Short Review

Role of miRNAs In Bone homeostasis- short review

Mahmood Ghafoor ¹, Aleena Sumrin ^{2,*}

¹ Affiliation 1; mahmood.phd18.camb.edu.pk

² Affiliation 2; aleena.camb@pu.edu.pk

* Correspondence: aleena.camb@pu.edu.pk; Tel.: (+92-333-4559503, +92 (42) 5293141-46 (Pakistan)

Abstract: Bone homeostasis depends on the interplay between bone resorption by osteoclasts and bone formation by osteoblasts. Any Imbalance of this tightly regulated process can cause diseases such as osteoporosis. Therefore, the knowledge about the factors that regulate communication between osteoclasts and osteoblasts are critical to bone cell biology. Osteoporosis is a progressive systemic skeletal disease characterized by low bone mass density and deterioration of bone tissue. Mature miRNAs are about 22 nucleotide long non coding RNA molecules that are involved in regulatory processes intracellularly. A number of scientific studies have revealed a comprehensive and evidential knowledge about miRNAs that affect the bone metabolism by influencing bone formation and resorption processes. In this short review we have summarized the regulatory role of some selective miRNAs in bone formation.

Keywords: osteoblasts; osteoclasts; miRNA

1. Introduction

MicroRNAs are a class of small non-coding RNA molecules (about 22-nucleotide long) that are involved in a wide range of physiological and pathological processes. It is evident in many recent studies that miRNAs affect bone metabolism by influencing bone formation and resorption processes. In the last few years, a number of promising studies suggested miRNAs as potential biomarkers for osteoporosis. A tightly regulated balance between bone resorption (mediated by osteoclasts) and bone formation (mediated by osteoblasts) ensure a normal bone remodeling process and ultimately a mature healthy bone. Proper balance is controlled by the coupling of bone formation to bone resorption, which involves a number of coordinated signaling mechanisms. Nonetheless, an imbalance between bone resorption and bone formation may occur under certain pathological conditions, which leads to abnormal bone remodeling and the development of bone disorders.[1]

2. miRNAs as gene regulatory elements.

MiRNAs were first reported in 1993 by Lee et al.[2] Mature miRNAs are about 22 nucleotide long non coding RNA molecules that are involved in regulatory processes intracellularly, but were detected also in the circulation and other body fluids .[3]They are one of the most studied regulators of gene expression in both physiological and pathological conditions.[4] miRNAs and noncoding RNAs are considered epigenetic regulators along with DNA methylation and histone modifications that regulate gene transcription.[5,6]

Regulatory tasks are executed at post-transcriptional level by the binding of miRNAs to the 3' UTR (untranslated region) of the target RNA and modulating their target in two possible, ways depending on the degree of the complementarity between the miRNA and the target sequences within 3' untranslated regions (UTRs) of mRNA.

If the miRNA bind perfectly with the complimentary sequence of targeted mRNA it will induce the RNA interface pathway that result in the degradation of mRNA.[7-10]

While miRNAs that bind with their targeted mRNAs at 3' UTRs with partial complementarity result repress gene expression post-transcriptionally at the level of translation, without degradation of mRNA.[11,12]

3. miRNAs AND OSTEOBLAST DIFFERENTIATION

Osteoblastic bone formation process occurs in different stages: osteoblast proliferation; Extracellular matrix(ECM) maturation; ECM mineralization; and osteoblast apoptosis. A number of key steps are involved in osteoblast maturation starting from mesenchymal stem cells differentiation to preosteoblasts that is regulated by a transcription factor Runx2; these then differentiated into osteoblasts, regulated by a combined regulatory mechanism of Runx2 and osteoblast-specific transcription factor (Osx).[13]

Multiple signaling pathways play role in osteogenic differentiation including Wnt/-catenin, bone morphogenetic protein (BMP), JAK/STAT, and MAPK.[14,15]

In addition, regulatory genes like Runx2, osterix, SMADs, TCF/LEF, NFATc1, Twist1, and AP-1 take part to activate Smad protein family and Wnt signal.[16] This activated Wnt pathway successively activates frizzled related protein (FRP) and promotes the expression of specific genes that result in the proliferation of stem cells, preosteoblasts, increasing osteoblasts numbers, and the resistance to apoptosis of osteoblasts.[17]

Many studies have reported that the expression of particular miRNAs in osteoblast differentiation appears unregulated or down regulated. miRNAs regulate the osteoblastogenesis by directly or indirectly affecting the above mentioned pathways. Some miRNAs have positive effect on osteoblast and promote their differentiation such as miR-29b, miR-2861 and miR-3960, miR-378 are important examples that enhance the osteoblastic differentiation. Conversely, while some miRNAs negatively regulate the osteoblast by inhibiting Osteoblastic Differentiation. Some important examples of osteoblastic inhibitory miRNAs are miR-204/miR-211, miR-133 and miR-135, miR-125b, miR-206.

Table. 1 shows the list of reported important miRNAs with their target genes/pathways, effect on osteoblastogenesis and the reference study in detail.

