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Review

MicroRNA and pediatric tumors: Future perspectives

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ABSTRACT

A better understanding of pediatric tumor biology is needed to allow the development of less toxic and more efficient therapies, as well as to provide novel reliable biomarkers for diagnosis and risk stratification. The emerging role of microRNAs in controlling key pathways implicated in tumorigenesis makes their use in diagnostics a powerful novel tool for the early detection, risk assessment and prognosis, as well as for the development of innovative anticancer therapies. This perspective would be more urgent for the clinical management of pediatric cancer. In this review, we focus on the involvement of microRNAs in the biology of the main childhood tumors, describe their clinical significance and discuss their potential use as novel therapeutic tools and targets.

Introduction

The current challenges in the field of childhood cancer diseases include the identification of novel biomarkers that may allow non-invasive diagnosis, risk stratification and follow-up. Obviously, important advancements are also expected in pediatric cancer therapy. The survival rate of pediatric cancer patients has progressively improved over the last years as a result of the increased efficacy of diagnostic and therapeutic approaches. However, the tumor diagnosis is still based on symptoms, which may become evident at too late stages. One of the most promising approach involves the measurement as well as the functional characterization and targeting of microRNAs (miRNAs). Together with other molecular mechanisms, abnormal miRNAs expression could play a significant role at earlier stages in tumorigenesis. MiRNAs are short, non-protein coding RNAs acting through post-transcriptional regulation of mRNA. Several studies have so far demonstrated that miRNAs may behave as either oncogenes or tumor suppressors, depending on the specific cells and the target genes they modulate. Therefore, the detection of specific miRNAs in the tumor tissue and, more hopefully, in the serum would be a powerful diagnostic tool for pre-symptomatic cancer detection. Moreover, the elucidation of their specific function in tumorigenesis would provide a potential new therapeutic approach, as it may be possible to control miRNA expression by means of the delivery of synthetic pre-miRNA or antisense oligonucleotides. Although miRNA targeting has not yet been included in clinical trials, recent *in vitro* and *in vivo* studies have proven that this approach could be effective in combination with conventional therapy. It is also likely that tumors are characterized by the synthesis and possible release of specific miRNAs, which would be detectable as an aberrant serum profile compared with levels measured in normal subjects.

Here, we would like to review the state of the art about the involvement of miRNAs in the biology of pediatric tumors. Among the main childhood cancers, we have taken into account some of the principal brain tumors: medulloblastoma (MB), atypical teratoid/rhabdoid tumor (AT/RT) and neuroblastoma (NB), as well as some of the main pediatric blood malignancies: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and Burkitt's lymphoma (BL). Moreover, we have reviewed the role of miRNAs in the two pediatric sarcomas: rhabdomyosarcoma (RMS) and osteosarcoma (OS). We have summarized the differential expression of specific miRNAs in the tumor tissue as well as their possible use as cancer biomarkers and/or as therapeutic targets.

Definition of miRNAs

Earlier studies on this small (20–24 nucleotides) RNAs suggested that miRNAs are able to repress translation without affecting their

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AT/RT, atypical teratoid/rhabdod tumors; BL, Burkitt lymphoma; MB, medulloblastoma; NB, neuroblastoma; OS, osteosarcoma; RMS, rhabdomyosarcoma.

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target mRNAs abundance (Lee et al., 1993; Wightman et al., 1993). It has been later demonstrated that they can also induce significant mRNA degradation, and that both pathways, globally contribute to the negative regulation of target protein function. (Baek et al., 2008; Bagga et al., 2005; Eulalio et al., 2007; Selbach et al., 2008).

MicroRNAs have been identified in 1993 in the nematode *Caenorhabditis elegans* (Lee et al., 1993). In their work, researchers reported that the lin-4 gene does not encode for a protein product, but instead produces a 61 nucleotides RNA precursor that is subsequently processed into a more abundant 22 nucleotides mature form. Sequence analysis of these genes showed that the short lin-4 transcript contain a nucleotide region that is complementary to a repeated sequence element in the 3′ untranslated region (UTR) of lin-14 mRNA. These finding suggested a model of RNA interference controlled by Watson–Crick base pairing. Later in the 1993, it has been demonstrated that the down-regulation of lin-14 exerted by lin-4 occurs at post-transcriptional level (Wightman et al., 1993) and that only the 3′UTR is required for miRNA-mRNA binding. Then, a number of ortholog genes started to be identified in Drosophila (Hutvágner et al., 2001), plants (Reinhart et al., 2002), vertebrates (Lagos-Quintana et al., 2001) and also in virus (Pfeffer et al., 2004). Interestingly, it has been suggested that viral miRNA could modulate cell growth and differentiation to promote cell infection and virus survival (Laganà et al., 2010), and that a consequence of this interference may be the promotion of tumor insurgence as a side effect of apoptosis inhibition (Carl et al., 2013).

Since the first evidence of a miRNA based gene regulation in the worm, 28645 miRNAs has been described so far (Kozomara and Griffiths-Jones, 2014) with 2588 mature miRNAs reported for H. sapiens in the last release of the miRbase database.

