

Research Progress on the Mechanism of miRNA-378 Osteogenesis and Angiogenesis

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Abstract: Bone tissue defect is mainly caused by trauma, tumor and infection, which will not only cause serious dysfunction and malformation but also cause serious psychological damage to patients. Bone defect repair is a complex process. The proposal of bone tissue engineering provides more possibilities for clinical bone defect related diseases. The formation of vascularization is also a key element in bone tissue engineering. Bone regeneration requires the interaction between osteogenesis and angiogenesis in order to form bone and reconstruct tissue. In bone tissue engineering, microRNA (miRNA) can mediate bone metabolism and affect bone development. microRNA-378(miR-378) can promote osteoblast differentiation through different mechanisms, showing a good application prospect in bone defect repair. This paper reviews the mechanism of osteogenesis and angiogenesis of miR-378, as a result of which may provide a new idea for the study of bone tissue regeneration.

Keywords: microRNA, miR-378, Bone tissue engineering, Bone marrow mesenchymal stem cells, Osteogenic differentiation, Angiogenic differentiation

1. Introduction

At present, the most studied molecule in miR-378 family is miR-378a, including miR-378a-3p (usually recognized as miR-378) and miR-378a-5p (usually recognized as miR-378 *)^[1]. Now miR-378a-3p and miR-378a-5p are collectively referred to as miR-378a. Besides, there are more than 10 molecular sub-types of the miR-378 family. In addition to miR-378a, miR-378 family also includes miR-378b, miR-378c, miR-378d, miR-378e, etc. This paper will briefly review the mechanism of miR-378 and its main molecule miR-378a in osteogenesis and angiogenesis.

2. miRNA overview

2.1. miRNA definition and basic functions

MicroRNA (miRNA) is a kind of non-coding RNA with regulatory function, stem ring structure and highly conserved in evolution, which was first discovered in 1993. It is a small molecule RNA composed of 20-24 nucleotides long and single strand. It can regulate gene expression by identifying homologous sequences and interfering with transcription, translation or expression of genetic processes^[2-4]. According to the origin, function and structure of RNA, three short RNA types are found, including siRNA (small interfering RNA), piRNA (PiwiRNA) and miRNA^[5]. miRNA comes from two aspects, which are as follows. On the one hand, most miRNAs are transcribed from the spacer of the genome. On the other hand, about 1/4 of human miRNAs are located in the intron of the coding gene, and the transcription direction is consistent with the introns^[6]. miRNA has been proved to be involved in many biological processes, such as signal transduction, differentiation and etc^[5].

2.2. miRNA osteogenesis and angiogenesis

Bone is a mineralized mesenchymal tissue. It is not only a support against mechanical force, but also

an endocrine organ that mainly regulates mineral homeostasis and energy metabolism. Osteoblasts are the key to maintain appropriate bone mass and maintain calcium homeostasis. Osteoblasts have three consecutive stages: proliferation and growth, matrix maturation and mineralized nodule formation, which are characterized by gene expression in a temporal and spacial manner as well as fine-tuning by a large number of miRNAs^[7]. miRNA is produced by a large precursor after cleavage of RNA enzyme Dicer, and becomes a key transcriptional regulator of gene expression. They act by binding the specific miRNA recognition sequence (seed sequence) in the 3'untranslated region (3'UTR) of target mRNA^[8]. miRNA can be expressed in a specific period of cells, and can target and regulate the differentiation, proliferation, apoptosis and migration of tissue cells^[9]. miRNA plays an important regulatory role in the growth, differentiation and function of osteoblasts. For example, the increased expression level of miR-218 can be observed in the osteogenic differentiation of human adipose mesenchymal stem cells (hADSCs)^[10]. Now miRNA has been used as a therapeutic strategy to promote bone regeneration and bone repair. miR-26a was found by Li y et al. To be a coupling gene that positively regulates angiogenesis and osteogenesis in BMMSCs^[11]. Zhang et al.^[12] studied that miR-218 was added to the osteoblast culture experiment in vitro. Western blot showed that miR-218 could promote the expression of Runx2 and promote the maturation of osteoblasts. In addition to being up-regulated, some miRNAs are reduced during osteogenesis. Down regulation of miR-433 level was observed in BMP-2-induced osteogenesis of mouse C3H10T1/2 cells^[13]. Chen et al.^[14] found that the proliferation and differentiation of osteoblasts can be inhibited by miR-135-5p by regulating bone specific transcription factor Runx2.

Angiogenesis is generated from new blood vessels developed by existing blood vessels, which is regulated by cytokines and growth factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and angiopoietin-1 (Ang-1). Some miRNAs such as miR-21, miR-126, miR-221 and miR-222 are highly expressed in endothelial cells, Their expression can be controlled after miRNA transcription^[15]. miRNA can regulate all stages of angiogenesis, and miRNA has targeting to multiple genes in the signal pathway, making it a target for the development of second-generation anti angiogenesis drugs^[16]. All known specific miRNAs (angiomirs) basically have two functions: one is to promote angiogenesis by targeting negative regulators of angiogenesis, which is called Pro vascular miRs, such as miR-126, miR-17-92 cluster, let-7b, 7f, miR-130, miR-210, miR-378 and miR-296. They target various inhibitory signal molecules of angiogenesis and promote capillary formation. The other function is to inhibit angiogenesis by targeting positive regulators of angiogenesis, which is called anti angiomiRs, such as miR-221/222, miR-328, miR-15b/ miR-16 and miR-20a/20b, which have the effect of anti angiomiR^[17].