Table 1. miRNAs related to osteoblastogenesis

Mirna	Target protein/ Pathway	Effect on osteoblastogenesis	Reference
let-7f	<i>Axin2</i>	Promote	[18]
miR-10a	<i>KLF4</i>	promote	[19]
miR-15b	<i>Smurf1</i>	promote	[20]
miR-20a	<i>PPARγ Bambi Crm1</i>	promote	[20]
miR-23a	<i>Runx2</i>	Suppress	[21]
miR-23a~27a~24-2	<i>SATB2</i>	suppress	[22]
miR-26a	<i>Smad-1</i>	suppress	[23]
miR-27	<i>APC,SFRP1</i>	promote	[24,25]
miR-29a	<i>DKK1 Kremen2 SFRP2</i> <i>HDAC4,osteonectin</i>	promote	[26] ,[27] ,[28]
miR-29b	<i>DUSP2 ,TGF-β3</i> <i>HDAC4,AcvR2a,CTNNBIP1</i>	promote	[29]
miR-29c	<i>osteonectin</i>	promote	[26]

miR-30c	<i>Runx2</i>	suppress	[21]
miR-31	<i>Osx, SATB2</i>	suppress	[30],[31],[32],
miR-34a	<i>JAG1</i>	suppress	[33]
miR-34b	<i>SATB2</i>	promote	[34]
miR-34c	<i>SATB2, Runx2</i>	suppress	[34],[21]
miR-93	<i>Osx</i>	suppress	[35]
miR-103a	<i>Runx2</i>	suppress	[21]
miR-125b	<i>Osx, Smad-4, Cbfb</i>	suppress	[36]
miR-133	<i>Runx2</i>	suppress	[37],[21]
miR-135a	<i>Smad-5, Runx2</i>	suppress	[37],[21]
miR-137	<i>Runx2</i>	suppress	[21]
miR-138	<i>FAK</i>	suppress	[38]
miR-140-5p	<i>BMP-2</i>	suppress	[39]
miR-141	<i>Dlx5</i>	suppress	[40]
miR-143	<i>Osx</i>	suppress	[41]
miR-145	<i>Osx</i>	suppress	[42]
miR-146a	<i>JMJD3, Smad-2, Smad-3</i>	suppress	[43],[44]
miR-181a	<i>Tgfb1, Tbr1, Rgs4</i> <i>Gata6</i>	promote	[45]
miR-196a	<i>HOXC8</i>	promote	[46]
miR-199a	<i>Smad-1</i>	suppress	[47]
miR-200a	<i>Dlx5</i>	suppress	[40]
miR-206	<i>Connexin43</i>	suppress	[48]
miR-204	<i>Runx2</i>	suppress	[49],[21]
miR-205	<i>Runx2</i>	suppress	[21]
miR-210	<i>AcvR1b</i>	promote	[50]
miR-211	<i>Runx2</i>	suppress	[49]
miR-214	<i>Osx, ATF4</i>	suppress	[51],[52]
miR-217	<i>Runx2</i>	promote	[21]
miR-218	<i>SOST DKK2 SFRP2</i> <i>Runx2</i>	promote suppress	[53],[54], [55]
miR-335-5p	<i>DKK1</i>	promote	[56]
miR-338	<i>Runx2</i>	suppress	[21]
miR-338-3p	<i>Runx2 Fgfr2</i>	promote	[57]
miR-346	<i>GSK-3β</i>	suppress	[52]
miR-378	unknown	promote	[58]
miR-433	<i>Runx2</i>	suppress	[59]
miR-542-3p	<i>BMP-7</i>	suppress	[60]
miR-548d-5p	<i>PPARγ</i>	promote	[61]
miR-637	<i>Osx</i>	suppress	[62]
miR-654-5p	<i>BMP-2</i>	suppress	[63]
miR-2861	<i>HDAC5</i>	promote	[64]

miR-3960	<i>HOXA2</i>	promote	[65]
----------	--------------	---------	------

4. miRNAs and Osteoclast

Multinucleated osteoclast primarily originate from mononuclear macrophages. The process comprises the complex interaction of different pathway and receptors and regulated by a variety of cytokines. Mononuclear cells are stimulated by a number of cyto-regulatory elements ,most importantly osteoclast differentiation factor (RANKL), macrophage colony stimulating factor (M-CSF) and other positive stimulating factor, that leads to differentiate into osteoclasts.[66] Although different miRNAs contribute in regulating osteoblast differentiation however their effects on osteoclastogenesis have been less studied as compared to osteoblast. In Table 02 some selected miRNAs are listed with their target proteins/pathways, effects and reference studies.

