The miRNAs in the pathgenesis of osteosarcoma

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1. ABSTRACT

Osteosarcoma is an aggressive sarcoma of the bone characterized by a high level of genetic instability and recurrent DNA deletions and amplifications. MicroRNAs (miRNAs) are non-coding small RNAs, usually 18–25 nucleotides in length, which repress translation and cleave mRNA by base-pairing to the 3 untranslated region of the target genes. miRNAs have demonstrated far-reaching effects on the cellular biology of development and cancer. Their role in osteosarcomagenesis remains largely unexplored. A number of reports have investigated the role of microRNAs in osteosarcoma. This review summeizes the recent research progress of miRNA in the osteosarcome.

2. INTRODUCTION

Osteosarcoma is the third most common cancer in childhood and adolescents and the most common cancer of bone (1). With combination treatment (neoadjuvant chemotherapy, surgery and adjuvant chemotherapy), the 5-year survival for patients who do not have metastatic disease at diagnosis is 60 to 70% (2-4). However, for patients who present with metastatic disease or whose tumor recurs, outcomes are far worse: less than 30% and less than 20% survival, respectively (5). Pulmonary metastasis is the predominant site of osteosarcoma recurrence and the most common cause of death. Unfortunately, survival has not

improved for twenty years despite multiple clinical trials with increased intensity, and further gains with refinements in cytotoxic chemotherapy regimens alone are unlikely. Thus new therapeutic targets and approaches must be sought.

miRNAs (microRNAs) are a class of endogenous, non-coding, single stranded small regulatory RNA molecules, which are approximately 22 nucleotides in length (6). Their coding genes, which are mainly located in cancer associated genomic regions or in fragile sites, account for approximately 1% of the entire genome (7). miRNAs play an important role in the regulation of gene expression at the post-transcriptional level. Unlike short interfering RNAs (siRNAs), miRNAs mainly silence the expression of multiple genes instead of a single gene. It is estimated that miRNAs have the potential to regulate at least 20%–30% of all human genes(8), and that an average miRNA have more than 100 targets (9).

However, their biological function remains largely unknown and only a few mRNAs that are directly regulated by miRNAs in animals have been verified empirically. miRNAs are often deregulated in human malignancies and correlated to the regulation of many cellular processes including proliferation, differentiation, apoptosis and metastasis. miRNAs can function as either oncogenes or tumor suppressors by specifically regulating the expression of their target genes(10). Those miRNAs whose expression is increased in tumors may be considered as oncogenes. These oncogene miRNAs usually promote tumor development by negatively regulating tumor suppressor genes. Meanwhile, some miRNAs whose expression is decreased in tumor are considered as tumor suppressor genes. Tumor suppressor miRNAs usually prevent tumor development by negatively regulating oncogenes. Recently, mounting evidence has indicated that miRNAs are attractive candidates of upstream regulators in metastatic progression, because they may regulate a number of invasion and metastasis-related genes (11-15), suggesting that miRNAs may be used as a potential therapeutic avenue in preventing tumor metastasis. Herein, we give an breif overview about the recent research progress of miRNA in the Osteosarcome.

3. THE miRNAs EXPRESSION PROFILE IN THE OSTEOSARCOMA

miRNAs are endogenous RNAs that are highly conserved in the genomes of most species and can influence various biological processes, including the development and differentiation of tumors. To determine the expression pattern of miRNAs, a miRNA microarray approach has been developed. It has been reported that the miRNA expression profiles are able to classify human cancers successfully, whereas the messenger RNA (mRNA) expression profiles are inaccurate when applied to the same samples (16). This result indicates that the miRNA expression profiles may be more closely linked to the differentiation of the tumors. Several studies have already demonstrated that there are unique and differential miRNA expression patterns for

several cancers, which are promising for their diagnoses(17). To identify novel miRNA-based biomarkers, the significance of miRNA expression profiles has been extensively studied in a diverse group of human sarcomas.

Subramanian et al. obtained the miRNA expression profiles of 27 soft tissue sarcoma samples from 5 histological subtypes (synovial sarcoma, rhabdomyosarcoma, leiomyosarcoma, gastrointestinal stromal tumor (GIST), and liposarcoma) and 7 normal tissue samples(18). In these expression profiles, different histological subtypes of sarcoma had distinct miRNA expression signatures, reflecting the apparent lineage and differentiation status of the tumors.

