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Invited critical review

MicroRNAs in osteosarcoma

Jian Zhang [a], Yi-Guo Yan [a], Cheng Wang [a], Shu-Jun Zhang [a], Xiao-Hua Yu [b,*], Wen-Jun Wang [a,**]

[a] Department of Spine Surgery, The First Affiliated Hospital, University of South China, Hengyang, Hunan 421001, China
[b] Life Science Research Center, University of South China, Hengyang, Hunan 421001, China

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ABSTRACT

Osteosarcoma (OS) is a primary malignant bone tumor with high morbidity that principally emerges in children and adolescents. Presently, the prognosis of OS patients remains poor due to resistance to chemotherapy, highlighting the need for new therapeutic approaches. MicroRNAs (miRNAs), a class of small noncoding RNA molecules, can negatively modulate protein expression at the post-transcriptional level. miRNAs regulate a variety of normal physiologic processes and are involved in tumorigenesis and development of multiple malignancies, including OS. Some miRNAs are differentially expressed in OS tissues, cell lines and serum, and have been shown to correlate with the malignant phenotype and prognosis. These altered miRNAs function as oncogenes or tumor suppressor genes in this process. Moreover, restoration of miRNA expression has shown promise for the treatment of OS. Here, we describe miRNA biochemistry with a focus on expression profile, role and therapeutic potential in OS. A better understanding will facilitate the identification and characterization of novel biomarkers and development of miRNA-targeted therapies.

Contents

Abbreviations: OS, osteosarcoma; miRNAs, microRNAs; CSCs, cancer stem cells; RNA pol, RNA polymerase; DGCR8, DiGeorge syndrome critical gene 8; RISC, RNA-induced silencing complex; Exp 5, exportin 5; Ago, argonaute; MREs, miRNA recognition elements; APE1, apurinic/apyrimidinic endonuclease 1; MDM, murine double minute; TP53, tumor suppressor p53; mTOR, mammalian target of rapamycin; Eag1, ether à go-go 1; PI3K, phosphatidylinositol-3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome ten; Hsp90B1, heat shock protein 90B1; BMP2, bone morphogenetic protein 2; IGF1R, insulin-like growth factor 1 receptor; FAK, phospho-Akt and focal adhesion kinase; LPAATβ, lysophosphatidic acid acyltransferaseβ; HMGB1, high-mobility group box 1; FASN, fatty acid synthase; LZTS1, leucine zipper putative tumor suppressor 1; MMP, matrix metalloproteinase; LNA, locked nucleic acid; DATS, diallyl trisulfide.

* Corresponding author. Tel./fax: +86 734 8281852.
** Corresponding author. Tel./fax: +86 734 8578579.
E-mail addresses: yxh167@qq.com (X.-H. Yu), wwwwjj167@qq.com (W.-J. Wang).

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1. Introduction

Osteosarcoma (OS) is the most common primary malignant bone tumor in children and adolescents, comprising 2.4% of all malignancies in pediatric patients. This tumor is highly aggressive and metastasizes primarily to the lung. It usually occurs in the metaphyseal regions of distal femur, proximal tibia and proximal humerus, with a male predominance. Despite its obscure etiology, accumulating evidence has suggested that OS may be associated with cancer stem cells (CSCs), defects of genes involved in DNA repair and tumor suppressor pathways and genetic alterations [1]. Over the past decades, the five-year survival rate of OS patients has significantly improved to approximately 60–70% due to the introduction of neo-adjuvant chemotherapy and radiotherapy [2]. However, these conventional therapeutic methods have reached a survival plateau, and chemotherapy can lead to drug-resistance and produce life-threatening side effects, such as cardiotoxicity and nephrotoxicity. In addition, patients with metastasis or recurrence have a <20% chance of long-term survival despite the use of chemotherapeutic drugs [3]. Moreover, it is still difficult for early diagnosis of OS. Thus, a greater understanding of OS pathology is urgent to identify biomarkers, optimize treatment strategies, and develop novel anti-OS drugs.

MicroRNAs (miRNAs) are a class of endogenously expressed, small noncoding RNAs, which can inhibit gene expression by targeting mRNAs for translational repression and/or cleavage. They play critical roles in a number of normal biological processes, including embryogenesis, lineage determination, and regulation of cell differentiation, proliferation, apoptosis [4]. Nevertheless, there is growing evidence that miRNAs can serve as either oncogenes or tumor suppressor, depending on their target genes. Dysregulation of miRNAs has been associated with multiple malignancies, such as breast cancer, hepatocellular cancer, lung cancer, colon cancer, cervical cancer and OS [5,6]. It has been already reported that many miRNAs are overexpressed or underexpressed in OS tissues and cell lines, and these miRNAs are deeply implicated in multiple steps of disease occurrence and development, including proliferation, adhesion, invasion and metastasis [7]. Moreover, miRNA-targeted treatment approach has shown enormous potential in controlling aggressive biological behavior of OS [8]. In the current review, we summarize the expression pattern and roles of miRNAs in OS, and describe recent progress regarding their usage as a novel therapeutic approach.