Table 02. miRNAs related to Osteoclastogenesis

Mirna	Target protein/ Pathway	Effect on osteoclastogenesis	Reference
miR-21-5p	FasL, PDCD4	Inhibits/promotes	[67],[68],[69],[70]
miR-29	CDC42, SRGAP2, NFIA, CD93,CALCR	promotes	[71]
miR-31-5p	RhoA	promotes	[72]
hsa-miR-133a-3p	CXCL11, CXCR3 and SLC39A1	Inhibit/promotes	[73]
hsa-miR-422a	CBL, CD226,IGF1, PAG1,TOB2	promotes	[74]
hsa-miR-148a-3p	MAFB	Promotes	[75],[76]
miR-183-5p	HO-1	Promotes	[77]
miR-214-3p	Pten	Promotes	[52],[78],
miR-223-3p	NFIA, IKK α	Inhibits/promotes	[78],[79],[80],[81],[82] [83],[84],[85],[86],
miR-9718	PIAS3	promotes	[87]
miR-7b-5p	DC-STAMP	Inhibits	[88]
miR-26a-5p	CTGF	Inhibits	[89]
miR-124-3p	NFATc1, RhoA, Rac1	Inhibits	[90]
miR-125a-5p	TRAF6	Inhibits	[91]
miR-146a-5p	TRAF6, Stat1	Inhibits	[92],[93],[94]
miR-218-5p	p38MAPK-c-Fos- NFATc1	Inhibits	[95]
miR-503-5p	RANK	Inhibits	[96]
miR-34a-5p	Tgif2	Inhibits	[97]

5. Conclusion

Despite the limitation of unclear role of miRNAs in bone disorders there is substantial information about some specific miRNAs relative to bone metabolism now emerging and making their use as a new tool for the early diagnosis of osteoporosis.

Funding: "This research received no external funding"

Conflicts of Interest: "The authors declare no conflict of interest."

References

1. Feng, X.; McDonald, J.M. Disorders of bone remodeling. *Annual Review of Pathology: Mechanisms of Disease* **2011**, *6*, 121-145.
2. Lee, R.C.; Feinbaum, R.L.; Ambros, V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *cell* **1993**, *75*, 843-854.
3. MacFarlane, L.-A.; R Murphy, P. MicroRNA: biogenesis, function and role in cancer. *Current genomics* **2010**, *11*, 537-561.
4. Gennari, L.; Bianciardi, S.; Merlotti, D. MicroRNAs in bone diseases. *Osteoporosis International* **2017**, *28*, 1191-1213.
5. Jacquier, A. The complex eukaryotic transcriptome: unexpected pervasive transcription and novel small RNAs. *Nature Reviews Genetics* **2009**, *10*, 833.
6. Tay, Y.; Rinn, J.; Pandolfi, P.P. The multilayered complexity of ceRNA crosstalk and competition. *Nature* **2014**, *505*, 344.
7. Tang, G.; Reinhart, B.J.; Bartel, D.P.; Zamore, P.D. A biochemical framework for RNA silencing in plants. *Genes & development* **2003**, *17*, 49-63.
8. Palatnik, J.F.; Allen, E.; Wu, X.; Schommer, C.; Schwab, R.; Carrington, J.C.; Weigel, D. Control of leaf morphogenesis by microRNAs. *Nature* **2003**, *425*, 257.
9. Llave, C.; Xie, Z.; Kasschau, K.D.; Carrington, J.C. Cleavage of Scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA. *Science* **2002**, *297*, 2053-2056.
10. Yekta, S.; Shih, I.-h.; Bartel, D.P. MicroRNA-directed cleavage of HOXB8 mRNA. *Science* **2004**, *304*, 594-596.
11. Hutvagner, G.; Zamore, P.D. A microRNA in a multiple-turnover RNAi enzyme complex. *Science* **2002**, *297*, 2056-2060.
12. Pillai, R.S.; Bhattacharyya, S.N.; Artus, C.G.; Zoller, T.; Cougot, N.; Basyuk, E.; Bertrand, E.; Filipowicz, W. Inhibition of translational initiation by Let-7 MicroRNA in human cells. *Science* **2005**, *309*, 1573-1576.
13. Zhang, C. Transcriptional regulation of bone formation by the osteoblast-specific transcription factor *Osx*. *Journal of orthopaedic surgery and research* **2010**, *5*, 37.
14. Yang, B.; Guo, H.; Zhang, Y.; Chen, L.; Ying, D.; Dong, S. MicroRNA-145 regulates chondrogenic differentiation of mesenchymal stem cells by targeting Sox9. *PloS one* **2011**, *6*, e21679.
15. Joung, Y.H.; Lim, E.J.; Darwin, P.; Chung, S.C.; Jang, J.W.; Do Park, K.; Lee, H.K.; Kim, H.S.; Park, T.; Yang, Y.M. MSM enhances GH signaling via the Jak2/STAT5b pathway in osteoblast-like cells and osteoblast differentiation through the activation of STAT5b in MSCs. *PloS one* **2012**, *7*, e47477.
16. Wang, R.N.; Green, J.; Wang, Z.; Deng, Y.; Qiao, M.; Peabody, M.; Zhang, Q.; Ye, J.; Yan, Z.; Denduluri, S. Bone Morphogenetic Protein (BMP) signaling in development and human diseases. *Genes & diseases* **2014**, *1*, 87-105.
17. Miclea, R.L.; van der Horst, G.; Robanus-Maandag, E.C.; Löwik, C.W.; Oostdijk, W.; Wit, J.M.; Karperien, M. Apc bridges Wnt/ β -catenin and BMP signaling during osteoblast differentiation of KS483 cells. *Experimental cell research* **2011**, *317*, 1411-1421.