Many studies have showed so far that different miRNA was expressed in the Osteosarcoma. Sarver et al. (19) have generated miRNA expression profiles for over 300 sarcoma tissue samples representing 22 different sarcoma subtypes (including 15 OS samples) and developed a sarcoma miRNA expression database (SMED) (20). Interestingly, an unsupervised clustering analysis of the miRNA expression profiles showed that OS formed a single cluster that was distinct from other sarcomas, such as synovial sarcoma, fibrosarcoma, GIST, and malignant fibrous histiocytoma (MFH) (20).

Jonee et al.(21) identifed that an miRNA signature reflecting the pathogenesis of osteosarcoma from surgically procured samples from human patients. The signature includes high expression of miR-181a, miR-181b, and miR-181c as well as reduced expression of miR-16, miR-29b, and miR-142-5p. They also demonstrate that miR-181b and miR-29b exhibit restricted expression to distinct cell populations in the tumor tissue. Further, higher expression of miR-27a and miR-181c in pre-treatment biopsy samples characterized patients who developed clinical metastatic disease. In addition, higher expression of miR-451 and miR-15b in pre-treatment samples correlated with subsequent positive response to chemotherapy. In vitro and in vivo functional validation in osteosarcoma cell lines confirmed the tumor suppressive role of miR-16 and the pro-metastatic role of miR-27a. Furthermore, predicted target genes for miR-16 and miR-27a were confirmed as down-regulated by real-time PCR. Affymetrix array profiling of cDNAs from the osteosarcoma specimens and controls were interrogated according to predicted targets of miR-16, miR142-5p, miR-29b, miR-181a/b, and miR-27a. This analysis revealed positive and negative correlations highlighting pathways of known importance to osteosarcoma, as well as novel genes.

Recently, Gougelet et al. (22) examined miRNA expression profiles to determine the relevance of miRNA expression on the chemoresistance in 27 OS paraffin-embedded samples, cell lines and samples from a rat OS model. They showed that miRNA profiles were determined using microfluidic cards performing high-throughput TaqMan(®) -based PCR assays, called TaqMan(®) Low Density Arrays. Osteosarcoma of rat and human origins showed a miRNA signature, which could discriminate good from bad responders. In particular, we identified five
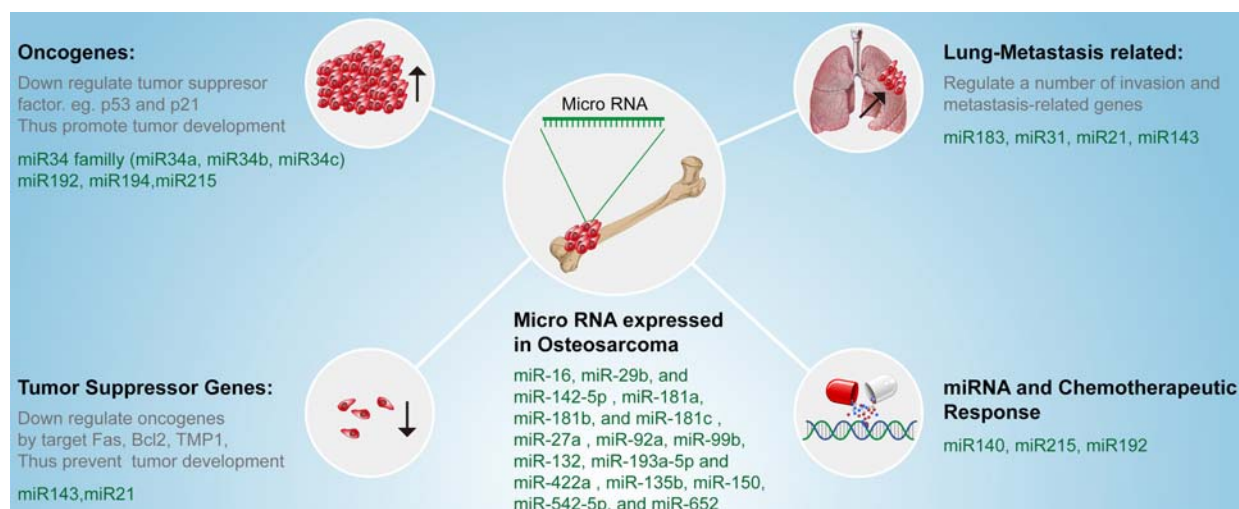
Figure 1. The miRNA experssed in the osteosarcoma and their functions. miRNAs can function as either oncogenes or tumor suppressors by specifically regulating the expression of their target genes. There are also miRNAs which related to Lung-Metastasis and others invovled in the chemotherapeutic response.