3. Structure and function of miRNA-378

miR-378 plays an important role in cell proliferation, division, apoptosis, invasion and metastasis through different mechanisms. Its mechanism is as follows: in miRNA induced silencing complex MIRISC, miRNA can recruit Argonaute (ago) to specific target sites through base complementary pairing, and ago can degrade target RNA in nucleus after base complementary pairing; When miRNA is completely complementary to the target site, it will lead to the degradation of target mRNA, especially in plants; However, it is common in mammals that miRNAs that are not completely complementary to the target mRNA inhibit the expression at the protein translation level^[18]. The microRNA precursor has hairpin structure. During maturation, the hairpin structure will be cut off to form miRNA-378 (called sense chain or left arm) and miRNA-378* (called antisense chain or right arm). They are the products of a gene. Usually, the sense chain has function and the antisense chain is degraded without function. But now it is found that antisense chains sometimes have functions, which can help the justice chain function or antagonize the justice chain^[19].

4. Osteogenesis and angiogenesis of miRNA-378

Bone regeneration is closely related to osteogenesis and angiogenesis. Only the growth factors of osteogenesis and angiogenesis can enhance bone tissue regeneration. miRNA can regulate a variety of metabolic processes through endogenous signal transduction pathways at the gene level^[20]. According to previous reports, miR-378, miR-214 and miR-155 are greatly affected in the process of osteogenesis and angiogenesis. Bo Zhang et al.^[20] identified miR-378 as a positive regulator of osteogenesis and angiogenesis at the same time. They observed that the expression of miR-378 in human bone marrow mesenchymal stem cells (BMMSCs) increased after osteoblast induction. When miR-378 was overexpressed, the expression of osteogenic and angiogenic genes increased at the same time, which was

predicted by target genes, miR378 can be used as a key factor in regulating osteogenesis and angiogenesis^[20]. Li Jun^[21] and others infected bone marrow mesenchymal stem cells with miR-378a lentivirus vector and negative control virus with appropriate titer respectively, and detected the expression of osteogenic gene and angiogenic gene mRNA of miR-378a by RT-PCR and Western blot. The group of BMMSCs was infected with miR-378a, as a result of which vascular endothelial growth factor, platelet growth factor, Runx2 and type I collagen were higher than those in negative control virus group and blank group. The test results of Western blot showed that the relative expressions of Runx2 and vascular endothelial growth factor protein in the BMMSCs group intervened by miR-378a were significantly higher than those in the negative control virus group and the blank group. Nan K et al.^[22] experiments showed that the administration of exosomes with miR-378 overexpression of ASCs (miR-378-ascs exos) improved the potential of osteogenic and angiogenic BMSCs and HUVECs. In vivo experiments showed that miR-378-ascs exos significantly accelerated bone regeneration and angiogenesis, thereby inhibiting femoral head necrosis.

4.1. miRNA-378 osteogenic mechanism

Bone formation is a complex process controlled by hormones, growth factors, signal transduction factors and environmental factors. The expression of runt related transcription factor-2 (Runx2) is very important for osteogenesis^[23]. Hupkes M et al.^[24] found that miR-378 was overexpressed in BMP2 induced osteogenic differentiation of mesenchymal stromal cells. miR-378 base is well paired with the 3'UTR of renin from nucleotides 1893-1913 and 1903-1923, as a result of which is selected as a hypothetical renin regulator during osteoblast development. Shireen kahai et al.^[25] observed the cell differentiation in MC3T3-E1 osteoblasts under the condition of differentiation and endogenous expression of miR-378. The experiment showed that the cell condensation process of miR-378 expression increased significantly around the 10th day, and then decreased sharply in the process of cell differentiation. It can be seen that miR-378 is involved in the process of MC3T3-E1 cell differentiation. Bo Zhang et al.^[20] tested the effect of miR-378 on osteogenesis and transfected bone marrow mesenchymal stem cells (BMMSCs) with miR-378 simulant. After 2, 7 and 14 days of miR-378 simulant transfection, the level of miR-378 detected by RT-PCR was at least 90 times higher than that of the control group. At the same time, the expression of osteogenic genes including Runx2, BMP-2, Col-I and OCN was determined by RT-PCR. The results showed that the expression of all osteogenic genes was significantly higher than that of the control group, especially the expression of OCN was 60 times higher than that of the control group.