18. Egea, V.; Zahler, S.; Rieth, N.; Neth, P.; Popp, T.; Kehe, K.; Jochum, M.; Ries, C. Tissue inhibitor of metalloproteinase-1 (TIMP-1) regulates mesenchymal stem cells through let-7f microRNA and Wnt/ β -catenin signaling. *Proceedings of the National Academy of Sciences* **2012**, *109*, E309-E316.
19. Li, J.; Dong, J.; Zhang, Z.h.; Zhang, D.C.; You, X.Y.; Zhong, Y.; Chen, M.S.; Liu, S.M. miR-10a restores human mesenchymal stem cell differentiation by repressing KLF4. *Journal of cellular physiology* **2013**, *228*, 2324-2336.
20. Zhang, J.-f.; Fu, W.-m.; He, M.-l.; Xie, W.-d.; Lv, Q.; Wan, G.; Li, G.; Wang, H.; Lu, G.; Hu, X. MiRNA-20a promotes osteogenic differentiation of human mesenchymal stem cells by co-regulating BMP signaling. *RNA biology* **2011**, *8*, 829-838.
21. Zhang, Y.; Xie, R.-l.; Croce, C.M.; Stein, J.L.; Lian, J.B.; Van Wijnen, A.J.; Stein, G.S. A program of microRNAs controls osteogenic lineage progression by targeting transcription factor Runx2. *Proceedings of the National Academy of Sciences* **2011**, *108*, 9863-9868.
22. Hassan, M.Q.; Gordon, J.A.; Beloti, M.M.; Croce, C.M.; Van Wijnen, A.J.; Stein, J.L.; Stein, G.S.; Lian, J.B. A network connecting Runx2, SATB2, and the miR-23a~ 27a~ 24-2 cluster regulates the osteoblast differentiation program. *Proceedings of the National Academy of Sciences* **2010**, *107*, 19879-19884.
23. Luzi, E.; Marini, F.; Sala, S.C.; Tognarini, I.; Galli, G.; Brandi, M.L. Osteogenic differentiation of human adipose tissue-derived stem cells is modulated by the miR-26a targeting of the SMAD1 transcription factor. *Journal of bone and mineral research* **2008**, *23*, 287-295.
24. Wang, T.; Xu, Z. miR-27 promotes osteoblast differentiation by modulating Wnt signaling. *Biochemical and biophysical research communications* **2010**, *402*, 186-189.
25. Guo, D.; Li, Q.; Lv, Q.; Wei, Q.; Cao, S.; Gu, J. MiR-27a targets sFRP1 in hFOB cells to regulate proliferation, apoptosis and differentiation. *PloS one* **2014**, *9*, e91354.
26. Kapinas, K.; Kessler, C.B.; Delany, A.M. miR-29 suppression of osteonectin in osteoblasts: regulation during differentiation and by canonical Wnt signaling. *Journal of cellular biochemistry* **2009**, *108*, 216-224.
27. Kapinas, K.; Kessler, C.; Ricks, T.; Gronowicz, G.; Delany, A.M. miR-29 modulates Wnt signaling in human osteoblasts through a positive feedback loop. *Journal of Biological Chemistry* **2010**, *285*, 25221-25231.
28. Ko, J.-Y.; Chuang, P.-C.; Chen, M.-W.; Ke, H.-C.; Wu, S.-L.; Chang, Y.-H.; Chen, Y.-S.; Wang, F.-S. MicroRNA-29a ameliorates glucocorticoid-induced suppression of osteoblast differentiation by regulating β -catenin acetylation. *Bone* **2013**, *57*, 468-475.
29. Li, Z.; Hassan, M.Q.; Jafferji, M.; Aqeilan, R.I.; Garzon, R.; Croce, C.M.; Van Wijnen, A.J.; Stein, J.L.; Stein, G.S.; Lian, J.B. Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. *Journal of Biological Chemistry* **2009**, *284*, 15676-15684.
30. Baglio, S.R.; Devescovi, V.; Granchi, D.; Baldini, N. MicroRNA expression profiling of human bone marrow mesenchymal stem cells during osteogenic differentiation reveals Osterix regulation by miR-31. *Gene* **2013**, *527*, 321-331.
31. Deng, Y.; Wu, S.; Zhou, H.; Bi, X.; Wang, Y.; Hu, Y.; Gu, P.; Fan, X. Effects of a miR-31, Runx2, and Satb2 regulatory loop on the osteogenic differentiation of bone mesenchymal stem cells. *Stem cells and development* **2013**, *22*, 2278-2286.
32. Deng, Y.; Zhou, H.; Zou, D.; Xie, Q.; Bi, X.; Gu, P.; Fan, X. The role of miR-31-modified adipose tissue-derived stem cells in repairing rat critical-sized calvarial defects. *Biomaterials* **2013**, *34*, 6717-6728.
33. Chen, L.; Holmstrøm, K.; Qiu, W.; Ditzel, N.; Shi, K.; Hokland, L.; Kassem, M. MicroRNA-34a inhibits osteoblast differentiation and in vivo bone formation of human stromal stem cells. *Stem cells* **2014**, *32*, 902-912.