discriminating miRNAs (miR-92a, miR-99b, miR-132, miR-193a-5p and miR-422a) in patient tumors, which could be easily transferable to diagnosis. These discriminating miRNAs, as well as those identified in rat, targeted the TGFβ, the Wnt and the MAP kinase pathways.

Lulla *et al.* (23) performed miRNA expression profiling of osteosarcoma cell lines, tumor samples, and normal human osteoblasts. Twenty-two differentially expressed microRNAs were identified using high throughput real-time PCR analysis, and 4 (miR-135b, miR-150, miR-542-5p, andmiR-652) were confirmed and validated in a different group of tumors. Both miR-135b and miR-150 have been previously shown to be important in cancer.

Maire *et al.* performed miRNA expression profiling for 723 human miRNAs in 7 osteosarcoma tumors, and 38 miRNAs differentially expressed >/=10-fold (28 under- and 10 overexpressed) were identified. To develop a comprehensive molecular genetic map of osteosarcoma, the miRNA profiles were integrated with previously published array comparative genomic hybridization DNA imbalance and mRNA gene expression profiles from a set of partially overlapping osteosarcoma tumor samples. Many of the predicted gene targets of differentially expressed miRNA are involved in intracellular signaling pathways important in osteosarcoma, including Notch, RAS/p21, MAPK, Wnt, and the Jun/FOS pathways. These data collectively suggest that miRNAs provide a novel post-transcriptional mechanism for fine-tuning the expression of specific genes and pathways relevant to osteosarcoma. Thus, the miRNA identified in this manner may provide a starting point for experimentally modulating therapeutically relevant pathways in this tumor.

4. THE ROLE OF miRNA PLAYED IN THE OSTEOSARCOMA

A number of reports have investigated the role of miRNAs in osteosarcoma. It has been shown that miRNAs can function as either oncogenes or tumor suppressors by specifically regulating the expression of their target genes. Those miRNAs whose expression is increased in tumors may be considered as oncogenes. These oncogene miRNAs usually promote tumor development by negatively regulating tumor suppressor genes. Meanwhile, some miRNAs whose expression is decreased in tumor are considered as tumor suppressor genes. Tumor suppressor miRNAs usually prevent tumor development by negatively regulating oncogenes (Figure 1).

Recently, mounting evidence has indicated that miRNAs are attractive candidates of upstream regulators in metastatic progression, because they may regulate a number of invasion and metastasis-related genes suggesting that miRNAs may be used as a potential therapeutic avenue in preventing tumor metastasis. Here we give a brief overview of the mechanisms of miRNA in Osteosarcoma.

5. miRNAs FUNCTION AS TUMOR SUPPRESSOR GENES (TARGETING FAS,BCL-2)

In the study done by *Zhang,et al.*, (24) it was found that miR-143 was down-regulated in osteosarcoma cell lines and primary tumor samples, and miR-143 was further identified to be a tumor suppressor, as restoration of miR-143 expression in osteosarcoma cell lines was able to reduce cell viability and promote cell apoptosis *in vitro*, and suppress tumorigenicity *in vivo*. Additionally, Bcl-2, an important antiapoptotic molecule, was identified to be a novel direct target of miR-143, and the proapoptotic function of miR-143 is further suggested to be mainly through targeting Bcl-2 expression.