2. miRNA biogenesis and functions

miRNAs are the product of miRNA genes that are found as independent transcripts or within the introns of another gene. As short (19–23 nt) non-coding RNAs, miRNAs are transcribed from both intergenic and genic regions of the genome. They can be co-transcribed with host-gene promoters or possess their own specific promoters. These small regulatory RNA molecules can be synthesized through two distinct mechanisms known as canonical or miRNA pathway, and non-canonical or mirtron pathway. In miRNA pathway, most miRNAs are transcribed by RNA polymerase (RNA pol) II as a pri-miRNA, a primary transcript with several hundred nucleotides in length; however, a small group of miRNAs are also transcribed by RNA pol III [9]. These pri-miRNAs bear a hairpin-shaped structure that temporarily receives a 5′-cap and a 3′-poly (A) tail. Intergenic miRNA promoters, particularly transcriptional start sites, have been mapped at distances from 1 kb to as much as 100 kb away from mature miRNA loci [10]. A critical aspect of initial pri-miRNA processing is the folding of specific regions into hairpin structures, because these long transcripts likely have extensive secondary structures. Notably, many examples of polycistronic miRNA clustering or multiple miRNAs on a single pri-miRNA have been reported [11]. The essential hairpin structural domains of pri-miRNAs were cleaved by an RNA specific ribonuclease enzyme complex (Drosha) and its cofactor DiGeorge syndrome critical gene 8 (DGCR8) to produce precursor-miRNAs (pre-miRNAs), a single double-stranded RNA (dsRNA) hairpin containing approximately 65–75 nucleotides [12]. On the other hand, the non-canonical pathway is involved in a group of short introns, termed mirtron. Mirtron biogenesis needs the spliceosomal machinery and is initiated by splicing and debranching into a pre-miRNA hairpin, which is suitable for Dicer cleavage and is incorporated into RNA-induced silencing complex (RISC) [13]. Mirtron-derived miRNAs not only occur in mammals, but also in avians and plants, suggesting the evolutionary conservation of this mechanism of gene regulation *in vivo* [14–16].

Pre-miRNAs are then translocated from cell nucleus to cytoplasm under the aid of exportin 5 (Exp 5). Once in the cytoplasm, these pre-miRNAs are further processed into a miRNA duplex by the Dicer, an endonuclease that specifically recognizes dsRNA complexes and cleaves ~22 nucleotides (two-full helical turns) from the non-stem-loop end of the pre-miRNA [17]. Although Dicer-mediated cleavage of the pre-miRNA hairpin plays a crucial role in the production of viable mature miRNA, Dicer-independent mechanism is also found in vertebrates and fission yeast [18,19]. The miRNA duplex consists of a mature miRNA strand (miRNA) and a passenger miRNA strand (miRNA*). After unwinding, the passenger miRNA strand is usually degraded, whereas the mature miRNA strand is incorporated into a RISC by interaction with argonaute (Ago) proteins [20]. In general, target sites of miRNAs are present in the 3′-untranslated region (UTR) of mRNAs that contain complementary sequences, known as miRNA recognition elements (MREs). However, miRNAs can also bind to 5′-UTR or open reading frame (ORF) [21]. Of note, endogenous ORF targeting seems to be less frequent and effective than 3′-UTR targeting but still more frequent than 5′-UTR targeting [22]. Targeting occurs through both perfect and imperfect complementarity between mature miRNAs and MREs. Perfect complementarity results in direct cleavage and degradation of target mRNAs, and imperfect complementarity causes a mRNA blockade and subsequent inhibition of protein translation (Fig. 1). The overall effect of a miRNA displays a significant reduction in target gene-encoded protein content. Additionally, miRNA targeting is regulated by multiple factors, including the flanking sequence of the target site, the location within the 3′-UTR, and accessibility of the mRNA target site due to secondary structure and protein interference [23]. Thus, the interplay between miRNAs and MREs is complex, further studies are required to clarify how miRNAs recognize their targets.

3. miRNA expression profiles in OS

Since miRNAs were firstly identified by Lee et al. in *Caenorhabditis elegans* in 1993 [24], over 1000 human miRNAs have been found in the past two decades. By using miRNA microarray analysis, a number of miRNAs have been demonstrated to be differentially expressed in OS tissues and cells. These miRNA expression profiles are discussed in detail below.

Recently, Hu et al. identified a total of 268 miRNAs that were significantly dysregulated in human OS cell line MG-63. Among these miRNAs, miR-9, miR-99, miR-195, miR-148a and miR-181a have been validated as overexpressed, whereas miR-143, miR-145, miR-335 and miR-539 are confirmed to be downregulated [25]. Dai and co-workers found that 13 miRNAs were significantly changed (>2-fold) in MG-63 cells with knockdown of apurinic/apyrimidinic endonuclease 1 (APE1), among which miR-451, miR-1290, miR-765, miR-483-5p, miR-513a-5p, hsa-miR-129-5p and hsa-miR-31 were upregulated, while miR-29b, miR-197, let-7b, miR-324-5p, let-7i and miR-484 were downregulated, suggesting APE1 as a regulator of miRNAs involved in OS [26]. Won and colleagues utilized miRNA microarray analysis to examine miRNA expression in 8 formalin-fixed, paraffin-embedded OS tissue samples and the corresponding adjacent normal bone tissues. They reported that ten miRNAs including miR-199b-5p, miR-338-3p, miR-891a, miR-10b, miR-223, miR-150, miR-451, HS-169, HS-29, and olexa-578-1915 enhanced by over 10-fold in OS samples when compared with normal controls [27]. Moreover, miR-199b-5p, miR-338-3p