MiRNA genes are transcribed by RNA Polymerase II (or more rarely by RNA Polymerase III) producing long (up to thousands nucleotides) precursors called primary miRNA (pri-miRNA) (Fig. 1). After their synthesis, these precursors are subjected to a maturation process that takes place in both nucleus and cytoplasm. Pri-miRNAs, that are usually several kilobases long, are enzymatically processed into smaller (70–80 nucleotides) molecules called pre-miRNAs. These shorter RNAs form hairpin structure due to reverse complementary regions in their sequence. Pri-miRNA cleavage is performed by the microprocessor, a multicomponent complex that comprises the Drosha and the DiGeorge syndrome critical region gene (DGCR8) enzymes (Quick-Cleveland et al., 2014). DGCR8 acts stabilizing the pri-miRNA while Drosha, exerts the specific endonucleolitic cut based on the tertiary structure of pri-miRNAs (Lee et al., 2003; Zeng et al., 2005) (Fig. 1).

Pre-miRNAs are then exported to the cytoplasm by the exportin-5, which uses nuclear Ran-GTP (Bohnsack et al., 2004; Yi et al., 2003). These findings suggest that pre-miRNA might be relatively unstable and can be stabilized through exportin-5 binding.

In the cytoplasm, the pre-miRNA is further processed by the RNAse III enzyme Dicer (Fig. 1), which recognizes the terminal loop and cleaves the molecules releasing a small duplex RNA (Ketting et al., 2001). Then, only one of its strands is eventually loaded into the RNA-Induced Silencing Complex (RISC), which is the functional effector of post-transcriptional silencing (Fig. 1).

Many studies revealed that the 5′ end of miRNAs plays a critical role in target recognition and deeply influence the level of post-transcriptional repression (Doench and Sharp, 2004; Grimson et al., 2007). This 6–8 nucleotides long end is defined as the “seed” of the miRNA. MiRNA-mRNA interaction is specific and controlled by sequence complementarity: the miRNA seed region binds to its corresponding sequence on target mRNA often forming an almost perfect duplex. Despite few controversial evidences, the seed complementarity is currently considered as the major molecular mechanism for miRNA target recognition in cell (Baek et al., 2008; Selbach et al., 2008). This recognition system allows a single miRNA to control multiple targets and many mRNAs to be regulated simultaneously by more than one miRNA.

Together, these findings indicate that miRNAs mediated regulation involves an highly coordinated and diffused set of processes that is estimated to control more than one third of human genes and almost all cellular processes.

Role of miRNAs in the human cancer

An increasing number of evidences suggest that miRNAs aberrant functioning is among the most important causes of cancer. It has been estimated that about 50% of miRNA genes in human genome are located in fragile sites or cancer-associated genomic regions (Calin et al., 2002). This supports their role as key drivers of neoplastic transformation that can occur as consequence of miRNAs deregulation. This can be caused by several mechanisms including mutation, deletion, amplification or loss transcriptional regulation. Different authors reported also that mutations in miRNA binding sites in target mRNA may promote cancer initiation caused by the loss of post transcriptional control (Mayr et al., 2007; Veronese et al., 2011).

During the last decades, the use of high throughput technologies like oligonucleotide miRNA microarrays and NGS, allowed the analysis of the entire known miRNA expression layer in a wide range of neoplastic diseases and altered miRNA expression has been reported in almost all types of cancer investigated (Li et al., 2009a,b).

The first evidence suggesting a causal role of miRNAs in cancer has been reported in 2002 for chronic lymphocytic leukemia, where the genomic region producing miR-15/miR-16 cluster is frequently deleted or down-regulated in cancer cells (Calin et al., 2002). It was later demonstrated that miR-15 and miR-16 are fundamental regulators of apoptosis by negatively regulating the anti-apoptotic BCL2 mRNA (Cimmino et al., 2005). The first *in vivo* evidence supporting miRNA contribution in tumor development was published in 2005, when researchers artificially overexpressed the miR-17/miR-22 cluster, which is upregulated in human lymphoma, thus promoting lymphomagenesis in a rodent B-cell lymphoma model (He et al., 2005; Pagliuca et al., 2013).

As metastasis is the principal cause of death in cancer patients, the involvement of miRNAs in metastasis development has been frequently investigated. The metastatic process requires multiple steps like cell mobilization, invasion of adjacent stroma, dissemination through blood or lymph and colonization of distant tissues producing the secondary tumor development. These steps involve multiple molecular pathways and miRNAs may have roles in both promoting or inhibiting metastasis (Huang et al., 2008; Tavazoie et al., 2008; Zhu et al., 2008). For example, it has been shown that the human miR-10b positively regulates tumor invasion and metastasis in breast cancer. In cancer cells, miR-10b binds to the Homeobox D10 (HOXD10) mRNA inhibiting its translation. HOXD10 is a transcriptional repressor of Ras homolog gene family member C (RhoC) which controls cytoskeleton reorganization and cell motility. Therefore, the observed increase of miR-10b in metastatic breast tumors, when compared to metastasis-free tumors or normal breast tissues, causes an increase of RhoC activity through HOXD10 control escape. On the other hand, it has been observed that breast cancer patients with low expression of miR-335, miR-126 and miR-206 had a shorter median time to metastatic relapse, suggesting that these miRNAs may act by suppressing metastasis (Tavazoie et al., 2008). Moreover, it has been suggested, also by our research group, that the human miR-21 may promote tumor invasion and metastasis by targeting tumor suppressor genes and cell cycle regulators (Forte et al., 2013; Zhu et al., 2008).

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