Zhu *et al.*(34) found that, as an oncogenic miRNA, mir-21affects not only tumor growth but also invasion and metastasis. They showed that suppression of mir-21 in metastatic breast cancer MDA-MB-231 cells significantly reduced invasion and lung metastasis. Consistent with this,

overexpression of tumor suppressor gene tropomyosin1(TPM1) remarkably reduced cell invasion. Furthermore, we identified two additional direct mir-21 targets, programmed cell death 4 (PDCD4) and maspin, both of which have been implicated in invasion and metastasis. Finally, the expression of PDCD4 and maspin inversely correlated with mir-21expression in human breast tumor specimens, indicating the potential regulation of PDCD4 and maspin by mir-21 in these tumors.

6. miRNA FOUNCTION AS ONCOGENES (TARGET P53 AND P21)

The TP53 is a tumor supressor gene which induces apoptosis, cell cycle arrest, and senescence. It is mutated in more than 20% of OS, and its mutations have been demonstrated to be involved in the tumorigenesis of OS(25). In addition, Li-Fraumeni syndrome, which is characterized by an autosomal dominant mutation of TP53, leads to the development of multiple malignancies, including OS(26). Recently, several miRNAs have been identified as direct targets of p53 (27).

Among them, the highly conserved miR-34 family (miR-34a, 34b and 34c) has been an important component of the p53 tumor suppressor pathway, and the expression of these miRNAs was induced by p53 in response to DNA damage or oncogenic stress in multiple cancers (28, 29). Although the current knowledge about the involvement of p53-related miRNAs in OS is limited, He et al.(30) reported that the miR-34 family induced G1 arrest and apoptosis via their targets, CDK6, E2F3, Cyclin E2, and BCL2, in a p53-dependent manner in OS cells(30). According to an examination of the expression of genetic and epigenetic alterations of miR-34 in 117 primary OS samples, the expression of miR-34 was decreased in OS, and miR-34 inhibited the p53-mediated cell cycle arrest and apoptosis in OS cells(31).

Additionally, p53 also induced the upregulation of miR-192, miR-194, and miR-215 in U2OS cells carrying wild-type p53(32). MiR-192 and miR-215 induce the expression of p21, and U2OS cells transfected with an expression vector for miR-192 formed significantly fewer colonies than those transfected with that for a control miR or miR-34a (31). The loss of miR-31was associated with defects in the p53 pathway, and over-expression of miR-31 significantly inhibited the proliferation of OS cell lines. Another study done by Creighton et al. (32) that ffunctional over-expression of miR-31 repressed predicted miR-31 gene targets including cell cycle regulator E2F2 (a member of the E2F family of transcription factors).

7. miRNAs AND METASTASIS (AS REGULATOR OF CELL INVISION AND PROLIFERATION)

Our knowledge of the mechanistic control of invasion and metastasis in osteosarcoma is limited. A recent study focused on the role of miR31 in regulating the development of metastatic disease. Valastyan et al.(33) demonstrated that miR-31 was able to inhibit multiple steps in the metastatic development of breast cancer. The silencing of the mRNA

targets of miR-31, integrin-α5 (ITGA5), radixin (RDX), and RhoA, reduced local invasion and motility *in vitro* and decreased the development of metastases in a xenograft mouse model of breast cancer (33). Another study done by Osaki *et al.*(34) also showed the downregulation of miR-143,which correlates with the lung metastasis of human osteosarcoma cells by promoting cellular invasion via MMP-13 upregulation.

Ezrin and Fas have been linked mechanistically to osteosarcoma metastasis. Ezrin is a cell membrane-cytoskeleton linking protein that allows the cell to interact with themicroevironment and facilitates signal transduction (35). Impaired Fas signaling may allow osteosarcoma cells to evade host resistance in the lung (36). In this paired analysis done by Zhu *et al* (37)., it was found that miR-183 was markedly down-regulated in osteosarcoma cells and tissues compared with matching normal bone tissues using RT-qPCR. Statistical analyses revealed that the expression levels of miR-183 significantly correlated with lung metastasis as well as with local recurrence of osteosarcoma.miR-183 expression was inversely correlated with Ezrin mRNA and protein expression levels in osteosarcoma cells as well as in a subset of primary osteosarcoma. Ectopically expressed miR-183 inhibited migratory and invasive abilities of osteosarcoma cells, whereas knockdown of endogenous miR-183 significantly enhanced these abilities.

