BMB
Reports

BMB Rep. 2015; 48(6): 319-323
www.bmbreports.org

Contributed Mini Review

The role of microRNAs in cell fate determination of mesenchymal stem cells : balancing adipogenesis and osteogenesis

Hara Kang[1,*] *& Akiko Hata*[2]

[1]Division of Life Sciences, College of Life Sciences and Bioengineering, Incheon National University, Incheon 406-772, Korea, [2]Cardiovascular Research Institute, University of California, San Francisco, CA 94158, USA

Mesenchymal stem cells (MSCs) are multipotent stem cells capable of differentiating into adipocytes, osteoblasts, or chondrocytes. A mutually inhibitory relationship exists between osteogenic and adipogenic lineage commitment and differentiation. Such cell fate decision is regulated by several signaling pathways, including Wnt and bone morphogenetic protein (BMP). Accumulating evidence indicates that microRNAs (miRNAs) act as switches for MSCs to differentiate into either osteogenic or adipogenic lineage. Different miRNAs have been reported to regulate a master transcription factor for osteogenesis, such as Runx2, as well as molecules in the Wnt or BMP signaling pathway, and control the balance between osteoblast and adipocyte differentiation. Here, we discuss recent advancement of the cell fate decision of MSCs by miRNAs and their targets. [BMB Reports 2015; 48(6): 319-323]

INTRODUCTION

MSCs are capable of differentiate into several distinct cell types, including osteoblasts and adipocytes. The osteogenesis and adipogenesis of MSCs maintain a homeostasis under physiological conditions. It is often found that the signal, which promotes one cell fate, actively represses the alternative fate (1). The balance between osteogenic and adipogenic differentiation is tightly regulated by multiple signaling pathways. Dysregulation of this balance is known to lead to various human diseases, such as osteopotosis which is often associated with a significant increase in adipocytes accumulation at an expense of bone loss (2). On the contrary, patients with a high bone mass phenotype often exhibit reduced fat tissue volume (3). Therefore, clear understanding of the control mechanism of maintenance of this balance between osteogenic and adipogenic differentiation of MSCs is of great importance to elucidate the pathogenesis and a development of novel and effective therapies for bone diseases.

The adipogenic and osteogenic differentiation from MSCs is regulated by multiple regulatory factors and signaling pathways, such as the Wnt/β-catenin, TGFβ/BMPs/Smads, Notch, JAK/STAT, MAPK, phosphatidylinositol-3 kinase (PI3K)/Akt and Hedgehog pathways (4-6). Osteoblast development is governed by the activation of Wnt/β-catenin signaling. Wnt signaling through Frizzled and its co-receptors, low-density lipoprotein receptor-related protein (LRP) 5/6, inhibits the Axin /GSK3/APC complex, and β-catenin accumulates in the nucleus, which then directly regulates osteoblast activity (7). Transcription factors, such as Runt-related transcription factor 2 (Runx2) and Osterix (Osx), lead to the terminal osteoblast differentiation, which is characterized by the calcification of the extracellular matrix (5). Alkaline phosphatase (ALP), osteopontin (Opn) and osteocalcin (Ocn) are involved in the mineralization process. BMP signaling is also a central signaling pathway involved in the induction of osteogenic differentiation and regulation of bone formation. Specifically, BMP-2 is the most frequently studied ligand of BMPs that promotes osteogenic commitment and terminal osteogenic differentiation in MSCs. Gene regulation mediated by several transcription factors play a critical role to form mature adipocytes from MSCs (8, 9). CCAAT/enhancer binding protein (C/EBP) β and δ activate C/EBPα and peroxisome proliferator-activated receptor γ (PPARγ) to coordinate the expression of adipogenic genes characteristic of terminally differentiated adipocytes. PPARγ is regarded as a master transcriptional regulator of both adipocyte differentiation and lipid storage in mature adipocytes.

miRNAs are evolutionarily conserved short (19-25 nt) noncoding RNAs that mainly regulate gene expression in a posttranscriptional manner. miRNAs function via partially complementary base pairing with the 3′-untranslated region (UTR) of target mRNAs. miRNA and target mRNA pairing typically results in gene silencing via translational repression and/or destabilization of mRNA (10). Many studies suggest that miRNAs critically regulate fate decisions of stem cells, including self-renewal and differentiation. Conversely, miRNAs also critical during the reprogramming of differentiated somatic cells to generate induced pluripotent stem (iPS) cells (11). During a

