HHS Public Access

Author manuscript

Ann N Y Acad Sci. Author manuscript; available in PMC 2017 November 01.

Published in final edited form as:

Ann N Y Acad Sci. 2016 November ; 1383(1): 80–87. doi:10.1111/nyas.13206.

Runx2 and microRNA regulation in bone and cartilage diseases

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Abstract

The homeostasis of skeletal tissues requires tight regulation of a variety of signaling pathways, and the onset and progression of skeletal diseases are often caused by signaling abnormalities. MicroRNAs (miRNAs) are short non-coding RNA molecules that have emerged as a new dimension of gene regulation. MiRNAs have been shown to play an important role in the regulation of the differentiation of embryonic and hematopoietic stem cells. However, the role of specific miRNAs and their target genes has not been fully defined in the regulation of mesenchymal stem cells (MSCs). Runx2 is a key transcription factor controlling MSC differentiation and bone and cartilage function. This article reviews work on Runx2 and miRNA regulation in bone and cartilage diseases.

Keywords

Runx2; microRNA; bone; cartilage

Introduction

In recent years, microRNAs (miRNAs) have emerged as a new dimension of gene regulation. MiRNAs, which are ~22-nucleotide non-coding RNAs found in eukaryotic organisms, play an important role in the regulation of the differentiation of embryonic and hematopoietic stem cells.[1] However, specific miRNAs in the regulation of mesenchymal stem cells (MSCs) have not been fully characterized. The cells of mesenchymal origin in the skeleton include chondrocytes and osteoblasts. Several transcriptional factors have been found to be essential in directing the differentiation of MSCs toward osteochondral lineage, such as sex-determining region Y (SRY)-box 9 (Sox9), Runt-related 2 (Runx2), Osterix (Osx), and activating transcription factor-4 (ATF4).[2, 3] Among these transcription factors, Runx2 is unique in its multifunctional roles during both chondrogenesis and osteogenesis.

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Conflicts of interest

The authors declare no conflicts of interest.

Runx2 is the most potent inducer of osteoblastic differentiation, as well as the master transcriptional factor in chondrocyte hypertrophy. Therefore, it is important to precisely regulate Runx2 levels. Compared with transcriptional regulation of gene expression, control of exiting mRNA by miRNAs–mRNA interaction is faster and more moderate.[4] Understanding the regulatory mechanism underlying Runx2 by miRNAs may help determine the control mechanism of MSC differentiation in normal physiological processes and diseases.

Runx2

While the role of Runx2 in osteoblast differentiation has been well documented, less is known about the role of Runx2 in articular cartilage function. Recent findings demonstrated that Runx2 regulates the transcription of genes encoding for matrix degradation enzymes in chondrocytes,[5–10] suggesting that Runx2 plays an important role in osteoarthritis (OA) development and chondrocyte hypertrophy. In this review article, we discuss miRNA regulation of Runx2 in bone and cartilage diseases (Table 1).

MicroRNAs

The number of individual miRNAs is estimated to be more than 800, making up over 1% of the predicted genes in humans.[11, 12] As post-transcriptional regulators, miRNAs bind to their target mRNAs through complementarity between a “seed” region located at the 5′ end of the miRNA and the 3′-untranslated region (3′-UTR) of their target genes, resulting in translational repression or mRNA degradation.[11] Bioinformatic analysis based on searching conserved seed-matched sequences has predicted that miRNAs could target more than 60% of human protein-coding genes, including key transcription factors, receptors, and kinases.[13] In addition, quantitative, large-scale proteomic analyses have demonstrated that a single miRNA can repress the production of hundreds of proteins.[14, 15] Thus, miRNAs provide a vast, complex regulatory network to ensure precise molecular signaling transduction and confer robustness to genetic programs in response to internal or external perturbations.