34. Wei, J.; Shi, Y.; Zheng, L.; Zhou, B.; Inose, H.; Wang, J.; Guo, X.E.; Grosschedl, R.; Karsenty, G. miR-34s inhibit osteoblast proliferation and differentiation in the mouse by targeting SATB2. *J Cell Biol* **2012**, *197*, 509-521.
35. Yang, L.; Cheng, P.; Chen, C.; He, H.B.; Xie, G.Q.; Zhou, H.D.; Xie, H.; Wu, X.P.; Luo, X.H. miR-93/Sp7 function loop mediates osteoblast mineralization. *Journal of Bone and Mineral Research* **2012**, *27*, 1598-1606.
36. Goettsch, C.; Rauner, M.; Pacyna, N.; Hempel, U.; Bornstein, S.R.; Hofbauer, L.C. miR-125b regulates calcification of vascular smooth muscle cells. *The American journal of pathology* **2011**, *179*, 1594-1600.
37. Li, Z.; Hassan, M.Q.; Volinia, S.; Van Wijnen, A.J.; Stein, J.L.; Croce, C.M.; Lian, J.B.; Stein, G.S. A microRNA signature for a BMP2-induced osteoblast lineage commitment program. *Proceedings of the National Academy of Sciences* **2008**, *105*, 13906-13911.
38. Eskildsen, T.; Taipaleenmäki, H.; Stenvang, J.; Abdallah, B.M.; Ditzel, N.; Nossent, A.Y.; Bak, M.; Kauppinen, S.; Kassem, M. MicroRNA-138 regulates osteogenic differentiation of human stromal (mesenchymal) stem cells in vivo. *Proceedings of the National Academy of Sciences* **2011**, *108*, 6139-6144.
39. Hwang, S.; Park, S.-K.; Lee, H.Y.; Kim, S.W.; Lee, J.S.; Choi, E.K.; You, D.; Kim, C.-S.; Suh, N. miR-140-5p suppresses BMP2-mediated osteogenesis in undifferentiated human mesenchymal stem cells. *FEBS letters* **2014**, *588*, 2957-2963.
40. Itoh, T.; Nozawa, Y.; Akao, Y. MicroRNA-141 and -200a are involved in bone morphogenetic protein-2-induced mouse pre-osteoblast differentiation by targeting distal-less homeobox 5. *Journal of Biological Chemistry* **2009**, *284*, 19272-19279.
41. Li, E.; Zhang, J.; Yuan, T.; Ma, B. MiR-143 suppresses osteogenic differentiation by targeting Osterix. *Molecular and cellular biochemistry* **2014**, *390*, 69-74.
42. Jia, J.; Tian, Q.; Ling, S.; Liu, Y.; Yang, S.; Shao, Z. miR-145 suppresses osteogenic differentiation by targeting Sp7. *FEBS letters* **2013**, *587*, 3027-3031.
43. Huszar, J.M.; Payne, C.J. MIR146A inhibits JMJD3 expression and osteogenic differentiation in human mesenchymal stem cells. *FEBS letters* **2014**, *588*, 1850-1856.
44. Cheung, K.S.; Sposito, N.; Stumpf, P.S.; Wilson, D.I.; Sanchez-Elsner, T.; Oreffo, R.O. MicroRNA-146a regulates human foetal femur derived skeletal stem cell differentiation by down-regulating SMAD2 and SMAD3. *PLoS One* **2014**, *9*, e98063.
45. Bhushan, R.; Grünhagen, J.; Becker, J.; Robinson, P.N.; Ott, C.-E.; Knaus, P. miR-181a promotes osteoblastic differentiation through repression of TGF- β signaling molecules. *The international journal of biochemistry & cell biology* **2013**, *45*, 696-705.
46. Kim, Y.J.; Bae, S.W.; Yu, S.S.; Bae, Y.C.; Jung, J.S. miR-196a regulates proliferation and osteogenic differentiation in mesenchymal stem cells derived from human adipose tissue. *Journal of Bone and Mineral Research* **2009**, *24*, 816-825.
47. Lin, E.A.; Kong, L.; Bai, X.-H.; Luan, Y.; Liu, C.-j. miR-199a*, a bone morphogenetic protein 2-responsive microRNA, regulates chondrogenesis via direct targeting to Smad1. *Journal of Biological Chemistry* **2009**, *284*, 11326-11335.
48. Inose, H.; Ochi, H.; Kimura, A.; Fujita, K.; Xu, R.; Sato, S.; Iwasaki, M.; Sunamura, S.; Takeuchi, Y.; Fukumoto, S. A microRNA regulatory mechanism of osteoblast differentiation. *Proceedings of the National Academy of Sciences* **2009**, *106*, 20794-20799.
49. Huang, J.; Zhao, L.; Xing, L.; Chen, D. MicroRNA-204 regulates Runx2 protein expression and mesenchymal progenitor cell differentiation. *Stem cells* **2010**, *28*, 357-364.

