloblastoma patients. Since miR-199-5p targets HES1, the loss of this miRNA expression, such as from epigenetic silencing or even standard genetic mutations, can potentially be used as another biomarker for predicting the prognosis of high-risk medulloblastoma cases. Finally, elevated expression of ErbB2 correlates with poor survival among medulloblastoma patients. Of note, the oncogenic expression of ErbB2 downregulates the expressions of miR-10b, miR-135a/b, miR-125b, miR-153 and miR-199 in medulloblastomas, hence providing prospects for these miRNAs to be further considered as additional prognosis biomarkers.

Use for targeted therapies

Current treatment modalities for medulloblastoma patients, especially children up to three years of age, are confounded with poor efficacies and therapeutic complications. Such treatments can be hindered by severe side effects and by treatment resistance of medulloblastoma stem cells within the tumor mass. MiRNA therapy, using synthetic miRNAs and combined with elegant delivery systems, can become a novel improved approach for treating medulloblastoma patients, since miRNAs are biologically significant in influencing the proliferative, anti-apoptotic, pro-angiogenic and even pro-metastatic properties of tumor cells (Table 1, Figure 2). Moreover, some miRNAs are already identified as informative in predicting the sensitivity or efficacy of patients’ tumors to various anticancer (chemo- or radiotherapy) therapies. For instance, Smoothened receptor antagonists, such as KAAD-cyclopamine and SANT1-4, are a new promising class of antitumor agents for the treatment of medulloblastomas. In this context, miRNAs that target the SHH pathway, including the miR-17/92 cluster, miR-125b, miR-326 and miR-324-5p, are of therapeutic targeting importance. Furthermore, miR-100, which targets SUFU and which is down-regulated to induce the maximal activation of Gli in the presence of the SHH ligand, might also be a potential new effective therapeutic target.

The effectiveness of many chemotherapeutic drugs has been correlated with their ability to induce apoptosis. The inhibition of the Notch pathway leads to depletion of medulloblastoma tumor stem cells via induction of apoptosis. Hence, the therapeutic delivery of over-expressed miR-199-5p can be exploited to sensitize these medulloblastoma stem cells, resulting in significant tumor growth regression via the down regulation of Notch signaling. Targeting the Notch signaling pathway with the up-regulation of miR-9 and miR-125a or even with the silencing of miR-21 may also represent novel avenues for chemosensitizing the tumor cells by increasing the propensity for apoptosis. STAT3 activation, alone or concurrently with EGFR expression, also correlates with the histological grade of medulloblastoma and with the sensitivity of these tumors to chemotherapeutic agents, such as DNA-damaging alkylating agents. In this approach, let-7g, which targets STAT3, and miR-218, which targets EGFR, might be good candidates for future miRNA therapeutics via intervening oncogenic pathways mediated by for instance VEGF and EGFR. miR-218 also targets Bcl-2, which is an important biological functional marker for chemo- and radio-response therapies in brain tumors. Collectively, exploitation of the biological roles of miRNAs can be effective in designing improved therapies for medulloblastoma patients.

Potential novel approaches towards miRNA targeted therapies

Given that microRNA genes demonstrate important functional facets in promoting the survival and treatment responses of cancers, efforts need to be focused
on designing effective means of therapeutically delivering miRNA genes into the tumors. Considerations for such therapies must also account for either bypassing or crossing the blood-brain-barrier (BBB). In general, miRNA therapy shares many disadvantages with short interfering RNA (siRNA)-therapy, including delivery limitations, instability, and spurious targeted effects. Furthermore, miRNAs do not freely diffuse into cells, therefore the efficient in vivo delivery of therapeutic oligonucleotides may be a critical factor for the development of successful miRNA-based treatment modalities (Table 2). Two main therapeutic strategies must be considered with miRNA-based therapy. Firstly, tumor suppressor miRNAs can be restored by using miRNA-mimetics. miR-mimetics can bind specifically to the target genes resulting in post-transcriptional repression by emulating an endogenous functional miRNA. Furthermore, these miR-mimetics can be structurally modified to increase the binding and targeting efficiency and, therefore, to become more potent therapeutics. Secondly, oncogenic miRNAs can be inhibited with miRNA antagonists. Anti-miRNA antisense inhibitor oligo-ribonucleotide (AMO) technology has undergone many recent modifications to enhance the efficiency and specificity of miRNA interference. The modified AMO molecules, known as “antagomirs”, are chemically modified, cholesterol-conjugated single-stranded RNA analogues that are complementary to miRNAs. AMOs have been shown to be very effective in stably silencing miRNAs in transgenic mice in vivo. In support of the in vivo stability of AMOs, Scherr and colleagues reported that lentivirus-mediated expression of miRNAs and miRNA-specific antagonists can induce stable gain- and loss-of-function phenotypes for individual miRNAs. The use of Antisense oligolu-
cleotides (ASOs) and nucleic acid enzymes (antagomirzymes) are also valuable tools to specifically knock-down miRNAs in vitro and in vivo, and have been tried in glioma pre-clinical models. "miRNA-sponges", which are as effective as ASOs but do not degrade as rapidly, can also be used in targeting miRNAs within cells. As a means to enhance cellular uptake and lowest toxicity using nanotechnology, anti-miRNA oligonucleotides can be modified and cross-linked to nanoparticles. Alternatively, Cornsten and colleagues revealed that the delivery of LNA-anti-miRNA molecules, such as LNA-anti-mir-21, can similarly and efficiently regress glioma growth. In this manner, Locked nucleic acid (LNA)-anti-miRNAs possess the highest affinity for the complementary target RNA and with very high stability, increased nuclease resistance, and a lack of acute and subchronic toxicities.

Various therapeutic targeting modalities against miRNAs offer unique technical advantages (Table 2). With these methods, the risk of spurious gene targeting is likely to be lower than that associated with the use of artificial RNA interference. Furthermore, it must be noted that each miRNA targets genes of multiple pathways in comparison with a single siRNA that targets only one or more transcript isoforms of a gene. Viral delivery of molecules that targets miRNAs must also be considered. For instance, the use of adenoviruses permits long term transient targeting of miRNA genes, with high specificities and low toxicities; however, adenoviruses are only biased towards transducing dividing cells that express the CAR receptors. As an alternative, Amendola and colleagues developed a lentiviral platform to efficiently, stably and constitutively co-express one or more natural/artificial-miRNA together with a reporter gene to undertake quantitative assessment of the targeted miRNA and even delivered miRNA. One must also consider the ability to cross the blood-brain barrier when delivering miRNAs in brain tumors using a variety of methods. To circumvent this challenge, lipid encapsulation with nucleic acids has been very effective. In addition, receptor-specific pegylated immunoliposomes (PILs) are the recent and promising nontoxic alternatives to therapeutically deliver miRNA targeted genes to the brain via the transvascular route.

Concluding Remarks

miRNA profiling has allowed the distinction between brain tumors and non-neoplastic tissues with the identification of key miRNA genes that have unraveled novel insights into the pathogenesis of medulloblastomas. Since aberrant miRNA expressions contribute to molecular mechanisms underlying tumorigenesis, microRNA genes by themselves have oncogenic and tumor suppressive properties. Investigation of the role of miRNAs in brain tumors has lead to a wide spectrum of alternative translational applications that include redefining the way how brain tumors are classified or pathologically diagnosed, how patients’ overall survival is predicted based on administering current adjuvant therapies, and how these genes can be utilized to target therapeutics for restricting brain tumor growth. Combinations of these translational applications with miRNAs are anticipated to be highly effective towards improving the well-being of patients affected with these deadly brain tumors.

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