The development and homeostasis of skeletal tissues, including bone and cartilage, require tight and accurate regulation of a variety of genes in different signaling pathways, and the onset and progression of skeletal diseases, such as OA and osteoporosis, are often caused by signaling abnormalities. By virtue of their vast numbers and versatile regulatory functions, miRNAs also play instrumental roles in skeletal pathophysiology. A straightforward example to appreciate the potential of miRNAs is the *in vivo* study using mice with conditional ablation of the RNase III enzyme Dicer, which processes miRNA precursors into mature miRNAs: Dicer ablation in mature osteoblasts enhances osteoblast activity and therefore promotes bone formation, and Dicer-deficient chondrocytes show significant retardation in proliferation, resulting in severe defects of skeletal development.[16, 17] Nevertheless, the study of Dicer knockout (KO) mice could not fully reveal the scope, magnitude, and detail of miRNA regulation, as hundreds of miRNAs with different or even converse functions are integrated as a network to confer robustness to biological systems. Recent studies showed that miR-140 KO mice show profound skeletal phenotypes, including shorter limb bones, craniofacial deformities, and age-related OA-like changes,[18, 19]

underscoring the essential roles of miR-140 in skeletal development. Thus, more *in vivo* study of individual miRNAs would greatly deepen understanding of the miRNA regulatory network.

MiRNA regulation of Runx2 in MSC lineage determination

Given that MSCs are capable of differentiating into multiple lineages, the role of transcription factors in maintaining pluripotency and determining lineage has been identified. Runx2 is regarded as a master regulator in osteogenic commitment and differentiation, and genetic and epigenetic regulation of Runx2 expression has been well documented. Post-transcriptional control of Runx2 includes miRNA regulation, which negatively affects the translation or stability of Runx2 mRNA and subsequently modulates cell fates.

The miR-23a~27a~24-2 cluster and a panel of 11 miRNAs have been identified as negative regulators of Runx2 in various mesenchymal cells, which suppressed osteoblast differentiation.[20, 21] Among them, miR-204 and its homologue miR-211 were found to be upregulated during adipogenesis in progenitor/stem cells. In loss- or gain-of-function experiments, we have confirmed that miR-204/-211 can inhibit osteogenesis while promoting adipogenesis through the attenuation of Runx2.[22] MiR-320 also targets Runx2 and acts as a pro-adipogenic miRNA.[23] On the contrary, several miRNAs have been shown to promote osteoblast differentiation via indirect stimulation of Runx2 gene expression, including the miR-2861~3960 cluster, miR-22, and miR-449a, target inhibitors of Runx2.[24–26] An imbalance between adipogenesis and osteogenesis contributes to skeletal disorders, such as osteoporosis. Thus, emerging roles of switcher miRNAs as potential therapeutic targets and intervention tools have been suggested.

The differentiation of bi-potential osteochondral progenitors is under the control of two master transcription factors, Sox9 and Runx2, with dominance of Sox9 over Runx2 being pivotal for chondrogenic determination. A subset of Runx2-targeting miRNAs is highly expressed to repress Runx2 during onset of chondrogenesis but downregulated in hypertrophic chondrocytes.[21] Recently, miR-455-3p was demonstrated to act as a Runx2-targeting miRNA and to activate early chondrogenic differentiation.[27] MiR-1 is dominantly expressed in the hypertrophic zone of growth plate. The inhibitory effect of histone deacetylase 4 (HDAC4) on Runx2 during hypertrophy is mitigated as a result of miR-1 binding to HDAC4 mRNA.[28] The role of miRNAs in cartilage development, homeostasis, and pathology needs to be further investigated.

MiRNA regulation of Runx2 in osteoporosis

Osteoporosis is at least partially caused by the disorder of MSC lineage commitment during aging. Shift from osteogenesis to adipogenesis results in bone loss and fatty marrow. Among the dozens of Runx2-targeting miRNAs, miR-338-3p has been suggested as a potential modulator of osteoporosis. Increased miR-338-3p was observed in ovariectomized (OVX) mice. Knockdown of miR-338-3p in MSCs isolated from OVX mice could upregulate the expression of Runx2 and rescue the osteoblastic differentiation.[29] Conversely, Runx2

inhibitors targeting miRNAs are predicted to play a positive role in maintaining Runx2 function. For instance, transgenic mice overexpressing miR-29a show resistance to glucocorticoid-mediated osteoporosis by loss of HDAC4-induced repression of Runx2.[30] Accumulating evidence reveals the role of Runx2-related miRNAs in the treatment of osteoporosis.