*Corresponding author. Tel: +82-32-835-8238; Fax: +82-32-835-0763; E-mail: harakang@incheon.ac.kr

http://dx.doi.org/10.5483/BMBRep.2015.48.6.206

Received 24 September 2014

Keywords: Adipogenesis, Mesenchymal stem cells, MicroRNAs, Osteogenesis

ISSN: 1976-670X (electronic edition)

pluripotent state, transcription factors which are required to promote cellular differentiation are downregulated by miRNAs. Once decision to exit from a pluripotent state is made, lineage-specific miRNAs are induced, which inhibit transcription factors specific for the pluripotent state, such as Sox2, Oct4 and Nanog.

Emerging evidence suggests that miRNAs are involved in regulating the differentiation and cell fate decisions of MSCs (12). In human bone marrow-derived MSCs, silencing of Dicer or Drosha, two key enzymes in the miRNA biogenesis pathway, inhibits both osteogenic and adipogenic differentiation (13). Recently, miR-196a, -29b, -2861, -3960 and -335-5p are reported to enhance osteogenic differentiation (14-17), while miR-26a, -133, -135, -141 and -200a could impede osteogenic differentiation (18-20), and miR-143, -24, -31, -30c and -642a-3p are involved in regulating adipogenesis (21-24). Although many miRNAs have been identified to regulate either adipogenesis or osteogenesis, only a few were implicated in both processes and play a role in balancing these two cell fates.

This review focuses on miRNAs that function as mediators of the balance between the adipogenesis and osteogenesis of MSCs. These miRNAs determine the adipogenic versus osteogenic fates of MSCs by modulating Wnt or BMP signaling via the repression of components of the signaling pathway or regulating key transcription factors in the differentiation of MSCs, such as Runx2 (Table 1).

miRNAs THAT DETERMINE ADIPOGENIC DIFFERENTIATION

Each member of the miR-30 family (miR-30a-e) is differentially regulated during adipocyte and osteoblast differentiation (25). miR-30e is the most prominently regulated during adipogenesis and osteogenesis (26). miR-30e is induced in the mesenchymal cell line C3H10T1/2 and the pre-adipocyte 3T3-L1 in response to treatment of adipocyte-inducing medium. Conversely, the expression of miR-30e is reduced in the mouse stromal line ST2 and pre-osteoblast MC3T3-E1 after treatment of osteocyte-inducing medium. The overexpression of miR-30e promotes pre-adipocytes to differentiate into mature adipocytes, along with increased expression of adipocyte-specific transcription factors, such as PPARc, C/EBPα and C/EBPβ (26). The overexpression of miR-30e inhibits osteoblast differentiation, characterized by reduced expression of pro-osteogenic transcription factors, such as Runx2, Osx, Ocn, ALP and bone sialoprotein (BSP). The inhibition of the endogenous miR-30e represses the differentiation of pre-adipocytes and potentiates the osteoblast differentiation (26). LRP6 is shown to be a direct target of miR-30e (26). The knockdown of LRP6 in 3T3-L1 cells downregulates β-catenin/T-cell factor (TCF)-mediated gene expression and potentiates the differentiation into mature adipocytes. These results demonstrate that miR-30e controls the balance of adipocyte differentiation and osteoblast differentiation by modulating the canonical Wnt signaling (Fig. 1). The levels of miR-30c and miR-30d are also increased during adipocyte differentiation, but decreased during osteoblast differentiation similar to miR-30e (25). miR-30c and miR-30d are found to target Smad1, a signal transducer of BMP signaling pathway, and inhibit BMP-mediated osteoblast differentiation. Therefore, miR-30c and miR-30d are also mediators to balance the osteogenesis and adipogenesis via regulating BMP signaling (Fig. 1).

miRNA expression profiling in human adipose-derived mesenchymal stem cells (hADSCs) find that the miR-17 cluster of family of miRNAs, miR-17-5p, miR-106a and miR-20a, are downregulated when the cell undergoes osteogenic differentiation while upregulated during adipocyte differentiation (27). The overexpression of miR-17-5p and miR-106a inhibits the ALP activity, mineralization and expression of the osteogenic transcription factors, such as Runx2, Osx, Opn and Ocn. The downregulation of the endogenous miR-17-5p and miR-106a