50. Mizuno, Y.; Tokuzawa, Y.; Ninomiya, Y.; Yagi, K.; Yatsuka-Kanesaki, Y.; Suda, T.; Fukuda, T.; Katagiri, T.; Kondoh, Y.; Amemiya, T. miR-210 promotes osteoblastic differentiation through inhibition of AcvR1b. *FEBS letters* **2009**, *583*, 2263-2268.
51. Shi, K.; Lu, J.; Zhao, Y.; Wang, L.; Li, J.; Qi, B.; Li, H.; Ma, C. MicroRNA-214 suppresses osteogenic differentiation of C2C12 myoblast cells by targeting Osterix. *Bone* **2013**, *55*, 487-494.
52. Wang, Q.; Cai, J.; Cai, X.-h.; Chen, L. miR-346 regulates osteogenic differentiation of human bone marrow-derived mesenchymal stem cells by targeting the Wnt/ β -catenin pathway. *PloS one* **2013**, *8*, e72266.
53. Zhang, W.-B.; Zhong, W.-J.; Wang, L. A signal-amplification circuit between miR-218 and Wnt/ β -catenin signal promotes human adipose tissue-derived stem cells osteogenic differentiation. *Bone* **2014**, *58*, 59-66.
54. Gay, I.; Cavender, A.; Peto, D.; Sun, Z.; Speer, A.; Cao, H.; Amendt, B.A. Differentiation of human dental stem cells reveals a role for micro RNA-218. *Journal of periodontal research* **2014**, *49*, 110-120.
55. Hassan, M.Q.; Maeda, Y.; Taipaleenmaki, H.; Zhang, W.; Jafferji, M.; Gordon, J.A.; Li, Z.; Croce, C.M.; Van Wijnen, A.J.; Stein, J.L. miR-218 directs a Wnt signaling circuit to promote differentiation of osteoblasts and osteomimicry of metastatic cancer cells. *Journal of Biological Chemistry* **2012**, *287*, 42084-42092.
56. Zhang, J.; Tu, Q.; Bonewald, L.F.; He, X.; Stein, G.; Lian, J.; Chen, J. Effects of miR-335-5p in modulating osteogenic differentiation by specifically downregulating Wnt antagonist DKK1. *Journal of Bone and Mineral Research* **2011**, *26*, 1953-1963.
57. Liu, H.; Sun, Q.; Wan, C.; Li, L.; Zhang, L.; Chen, Z. MicroRNA-338-3p regulates osteogenic differentiation of mouse bone marrow stromal stem cells by targeting Runx2 and Fgfr2. *Journal of cellular physiology* **2014**, *229*, 1494-1502.
58. Hupkes, M.; Sotoca, A.M.; Hendriks, J.M.; van Zoelen, E.J.; Decherig, K.J. MicroRNA miR-378 promotes BMP2-induced osteogenic differentiation of mesenchymal progenitor cells. *BMC molecular biology* **2014**, *15*, 1.
59. Kim, E.-J.; Kang, I.-H.; Lee, J.W.; Jang, W.-G.; Koh, J.-T. MiR-433 mediates ERR γ -suppressed osteoblast differentiation via direct targeting to Runx2 mRNA in C3H10T1/2 cells. *Life sciences* **2013**, *92*, 562-568.
60. Kureel, J.; Dixit, M.; Tyagi, A.; Mansoori, M.; Srivastava, K.; Raghuvanshi, A.; Maurya, R.; Trivedi, R.; Goel, A.; Singh, D. miR-542-3p suppresses osteoblast cell proliferation and differentiation, targets BMP-7 signaling and inhibits bone formation. *Cell death & disease* **2014**, *5*, e1050.
61. Sun, J.; Wang, Y.; Li, Y.; Zhao, G. Downregulation of PPAR γ by miR-548d-5p suppresses the adipogenic differentiation of human bone marrow mesenchymal stem cells and enhances their osteogenic potential. *Journal of translational medicine* **2014**, *12*, 168.
62. Zhang, J.-f.; Fu, W.-m.; He, M.-l.; Wang, H.; Wang, W.-m.; Yu, S.-c.; Bian, X.-W.; Zhou, J.; Lin, M.C.; Lu, G. MiR-637 maintains the balance between adipocytes and osteoblasts by directly targeting Osterix. *Molecular biology of the cell* **2011**, *22*, 3955-3961.
63. Wei, J.; Chen, H.; Zheng, X.; Zhang, B.; Wang, Y.; Tang, P.; She, F.; Song, Q.; Li, T. Hsa-miR-654-5p regulates osteogenic differentiation of human bone marrow mesenchymal stem cells by repressing bone morphogenetic protein 2. *Nan fang yi ke da xue xue bao= Journal of Southern Medical University* **2012**, *32*, 291-295.
64. Li, H.; Xie, H.; Liu, W.; Hu, R.; Huang, B.; Tan, Y.-F.; Xu, K.; Sheng, Z.-F.; Zhou, H.-D.; Wu, X.-P. A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. *The Journal of clinical investigation* **2010**, *120*, 395-395.