MiRNA regulation of Runx2 in bone tumors

Osteosarcoma is a primary bone tumor that predominantly affects adolescents. The reduction of tumor suppressor p53 and elevated Runx2 expression correlate with the pathogenesis of osteosarcoma. As a Runx2-targeting miRNA, p53-dependent miR-34c is significantly downregulated in osteosarcoma, and the loss of miR-34c–mediated attenuation of Runx2 may promote the development of osteosarcoma.[31] In addition, both miR-23a and miR-30a function as tumor suppressors in osteosarcoma by targeting Runx2[32, 33] and could be potential diagnostic markers for osteosarcoma.

Bone metastasis is common in patients with advanced breast and prostate cancers. Highly expressed levels of Runx2 are associated with initiation and progression of tumor growth in bone,[34] and targeting Runx2 has been emphasized as a novel therapeutic strategy to treat bone metastasis. Furthermore, significant loss of miR-203 has been identified in prostate biopsies and cell lines. MiR-203 inhibits a cohort of metastatic genes, including Runx2, and re-introduction of miR-203 significantly reduced the migration and invasion abilities of prostate cancer cells and suppressed metastasis *in vivo*, suggesting an anti-metastasis role of miR-203 in prostate cancer.[35] Am absence of miR-135 and miR-203 was also observed in metastatic breast cancer. One study showed that administration of synthetic miRNAs could attenuate Runx2 and impede the development of bone metastasis.[36]

MiRNA regulation of Runx2 in vascular calcification

Aberrant expression of Runx2 in vascular smooth muscle cells (VSMCs) drives trans-differentiation of these cells into osteoblast-like cells, consequently inducing vascular calcification. A critical role of Runx2-targeting miRNAs during osteoblast differentiation has been demonstrated, suggesting their potential involvement in the pathogenesis of vascular calcification. MiR-204 is recognized as one of the most important regulators in osteogenesis,[22] and its role in the inhibition of VSMC calcification via repression of Runx2 was confirmed *in vitro* and *in vivo*.[37] Such studies enhance understanding of the mechanism underlying vascular calcification and encourage novel miRNA-based drug development. Recently, miR-30b/c, miR-133a, and miR-205 were found to act as negative regulators of VSMC osteogenesis, indicating a their possible application as biomarkers of vascular calcification.[38–40]

Runx2 in OA

In human osteoarthritic cartilage, the expression of Runx2 is elevated,[5, 41] which has consistently been shown to contribute to the progression of posttraumatic OA induced by the destabilization of knee joints.[42] Partial depletion of Runx2 in *Runx2*$^{+/-}$ mice attenuates

surgically induced cartilage destruction and osteophyte formation, suggesting a role of Runx2 in the pathogenesis of OA.[42] Hypertrophic differentiation of chondrocytes during OA has been well characterized and considered as a potential therapeutic target.[43] As the master transcriptional factor in chondrocyte terminal differentiation, Runx2 directly regulates both cell behaviors and enzymes responsible for the degradation of extracellular matrix. Type X collagen is the most representative marker of hypertrophic chondrocytes, and Runx2 binding sites were identified in the *Col10a1* promoter, indicating transcriptional control of the *Col10a1* gene by Runx2.[44] Two classes of enzymes, matrix metalloproteinases (MMPs), such as MMP9 and MMP13, and aggrecanases, such as ADAMTS4 (aggrecanase-1) and ADAMTS5 (aggrecanase-2), digest collagen or aggrecan individually and contribute to cartilage degradation during OA. We found that Runx2 can directly bind to its binding site (5-PyGPyGGTPy-3) at the rat *Mmp13* promoter in rat chondrosarcoma cells,[9] and numerous studies support the idea that MMPs and ADMATS are direct targets of Runx2.[45–49] Several signaling pathways have also been reported to play essential roles during OA initiation and progression. Considering the importance of TGF-β and canonical Wnt signaling pathways, we have investigated their interaction with Runx2 in OA using genetically modified mice. Interruption of these pathways led to OA-like phenomena, possibly in a Runx2-dependent manner,[50, 51] and *Mmp13* gene transcription in particular was subsequently upregulated. In general, there is increasing empirical evidence to support the regulatory roles of Runx2 in OA, including its complete control over chondrocyte hypertrophy, direct regulation of matrix-degrading enzymes, and cross-talk with essential signaling pathways. Given the importance of Runx2 in OA, it is not surprising that Runx2 expression and activity are tightly regulated.