Table 1. miRNAs that reciprocally regulate the differentiation of adipocytes and osteoblasts

	miRNA	Target mRNA
Osteogenic switch	miR-21	Sox2, Spry2
(Increased expression	miR-22	HDAC6
during osteogenesis)		
Adipogenic switch	miR-204	Runx2
(Increased expression	miR-211	Rnux2
during adipogenesis)	miR-17-5p	BMP2
	miR-106a	BMP2
	miR-30e	LPR6
	miR-637	Osx

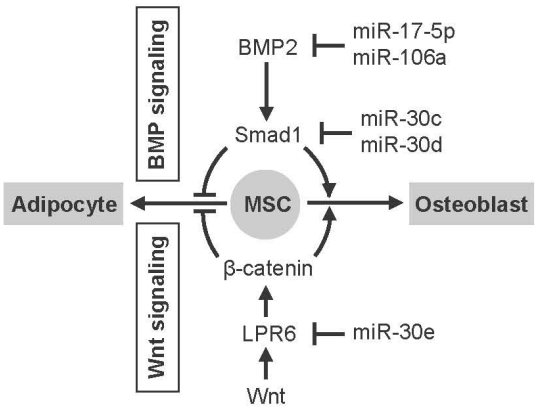

Fig. 1. miRNAs that control signaling governing osteogenesis and adipogenesis. BMP and Wnt signaling pathways have been demonstrated to preferentially induce the osteogenesis of MSCs at the expense of adipogenesis. miR-17-5p/miR-106a and miR-30c/miR-30d inhibit BMP signaling by targeting key components of the pathway, such as BMP2 and Smad1, respectively. miR-30e inhibits Wnt signaling via the repression of LPR6, a key coreceptor of Wnts.

promotes osteogenic differentiation and suppresses the adipogenic differentiation in hADSCs (27). BMP2 is identified as a direct target of miR-17-5p and miR-106a (27). Therefore, miR-17-5p and miR-106a balance the osteogenic and adipogenic lineage commitment in hADSCs by modulating BMP signaling (Fig. 1).

Runx2 is identified as a key transcription factor that regulates osteogenesis and chondrogenesis (28, 29). Regulation of Runx2 also affects the adipogenic potential of MSCs. miRNAs that regulate MSC differentiation via the modulation of Runx2 were investigated. miR-204 and miR-211 are induced during adipocyte differentiation, which downregulate Runx2 expression (30) (Fig. 2). miR-204 and miR-211 act as endogenous repressors of Runx2 in MSCs (30). The perturbation of miR-204 resultes in upregulation of osteogenesis and downregulation of adipogenesis, characterized by suppression of adipocyte marker genes, such as adipocyte protein 2 (aP2), adipsin and PPARγ (30). Conversely, when miR-204 was overexpressed, the expression levels of aP2, adipsin and PPARγ are increased, which adipocyte differentiation is promoted and osteoblast differentiation is inhibited (30). However, miR-204 inhibitor did not reverse the decrease of Runx2 levels during adipocyte differentiation, although miR-204 perturbation did significantly affect the Runx2 levels. This finding suggests that Runx2 expression is not exclusively regulated by miRNAs in MSC differentiation.

Osx, as a downstream of Runx2, is induced by BMP2 in MSCs and required for the differentiation of pre-osteoblasts into mature osteoblasts (31, 32). The cartilage is formed normally in Osx-null embryos, but they completely lack bone formation (33). miR-637 is shown to target Osx (34). The expression of miR-637 is increased during adipocyte differentiation, and decreased during osteoblast differentiation. The expression of adipogenic markers, such as PPARγ, C/EBPα and sterol regulatory element-binding protein 1c (SREBP-1c), are significantly increased in miR-637-overexpressing MSCs, but are decreased in response to a miR-637 inhibitor. Moreover, the levels of both BMP2 and Runx2 are downregulated by miR-637 and upregulated by inhibition of miR-637. These results indicate that miR-637 promotes the adipogenesis and suppresses the osteogenesis of MSCs, and maintains the balance of these two cell fates.