It has been reported that miR-21 is aberrantly overexpressed in various cancers and is involved in the pathogenesis of cancers(38). MiR-21 also induces cell proliferation, mi-gration, and invasion by inhibiting the expression of tumor suppressor proteins in several cancers, such as breast, hepatocellular, and colorectal cancer(12 39-40). The inhibition of miR-21 significantly reduced the lung metastasis of breast cancer cells*in vivo* (43). Ziyan *et al.* demonstrated that miR-21 was also significantly overexpressed in OS, and the sup-pression of miR-21 decreased the invasion and migration in MG-63 OS cell lines(44). RECK (reversion-inducing cysteine-rich protein with kazal motifs) was found to be a direct target that was negatively regulated by miR-21 in an OS cell line and human OS samples(40), and it suppressed the invasion of OS cells by decreasing the activity of matrix metalloproteinases (MMPs)(42).

8. miRNA AND CHEMOTHERAPEUTIC RESPONSE

Advances in chemotherapy have resulted in the most significant improvement in the outcomes for patients with OS. Without chemotherapy, the survival rate of patients with localized OS is less than 20% at 5 years, but systemic combined chemotherapy (doxorubicin (DOX), cisplatin (CDDP), methotrexate (MTX), or ifosfamide (IFO)) has enabled the 5-year survival rates to improve to approximately 60–70%(46). Although the response to chemotherapy is one of the most important prognostic factors, more than 40% of all OS patients still respond poorly to chemotherapy(43). Only a few therapeutic options have been established for these poor responders(43-45). Moreover, no biomarker has yet been identified that

discriminates between good and poor responders before the introduction of chemotherapy.

MiR-140 is the first reported miRNA candidate associated with drug sensitivity in OS tumor xenografts treated with the chemotherapeutic agents DOX, CDDP, and IFO. Song *et al.*(46) revealed that miR-140 showed consistently high expression levels across all three xenograft models treated with different drugs. The overexpression of miR-140 caused chemoresistance to MTX and 5-Fluorouracil (5-FU) and suppressed cell proliferation, inducing G1 and G2 arrest in both U2OS and MG-63 OS cells, thus indicating that slowly proliferating or quiescent cells were more resistant to DNA damaging agents. In addition, miR-140 could nega-tively regulate histone deacetylase 4 (HDAC4) which inter-acted with p21 expression, resulting in 5-FU resistance.

The same group reported another miRNA candidate that plays a significant role in the mechanism of chemoresistance. MiR-215 decreased the cell proliferation and induced G2 arrest and also increased the chemoresistance to MTX in U2OS cells and HCT116 (wt-p53) colon cancer cells(47). Denticleless protein homolog (DTL) was identified as one of the critical targeted proteins of miR-215 using a bioinformatics approach. The reintroduction of miR-215 suppressed the expression of the DTL protein. The suppression of DTL by a DTL-specific siRNA reduced cell proliferation by inducing G2 arrest, causing a poor response to MTX (47). Boni *et al.* reported that miR-192 and miR-215 influenced the sensitivity of colorectal cancer to 5-FU(48). These miRNAs induced cell cycle arrest with the accumulation of p53 and its target genes p21and p27(48).

9. CONCLUSIONS

Also a limited number of reports have investigated the role of microRNAs in osteosarcoma so far, and their role in osteosarcomagenesis remains largely unexplored. While with recently arising the intrestes of miRNA, such discoveries of new miRNAs may be used to predict a response to chemotherapeutic agents in OS thus can potentially be used to stratify patients so that they can be treated with different preoperative chemotherapy regimens in the future, more effective treatment can also been development for the clinicl use.

10. ACKNOWLEDGEMENT

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Abbreviations: miRNAs: MicroRNAs; siRNAs, short interfering RNAs; mRNA, messenger RNA; SMED, sarcoma miRNA expression database; MFH: malignant fibrous histiocytoma; TPM1: tropomyosin1; PDCD4: programmed cell death 4; ITGA5: integrin-α5; DOX: doxorubicin, CDDP: cisplatin, MTX: methotrexate, IFO: ifosfamide; 5-FU: 5-Fluorouracil; DTL: Denticleless protein homolog

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