MiRNA regulation of Runx2 in OA

Runx2 undergoes multiple levels of regulation, aberrance of which could cause OA pathogenesis. As Runx2 has been demonstrated to be targeted by multiple miRNAs, an interesting question would be whether these miRNAs play a role, especially in accordance with their functions in repressing Runx2, in OA onset and progression. Potential clinical application of miRNAs in the diagnosis and treatment of OA has been reviewed.[52] A recent report demonstrated that miR-105 binds and targets Runx2 in chondrocytes and the expression of miR-105 is downregulated in human OA cartilage, which is negatively correlated with Runx2, ADAMTS7, and ADAMTS12 expression.[53] The miRNA regulation of Runx2 can be exerted in an indirect fashion: miR-145 was reported to repress *Sox9* expression in normal human articular chondrocytes and overexpression of miR-145 induces the mRNA levels of Runx2, possibly through relieving *Sox9* repression on Runx2 expression.[54] During chondrocyte differentiation, the mechanosensitive miR-365 promotes the maturation of chondrocytes by targeting HDAC4 and relieving its suppression on Runx2. Recently, the abnormal elevation in miR-365 expression was found to be associated with human primary and traumatic OA, suggesting the role of miR-365 as a potential target of therapeutic intervention of OA.[55, 56] Similarly, miR-140 also targets HDAC4 in chondrocytes and indirectly affects Runx2 activity. MiR-140 is specifically expressed in cartilaginous tissues during mouse embryogenesis and became one of the hotspots of OA research.[57] Although it could promote chondrocyte hypertrophy by targeting HDAC4,

miR-140 has been reported to protect against OA progression because of its inhibitory effect on several cartilage destruction–related genes, such as *MMP13* and *ADMATS-5*.[18,58] Thus, one should keep in mind that single miRNAs usually target multiple genes and can regulate anabolism and catabolism simultaneously. While the Runx2–miRNA interaction is receiving increasing attention in the OA field, we expect that more interesting data, particularly from *in vivo* studies using genetically modified animals or gene therapy, will be available to advance understanding of the miR–Runx2 roles in OA pathogenesis.

Summary and future perspectives

Runx2 is a prerequisite for both intramembranous ossification and endochondral ossification during skeletogenesis. The deletion of the miRNA-processing enzyme Dicer leads to reduced expression of miRNAs and Runx2 and interrupted bone formation, suggesting a critical role of miRNA in skeleton development.[16, 17] A regulatory effect of Runx2-related miRNAs in chondrogenesis and osteogenesis has been described above; therefore, their involvement in embryonic or postnatal bone formation can be predicted. A complete understanding to date of the crosstalk between miRNA and Runx2 during skeletal development *in vivo* is still lacking. It is highly anticipated that miRNAs will be used as novel tools for clinical diagnosis and treatment. Previous miRNA profiling studies have indicated the potential of circulating miRNAs as biomarkers of skeletal and other diseases. Meanwhile, aberrant expression of miRNAs has been found in a wide variety of human diseases, suggesting that miRNAs may serve as therapeutic targets. Synthetic miRNA mimics or anti-miRNA oligonucleotides are employed to modulate miRNA activity. Despite a growing interest in identifying the functions of miRNAs, an understanding of the regulation of miRNAs themselves remains elusive. Data from miRNA expression profiling studies reveal that miRNAs are expressed in specific spatiotemporal patterns. The biogenesis and activity of miRNAs are under sophisticated control at both transcriptional and post-transcriptional levels, thereby restricting miRNA expression to particular tissues or developmental stages. Significant progress has been made in recent years in understanding the role of Runx2 and miRNA regulation during the process of MSC differentiation and bone and cartilage functions. However, the *in vivo* functions of individual miRNA in maintaining skeletal tissue homeostasis and in regulating initiation and progression of bone and cartilage diseases, the clinical applications of miRNAs, and the control of miRNAs expression require further investigation.