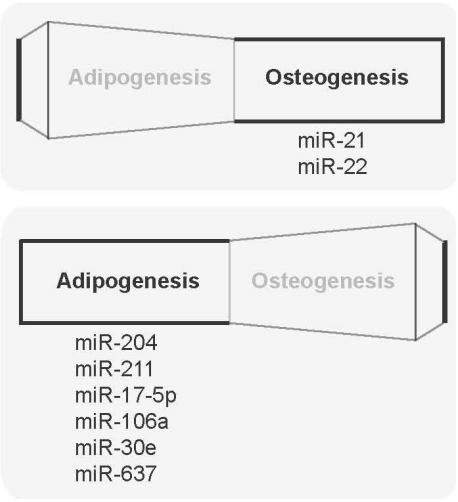

Fig. 2. miRNA switch of mesenchymal stem cell fate. The differentiation of an MSC into either an adipocyte or osteoblast can be controlled by miRNA switches. miR-21 and miR-22 switch on osteogenesis, while miR-204, miR-17-5p, miR-106a, miR-30e and miR637 switch on adipogenesis.

miRNAs THAT PROMOTE OSTEOGENIC DIFFERENTIATION

miR-22 is also found to regulate the adipogenic and osteogenic differentiation in hADSCs (35) (Fig. 2). The expression of miR-22 is decreased during adipogenic differentiation but increased during osteogenic differentiation. Consistently, the overexpression of miR-22 in hADSCs inhibits the accumulation of lipid droplets and represses the expression of adipogenic transcription factors and adipogenic-specific genes. Conversely, the enhanced ALP activity and matrix mineralization, as well as the increased expression of osteo-specific genes, indicate a positive role of miR-22 in regulating osteogenic differentiation. Histone deacetylase 6 (HDAC6), a co-repressor of Runx2 (36), is identified as a target of miR-22. Silencing endogenous HDAC6 expression in hADSCs enhances osteogenesis but represses adipogenesis, suggesting a role of the miR-22-HDAC6 axis which in turn activates Runx2 activity and osteogenic differentiation.

The ERK-MAPK signaling pathway plays a pivotal role in initiating and maintaining cell differentiation (37). The elimination of ERK activity is sufficient to maintain the self-renewal ability of embryonic stem cells, and the inhibition of MAPK signaling can convert terminally differentiated cells to a pluripotent state (37, 38). The ERK-MAPK signaling pathway has also been shown to be a major regulator of adipogenesis and osteogenesis in MSCs (39). Sprouty 1 and 2 (Spry1 and Spry2) are negative regulators of the ERK signaling pathway, and Spry2 is identified as a target of miR-21. miR-21 expression is elevated during adipogenesis and osteogenesis (40). These results suggest that miR-21 plays a critical role in maintaining the duration of the ERK-MAPK signaling pathway by repressing Spry2 expression to increase the differentiation potential of MSCs.

Furthermore, miR-21 targets Sox2 (41). Sox2 is one of four genes used to promote iPS cells and repress cell differentiation in concert with Oct4 and Nanog (42). The expression of osteogenic markers, such as Ocn and Runx2, is increased in MSCs when miR-21 is overexpressed. These results demonstrate that miR-21 not only suppresses the pluripotency but also accelerates osteogenic differentiation (Fig. 2).

CONCLUSIONS AND PERSPECTIVES

The differentiation of mesenchymal stem cells into a particular lineage is tightly regulated, and a malfunction in this regulation could lead to pathological consequences. Specifically, an inverse relationship exists between the osteogenic and adipogenic lineage commitment and differentiation, suggesting a switch between these two processes. Recent miRNA expression profiling studies during both the adipogenic and osteogenic differentiation of MSCs have found several miRNAs with an inverse expression pattern between adipogenesis and osteogenesis. These miRNAs act as switches during the fate determination of MSCs by regulating molecular signaling pathways, such as Wnt//β-catenin and BMP signaling, and multiple transcription factors. Therefore, modulation of levels of these miRNAs could serve as novel therapies for osteogenesis- or adipogenesis-related disorders. Further understanding of the miRNAs that modulate signaling pathways other than Wnt or BMP, including the TGFβ, Notch, JAK/STAT, PI3K/Akt and Hedgehog signaling pathways during MSC differentiation will provide more complete picture of the mechanisms of the cell fate decision in MSCs.