4.2. Mechanism of miRna-378 angiogenesis

In order to detect the effect of miR-378 on angiogenesis, Bo Zhang et al.^[20] respectively transfected bone marrow mesenchymal stem cells (BMMSCs) cells with miR-378 simulant, miR-378 inhibitor and negative control. The expression of VEGF was analyzed by Western blot. Compared with the control group, on the 2nd, 7th and 14th day after transfection of miR-378 simulant, in addition to osteogenic gene, The expression of angiogenic genes VEGF and Ang-1 also increased significantly. After 2, 7 and 14 days of miR-378 inhibitor transfection, the expression of VEGF and Ang-1 decreased almost twice, and it was confirmed that miR-378a affected VEGF-A in two ways. Matrigel test showed that after transfection of miR-378 simulant, the number of branches, branch length, number of nodes, grid and grid area were significantly higher than those of the negative control. On the contrary, after transfection of miR-378 inhibitor, its indexes were less than those of negative control. Cui Z et al.^[26] confirmed that KLK4 was the target gene of miR-378a-5p by double luciferase assay and found that down regulation of KLK4 inhibited the angiogenesis of miR-378a-5p silencing induced gene. In addition, the shRNA of the KLK4 gene blocked the activation of the Wnt / β -catenin signaling pathway in miR-378a-5p Antimir-transfected cells. The activation of catenin signaling pathway indicates that miR-378a-5p inhibits the angiogenesis of OSCC by regulating KLK4. Xing y et al.^[27] divided the cells into miR-378 group (MSCs transfected with miR-378) and control group (MSCs not transfected with miR-378). After 24 hours of transfection, the two groups of cells were cocultured medium with human umbilical vein endothelial cells. Western blot analysis showed that VEGF in MSC transfected with miR-378 under normoxic and hypoxic conditions VEGF α , PDGF β And TGF- β 1 they expression of was increased. Quantitative RT-PCR showed PDGF β the expression was up-regulated at the mRNA level, but VEGF was not found VEGF α And TGF β 1 gene expression difference. Nan K et al.^[22] found that exosomes overexpressing ASCs (miR-378-ASCs-Exos) can promote cell migration and angiogenesis. In addition, miR-378-ASCs-Exos enhanced angiogenesis related genes such as VEGF and ANG1. After immunohistochemical staining of CD31, vascular endothelial cells and neovascularization were observed around the newly

formed bone tissue in miR-378-ASCs-Exos group.

miR-378 is rich in CD34⁺ hematopoietic cells^[28], and its angiogenesis mechanism plays a key role in different tumor tissues. According to existing studies, miR-378 is differentially regulated in different types of cancer^{[29][30]}. For example, it is down regulated and plays the role of tumor suppressor gene in gastric cancer, oral cancer and colon cancer^{[31][32][33][34]}; The role of in breast cancer, renal cell carcinoma and lung cancer is increased^{[35][36]}, and the imbalance of miR-378 expression in tumor cells may affect its secretion of angiogenic factor^[37]. Studies have found that when human miR-378a-5p is overexpressed as an oncogene in tumor cell lines, it improves cell survival, reduces caspase-3 activity and enhances angiogenesis by inhibiting Sufu factor and tumor candidate gene TUSC2 (fus-1) ^[38]. It was also found that hsa-miR-378a-5p can directly affect VEGF-A upregulation by competing with hsa-miR-125a for the same seed region in VEGF-A3^[1], and can indirectly regulate VEGF-A affecting SHH signal through Sufu inhibition^[38]. Tiwari a et al.^[16] demonstrated that miR-378 promotes angiogenesis by inhibiting known Sufu factor and TUSC2 in U87 cell line. The data of Chan JK et al.^[39] show that miR-378 is overexpressed in ovarian cancer cells and tumors, and it may be a biomarker of anti angiogenesis treatment response in ovarian cancer and other cancers. In conclusion, miR-378 and its downstream targets can be used as markers of anti angiogenic therapeutic response.

5. Conclusion and Prospect

In conclusion, miRNA is involved in almost all metabolic processes and is also a key factor in various cellular and organic metabolic processes. We have learned that miR-378 has a positive regulatory effect on osteogenesis and angiogenesis of BMMSCs, and miR-378 is an expected factor to promote bone growth by increasing osteogenesis and angiogenesis at the same time. However, some scholars have put forward the opposite view. Feng l et al.^[40] used miR-378 transgenic TG mice to study the role of miR-378 in osteogenic differentiation and bone formation, showed abnormal bone tissue and damaged bone in miR-378 transgenic TG mice, and observed the delayed healing effect of bone in TG mice. Moreover, the osteogenic differentiation of the TG mouse derived mesenchymal stem cells was also inhibited. The author also found that the up-regulated miR-378 was inhibited, and the miR-378 inhibitor promoted the osteogenic effect of human bone marrow mesenchymal stem cells. It was concluded that miR-378 inhibited osteogenesis and bone formation by inactivating Wnt / β -catenin signaling. To summarize the previous research conclusions, whether overexpression of miR-378 or down-regulation of miR-378, the effect on osteogenesis and angiogenesis is an important discovery in the field of bone tissue engineering, and brings new opportunities for the clinical treatment of bone defect related diseases, but we still need to further explore the osteogenesis mechanism and angiogenesis mechanism of miR-378.

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