Acknowledgments

This work was supported by NIH Grants AR055915 and AR054465 to D.C. and was partially supported by a grant from the North American Spine Society (NASS) and by the Natural Science Foundation of China (Grant 81371999) to D.C.

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Table 1

Runx2-related miRNAs in skeleton

miRNA	Target gene	Study model	Function/activity
miR-23a~27a~24-2[20]	SATB2, Runx2	Primary rat osteoblasts, mouse pre-osteoblast cell line MC3T3-E1	Suppress osteogenic differentiation
A panel of 11 miRNAs (miR-23a, -30c, -34c, -133a, -135a, -137, -204, -205, -217, -218, -338)[21]	Runx2	MC3T3-E1, mouse chondroprogenitor cell line ATDC5	Suppress osteogenic differentiation; promote early chondrogenic differentiation and suppress chondrocyte maturation
miR-204/211[22, 37]	Runx2	Multiple mesenchymal cell lines, human MSCs, primary mouse bone marrow stromal cells; primary mouse vascular smooth muscle cells (VSMCs), aortic calcification mouse model	Suppress osteogenic differentiation, promote adipogenic differentiation; downregulated in calcified VSMCs, suppress VSMCs/artery calcification
miR-320[23]	Runx2	Human MSCs	Suppress osteogenic differentiation, promote adipogenic differentiation
miR-2861~3960[24]	HDAC5, Hoxa2	Primary mouse osteoblasts, mouse mesenchymal cell line ST2	Promote osteogenic differentiation
miR-22[25]	HDAC6	Human adipose tissue–derived MSCs	Promote osteogenic differentiation, suppress adipogenic differentiation
miR-449a[26]	HDAC1	Human-induced pluripotent stem cells (iPSCs)	Promote osteogenic differentiation
miR-455-3p[27]	Runx2	ATDC5	Promote early chondrogenic differentiation and suppress chondrocyte maturation
miR-1[28]	HDAC4	Primary chicken chondrocytes	Promote chondrocyte maturation
miR-338-3p[29]	Runx2, Fgfr2	Ovariectomy-induced osteoporosis mice	Upregulated in osteoporosis mice
miR-29a[30]	HDAC4	Transgenic mice overexpressing miR-29a	Mitigate glucocorticoid-induced osteoporosis
miR-34c[31]	Runx2	Human osteosarcoma cell line SAOS-2 and U2OS, human osteosarcoma biopsies	Downregulated in osteosarcoma
miR-23a[32]	Runx2, CXCL12	Multiple osteosarcoma cell lines, osteosarcoma nude mice model	Downregulated in osteosarcoma cell lines, suppress osteosarcoma tumor growth in nude mice
miR-30a[33]	Runx2	Multiple osteosarcoma cell lines	Downregulated in osteosarcoma cell lines
miR-203[35, 36]	Runx2	Multiple prostate/breast cancer cell lines, bone metastasis mice model	Downregulated in prostate/breast cancer cell lines, suppress prostate/breast cancer metastasis *in vivo*
miR-135[36]	Runx2	Multiple breast cancer cell lines, bone metastasis mice model	Downregulated in breast cancer cell lines, suppress breast cancer metastasis *in vivo*
miR-30b/c[38]	Runx2	Human coronary artery smooth muscle cells (CASMCs)	Downregulated in calcified CASMCs and human coronary artery atherosclerosis
miR-133a[39]	Runx2	Primary mouse VSMCs	Downregulated in calcified VSMCs
miR-205[40]	Runx2	Human aortic smooth muscle cells (HASMCs)	Downregulated in calcified HASMCs
miR-105[53]	Runx2	Primary human osteoarthritic chondrocytes/ biopsies	Downregulated in osteoarthritis
miR-145[54]	Sox9	Primary human healthy chondrocytes	Overexpressing miR-145 promotes Runx2 expression
miR-365[55, 56]	HDAC4	Primary human osteoarthritic chondrocytes/ biopsies	Upregulated in osteoarthritis