ACKNOWLEDGEMENTS

This work was supported by the Incheon National University Research Grant in 2013.

REFERENCES

1. Beresford JN, Bennett JH, Devlin C, Leboy PS, Owen ME (1992) Evidence for an inverse relationship between the differentiation of adipocytic and osteogenic cells in rat marrow stromal cell cultures. J Cell Sci 102 (Pt 2), 341-351
2. Justesen J, Stenderup K, Ebbesen EN, Mosekilde L, Steiniche T, Kassem M (2001) Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. Biogerontology 2, 165-171
3. Qiu W, Andersen TE, Bollerslev J, Mandrup S, Abdallah BM, Kassem M (2007) Patients with high bone mass phenotype exhibit enhanced osteoblast differentiation and inhibition of adipogenesis of human mesenchymal stem cells. J Bone Miner Res 22, 1720-1731
4. Rosen ED and MacDougald OA (2006) Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol 7, 885-896
5. Huang W, Yang S, Shao J, Li YP (2007) Signaling and transcriptional regulation in osteoblast commitment and differentiation. Front Biosci 12, 3068-3092
6. Stein GS, Lian JB, Stein JL, Van Wijnen AJ, Montecino M (1996) Transcriptional control of osteoblast growth and differentiation. Physiol Rev 76, 593-629
7. Chen J and Long F (2013) beta-catenin promotes bone formation and suppresses bone resorption in postnatal growing mice. J Bone Miner Res 28, 1160-1169
8. Rosen ED and Spiegelman BM (2000) Molecular regulation of adipogenesis. Annu Rev Cell Dev Biol 16, 145-171
9. Sethi JK and Vidal-Puig AJ (2007) Thematic review series: adipocyte biology. Adipose tissue function and plasticity orchestrate nutritional adaptation. J Lipid Res 48, 1253-1262
10. Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. Cell 136, 215-233
11. Lüningschrör P, Hauser S, Kaltschmidt B, Kaltschmidt C (2013) MicroRNAs in pluripotency, reprogramming and cell fate induction. Biochim Biophys Acta 1833, 1894-1903
12. Ivey KN and Srivastava D (2010) MicroRNAs as regulators of differentiation and cell fate decisions. Cell Stem Cell 7, 36-41
13. Oskowitz AZ, Lu J, Penfornis P et al (2008) Human multipotent stromal cells from bone marrow and microRNA: regulation of differentiation and leukemia inhibitory factor expression. Proc Natl Acad Sci U S A 105, 18372-18377
14. Hu R, Liu W, Li H et al (2011) A Runx2/miR-3960/miR-2861 regulatory feedback loop during mouse osteoblast differentiation. J Biol Chem 286, 12328-12339
15. Kim YJ, Bae SW, Yu SS, Bae YC, Jung JS (2009) miR-196a regulates proliferation and osteogenic differentiation in mesenchymal stem cells derived from human adipose tissue. J Bone Miner Res 24, 816-825
16. Li Z, Hassan MQ, Jafferji M et al (2009) Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. J Biol Chem 284, 15676-15684
17. Zhang J, Tu Q, Bonewald LF et al (2011) Effects of miR-335-5p in modulating osteogenic differentiation by specifically downregulating Wnt antagonist DKK1. J Bone Miner Res 26, 1953-1963
18. Li Z, Hassan MQ, Volinia S et al (2008) A microRNA signature for a BMP2-induced osteoblast lineage commitment program. Proc Natl Acad Sci U S A 105, 13906-13911
19. Itoh T, Nozawa Y and Akao Y (2009) MicroRNA-141 and -200a are involved in bone morphogenetic protein-2-induced mouse pre-osteoblast differentiation by targeting distal-less homeobox 5. J Biol Chem 284, 19272-19279
20. Luzi E, Marini F, Sala SC, Tognarini I, Galli G and Brandi ML (2008) Osteogenic differentiation of human adipose tissue-derived stem cells is modulated by the miR-26a targeting of the SMAD1 transcription factor. J Bone Miner Res 23, 287-295
21. Esau C, Kang X, Peralta E et al (2004) MicroRNA-143 regulates adipocyte differentiation. J Biol Chem 279, 52361-52365
22. Sun F, Wang J, Pan Q et al (2009) Characterization of function and regulation of miR-24-1 and miR-31. Biochem Biophys Res Commun 380, 660-665
23. Yang Z, Bian C, Zhou H et al (2011) MicroRNA hsa-miR-138 inhibits adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells through adenovirus EID-1. Stem Cells Dev 20, 259-267
24. Zaragosi LE, Wdziekonski B, Brigand KL et al (2011) Small RNA sequencing reveals miR-642a-3p as a novel adipocyte-specific microRNA and miR-30 as a key regulator of human adipogenesis. Genome Biol 12, R64
25. Wu T, Zhou H, Hong Y, Li J, Jiang X and Huang H (2012) miR-30 family members negatively regulate osteoblast differentiation. J Biol Chem 287, 7503-7511
26. Wang J, Guan X, Guo F et al (2013) miR-30e reciprocally