65. Hu, R.; Liu, W.; Li, H.; Yang, L.; Chen, C.; Xia, Z.-Y.; Guo, L.-J.; Xie, H.; Zhou, H.-D.; Wu, X.-P. A Runx2/miR-3960/miR-2861 regulatory feedback loop during mouse osteoblast differentiation. *Journal of Biological Chemistry* **2011**, *286*, 12328-12339.
66. Zhao, B.; Ivashkiv, L.B. Negative regulation of osteoclastogenesis and bone resorption by cytokines and transcriptional repressors. *Arthritis research & therapy* **2011**, *13*, 234.
67. Sugatani, T.; Vacher, J.; Hruska, K.A. A microRNA expression signature of osteoclastogenesis. *Blood* **2011**, *117*, 3648-3657.
68. Fujita, S.; Ito, T.; Mizutani, T.; Minoguchi, S.; Yamamichi, N.; Sakurai, K.; Iba, H. miR-21 Gene expression triggered by AP-1 is sustained through a double-negative feedback mechanism. *Journal of molecular biology* **2008**, *378*, 492-504.
69. Palacios, V.G.; Robinson, L.J.; Borysenko, C.W.; Lehmann, T.; Kalla, S.E.; Blair, H.C. Negative regulation of RANKL-induced osteoclastic differentiation in RAW264. 7 cells by estrogen and phytoestrogens. *Journal of Biological Chemistry* **2005**, *280*, 13720-13727.
70. Sugatani, T.; Hruska, K.A. Down-regulation of miR-21 biogenesis by estrogen action contributes to osteoclastic apoptosis. *Journal of cellular biochemistry* **2013**, *114*, 1217-1222.
71. Franceschetti, T.; Kessler, C.B.; Lee, S.-K.; Delany, A.M. miR-29 promotes murine osteoclastogenesis by regulating osteoclast commitment and migration. *Journal of Biological Chemistry* **2013**, *288*, 33347-33360.
72. Mizoguchi, F.; Murakami, Y.; Saito, T.; Miyasaka, N.; Kohsaka, H. miR-31 controls osteoclast formation and bone resorption by targeting RhoA. *Arthritis research & therapy* **2013**, *15*, R102.
73. Wang, Y.; Li, L.; Moore, B.T.; Peng, X.-H.; Fang, X.; Lappe, J.M.; Recker, R.R.; Xiao, P. MiR-133a in human circulating monocytes: a potential biomarker associated with postmenopausal osteoporosis. *PloS one* **2012**, *7*, e34641.
74. Cao, Z.; Moore, B.T.; Wang, Y.; Peng, X.-H.; Lappe, J.M.; Recker, R.R.; Xiao, P. MiR-422a as a potential cellular microRNA biomarker for postmenopausal osteoporosis. *PloS one* **2014**, *9*, e97098.
75. Cheng, P.; Chen, C.; He, H.B.; Hu, R.; Zhou, H.D.; Xie, H.; Zhu, W.; Dai, R.C.; Wu, X.P.; Liao, E.Y. miR-148a regulates osteoclastogenesis by targeting V-maf musculoaponeurotic fibrosarcoma oncogene homolog B. *Journal of Bone and Mineral Research* **2013**, *28*, 1180-1190.
76. Liu, J.; Dang, L.; Li, D.; Liang, C.; He, X.; Wu, H.; Qian, A.; Yang, Z.; Au, D.W.; Chiang, M.W. A delivery system specifically approaching bone resorption surfaces to facilitate therapeutic modulation of microRNAs in osteoclasts. *Biomaterials* **2015**, *52*, 148-160.
77. Ke, K.; Sul, O.-J.; Rajasekaran, M.; Choi, H.-S. MicroRNA-183 increases osteoclastogenesis by repressing heme oxygenase-1. *Bone* **2015**, *81*, 237-246.
78. Zhao, C.; Sun, W.; Zhang, P.; Ling, S.; Li, Y.; Zhao, D.; Peng, J.; Wang, A.; Li, Q.; Song, J. miR-214 promotes osteoclastogenesis by targeting Pten/PI3k/Akt pathway. *RNA biology* **2015**, *12*, 343-353.
79. Ell, B.; Kang, Y. MicroRNAs as regulators of bone homeostasis and bone metastasis. *BoneKEy reports* **2014**, *3*.
80. Sugatani, T.; Hruska, K.A. Impaired micro-RNA pathways diminish osteoclast differentiation and function. *Journal of Biological Chemistry* **2009**, *284*, 4667-4678.
81. Shibuya, H.; Nakasa, T.; Adachi, N.; Nagata, Y.; Ishikawa, M.; Deie, M.; Suzuki, O.; Ochi, M. Overexpression of microRNA-223 in rheumatoid arthritis synovium controls osteoclast differentiation. *Modern rheumatology* **2013**, *23*, 674-685.
82. Fukao, T.; Fukuda, Y.; Kiga, K.; Sharif, J.; Hino, K.; Enomoto, Y.; Kawamura, A.; Nakamura, K.; Takeuchi, T.; Tanabe, M. An evolutionarily conserved mechanism for microRNA-223 expression revealed by microRNA gene profiling. *Cell* **2007**, *129*, 617-631.