regulates the differentiation of adipocytes and osteoblasts by directly targeting low-density lipoprotein receptor-related protein 6. Cell Death Dis 4, e845
27. Li H, Li T, Wang S et al (2013) miR-17-5p and miR-106a are involved in the balance between osteogenic and adipogenic differentiation of adipose-derived mesenchymal stem cells. Stem Cell Res 10, 313-324
28. Komori T, Yagi H, Nomura S et al (1997) Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell 89, 755-764
29. Yoshida CA, Yamamoto H, Fujita T et al (2004) Runx2 and Runx3 are essential for chondrocyte maturation, and Runx2 regulates limb growth through induction of Indian hedgehog. Genes Dev 18, 952-963
30. Huang J, Zhao L, Xing L and Chen D (2010) MicroRNA-204 regulates Runx2 protein expression and mesenchymal progenitor cell differentiation. Stem Cells 28, 357-364
31. Ryoo HM, Lee MH and Kim YJ (2006) Critical molecular switches involved in BMP-2-induced osteogenic differentiation of mesenchymal cells. Gene 366, 51-57
32. Nishio Y, Dong Y, Paris M, O'Keefe RJ, Schwarz EM and Drissi H (2006) Runx2-mediated regulation of the zinc finger Osterix/Sp7 gene. Gene 372, 62-70
33. Zhang C, Cho K, Huang Y et al (2008) Inhibition of Wnt signaling by the osteoblast-specific transcription factor Osterix. Proc Natl Acad Sci U S A 105, 6936-6941
34. Zhang JF, Fu WM, He ML et al (2011) MiR-637 maintains the balance between adipocytes and osteoblasts by directly targeting Osterix. Mol Biol Cell 22, 3955-3961
35. Huang S, Wang S, Bian C et al (2012) Upregulation of miR-22 promotes osteogenic differentiation and inhibits adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells by repressing HDAC6 protein expression. Stem Cells Dev 21, 2531-2540
36. Westendorf JJ, Zaidi SK, Cascino JE et al (2002) Runx2 (Cbfa1, AML-3) interacts with histone deacetylase 6 and represses the p21(CIP1/WAF1) promoter. Mol Cell Biol 22, 7982-7992
37. Chen S, Do JT, Zhang Q et al (2006) Self-renewal of embryonic stem cells by a small molecule. Proc Natl Acad Sci U S A 103, 17266-17271
38. Li W and Ding S (2010) Small molecules that modulate embryonic stem cell fate and somatic cell reprogramming. Trends Pharmacol Sci 31, 36-45
39. Ge C, Xiao G, Jiang D and Franceschi RT (2007) Critical role of the extracellular signal-regulated kinase-MAPK pathway in osteoblast differentiation and skeletal development. J Cell Biol 176, 709-718
40. Mei Y, Bian C, Li J et al (2013) miR-21 modulates the ERK-MAPK signaling pathway by regulating SPRY2 expression during human mesenchymal stem cell differentiation. J Cell Biochem 114, 1374-1384
41. Trohatou O, Zagoura D, Bitsika V et al (2014) Sox2 suppression by miR-21 governs human mesenchymal stem cell properties. Stem Cells Transl Med 3, 54-68
42. Boyer LA, Lee TI, Cole MF et al (2005) Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 122, 947-956