83. Sugatani, T.; Hruska, K. MicroRNA-223 is a key factor in osteoclast differentiation. *Journal of cellular biochemistry* **2007**, *101*, 996-999.
84. Bunt, J.; Lim, J.W.; Zhao, L.; Mason, S.; Richards, L.J. PAX6 does not regulate Nfia and Nfib expression during neocortical development. *Scientific reports* **2015**, *5*, 10668.
85. O'Connell, R.M. MicroRNAs function on a new level. *Blood* **2012**, *119*, 3875-3876.
86. Fazi, F.; Rosa, A.; Fatica, A.; Gelmetti, V.; De Marchis, M.L.; Nervi, C.; Bozzoni, I. A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBP α regulates human granulopoiesis. *Cell* **2005**, *123*, 819-831.
87. Liu, T.; Qin, A.-P.; Liao, B.; Shao, H.-G.; Guo, L.-J.; Xie, G.-Q.; Yang, L.; Jiang, T.-J. A novel microRNA regulates osteoclast differentiation via targeting protein inhibitor of activated STAT3 (PIAS3). *Bone* **2014**, *67*, 156-165.
88. Dou, C.; Zhang, C.; Kang, F.; Yang, X.; Jiang, H.; Bai, Y.; Xiang, J.; Xu, J.; Dong, S. MiR-7b directly targets DC-STAMP causing suppression of NFATc1 and c-Fos signaling during osteoclast fusion and differentiation. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms* **2014**, *1839*, 1084-1096.
89. Kim, K.; Kim, J.H.; Kim, I.; Lee, J.; Seong, S.; Park, Y.-W.; Kim, N. MicroRNA-26a regulates RANKL-induced osteoclast formation. *Molecules and cells* **2015**, *38*, 75.
90. Lee, Y.; Kim, H.J.; Park, C.K.; Kim, Y.-G.; Lee, H.-J.; Kim, J.-Y.; Kim, H.-H. MicroRNA-124 regulates osteoclast differentiation. *Bone* **2013**, *56*, 383-389.
91. Guo, L.-J.; Liao, L.; Yang, L.; Li, Y.; Jiang, T.-J. MiR-125a TNF receptor-associated factor 6 to inhibit osteoclastogenesis. *Experimental cell research* **2014**, *321*, 142-152.
92. Nakasa, T.; Shibuya, H.; Nagata, Y.; Niimoto, T.; Ochi, M. The inhibitory effect of microRNA-146a expression on bone destruction in collagen-induced arthritis. *Arthritis & Rheumatism* **2011**, *63*, 1582-1590.
93. Lu, L.-F.; Boldin, M.P.; Chaudhry, A.; Lin, L.-L.; Taganov, K.D.; Hanada, T.; Yoshimura, A.; Baltimore, D.; Rudensky, A.Y. Function of miR-146a in controlling Treg cell-mediated regulation of Th1 responses. *Cell* **2010**, *142*, 914-929.
94. Yao, Y.; Jia, T.; Pan, Y.; Gou, H.; Li, Y.; Sun, Y.; Zhang, R.; Zhang, K.; Lin, G.; Xie, J. Using a novel microRNA delivery system to inhibit osteoclastogenesis. *International journal of molecular sciences* **2015**, *16*, 8337-8350.
95. Qu, B.; Xia, X.; Yan, M.; Gong, K.; Deng, S.; Huang, G.; Ma, Z.; Pan, X. miR-218 is involved in the negative regulation of osteoclastogenesis and bone resorption by partial suppression of p38MAPK-c-Fos-NFATc1 signaling: Potential role for osteopenic diseases. *Experimental cell research* **2015**, *338*, 89-96.
96. Chen, C.; Cheng, P.; Xie, H.; Zhou, H.D.; Wu, X.P.; Liao, E.Y.; Luo, X.H. MiR-503 regulates osteoclastogenesis via targeting RANK. *Journal of Bone and Mineral Research* **2014**, *29*, 338-347.
97. Krzeszinski, J.Y.; Wei, W.; Huynh, H.; Jin, Z.; Wang, X.; Chang, T.-C.; Xie, X.-J.; He, L.; Mangala, L.S.; Lopez-Berestein, G. miR-34a blocks osteoporosis and bone metastasis by inhibiting osteoclastogenesis and Tgif2. *Nature* **2014**, *512*, 431.