Histol Histopathol (2017) 32: 1-10

http://www.hh.um.es

Histology and Histopathology

From Cell Biology to Tissue Engineering

Review

Regulatory role of microRNAs in the proliferation and differentiation of adipose-derived stem cells

Doo Yeong Kim and Jong-Hyuk Sung

Yonsei Institute of Pharmaceutical Sciences, College of Pharmacy, Yonsei University, Incheon, Korea

Summary. Adipose-derived stem cells (ASCs) are multipotent mesenchymal stem cells obtained from stromal-vascular fraction of adipose tissue. ASCs are a promising resource for cell therapy due to their simple isolation, extensive expansion potential, and low immunogenicity. ASCs repair and regenerate damaged tissue by direct differentiation, whereas many other approaches rely on the secretion of paracrine factors. miRNAs target mRNAs for cleavage or translational repression, and have been shown to play critical roles in the regulation of stem cell proliferation and differentiation. The miRNA expression profile of ASCs varies according to the isolation and culturing method, and more than 40 different miRNAs have been reported to regulate ASC proliferation and differentiation. Therefore, this review summarizes the ASC-related miRNAs and their pivotal roles in regulating the proliferation and differentiation of ASCs. A comprehensive understanding of the effects of miRNAs on the proliferation and differentiation of ASCs is important and useful to enhance the regenerative potential of ASCs.

Key words: MicroRNAs, Adipose-derived stem cells, Proliferation, Differentiation

Offprint requests to: Jong-Hyuk Sung, Ph.D., College of Pharmacy, Yonsei University, 85 Songdogwahakro, Yeonsu-gu, Incheon, 406-840, Korea. e-mail: brian99@empas.com or brian99@yonsei.ac.kr

DOI: 10.14670/HH-11-798

Introduction

microRNAs (miRNAs) are short non-coding RNAs that are involved in the post-transcriptional regulation of gene expression and influence diverse biological activities (Lee et al., 1993; Wightman et al., 1993). Most mature miRNAs are produced from long pri-miRNAs, ranging from hundreds to thousands of nucleotides, through cleavage to hairpin pre-miRNAs by DROSHA/DGCR8 in the nucleus, and subsequent cleavage to the mature miRNA by DICER in the cytoplasm (Kim et al., 2009a-d; Winter et al., 2009). The mature miRNA is then incorporated into the RNA-induced silencing complex to regulate gene expression. Genomic studies have revealed that a single miRNA can regulate hundreds of targets (Lewis et al., 2005; Helwak et al., 2013). Therefore, miRNAs constitute a complex regulatory network, and emerging evidence suggests that they are closely involved in controlling the key steps of stem cell proliferation and differentiation (Schoolmeesters et al., 2009; Lakshmipathy et al., 2010; Zhang et al., 2012). In addition, miRNAs are crucial for self-renewal and act as important regulators of pluripotency in embryonic stem cells (Tay et al., 2008a,b; Lakshmipathy et al., 2010). For example, Tay et al. identified three miRNAs (miR-134, miR-296, and miR-470) that inhibit the expession of OCT4, SOX2, and NANOG, and as such, affect the differentiation of embryonic stem cells (Tay et al., 2008a,b).

As described, miRNAs have diverse roles in regulating stem cell function and pluripotency, but their roles in mesenchymal stem cells (MSCs) have been poorly understood. The global miRNA expression profile of MSCs varies depending on the tissue of origin,

species, and detection methodology, although certain miRNAs are consistently found in all types of MSCs (Schoolmeesters et al., 2009; Chen and Lim, 2013; Yoo et al., 2014). Of MSCs, adipose-derived stem cells (ASCs) are multipotent stem cells present in the stromal-vascular fraction of adipose tissue, and can differentiate into adipocytes, chondrocytes and osteoblasts (Zuk et al., 2001, 2002). ASCs are ideal candidates for cell-based therapies due to their ease of isolation, extensive expansion potential, and low immunogenicity. ASCs are expected to contribute to the replacement of damaged tissue (i.e., bone and cartilage) by direct differentiation, as they secret paracrine factors to modulate the immune response and promote angiogenesis (Gimble et al., 2007, 2011; Kim et al., 2007, 2009a-d; Park et al., 2008). A number of recent studies have reported that miRNAs have multiple functions in the proliferation and differentiation of ASCs (Kim et al., 2009c; Zhang et al., 2012b; Chen et al., 2014a; Martin et al., 2016). For example, miR-21 regulated the proliferation of ASCs and induced adipogenic differentiation (Kim et al., 2009c). To date there is only one review summarizing how miRNAs regulate ASCs, and it describes the role of miRNAs in ASCs differentiation (Chen et al., 2014a). Therefore, the present review focuses on miRNAs and their role in the regulation of both the proliferation and differentiation of ASCs, and provides updates on the recent advances in the miRNA-based regulation of ASCs.

miRNAs involved in the proliferation of ASCs

As described, many miRNAs have functions in both the proliferation and differentiation of ASCs, and miRNAs related to ASC proliferation are summarized in Fig. 1 and Table 1. Jung's group is a pioneer in the miRNA-dependent regulation of ASCs, and they reported several miRNAs that regulate the proliferation of ASCs (Kim et al., 2009a,b). For example, miR-196a decreased the proliferation of ASCs by targeting HOXC8 (Kim et al., 2009a-d), and miR-21 regulated the proliferation of human (h)ASCs acting through the SMAD3 pathway (Kim et al., 2009a-d). miR-486-5p induced replicative senescence by repression of SIRT1, and inhibited the proliferation of hASCs (Kim et al., 2012). Similarly, miR-137 also inhibited the proliferation of hASCs by directly targeting CDC42 (Shin et al., 2014). In addition, Chen et al., reported that miR-363 inhibited mitotic clonal expansion of ASCs through E2F3 inhibition (Chen et al., 2014b).

By contrast, miR-302d induced proliferation and inhibited the oxidant-induced cell death in ASCs through its targeting of CDKN1A and CCL5 (Kim et al., 2014). Transfection of ASCs with members of the miR-302 family upregulated the mRNA expression levels of pluripotency markers such as OCT4, NANOG, and SOX2 (Taha et al., 2014). Our group also found that miR-210 responded to hypoxia and reactive oxygen species (ROS), and increased the proliferation and migration of ASCs through PTPN2 downregulation (Kim et al., 2013). Furthermore, ROS generation increased phosphorylation of NF-κB and ELK1, and, in turn, these transcription factors regulated miR-210 expression in ASCs (Kim et al., 2013). As ASC expansion is required to increase the cell number prior to transplantation, modulation of these miRNAs could be used to increase the yield during large-scale production. Although their direct targets have not been identified, miR-34a and miR-1908 also reportedly regulated the proliferation of ASCs (Park et al., 2015; Yang et al., 2015).

miRNAs and differentiation

ASCs share many properties with other MSCs, such as the potential for differentiating into multiple cell types. ASCs can differentiate into adipogenic, osteogenic, chondrogenic, hepatogenic, and neurogenic

Table 1. miRNAs and ASC proliferation.

miRNA	Target	Function	Reference
miR-34a	not identified	miR-34a decreased ASC proliferation Expression of miR-34a was increased as the cell passage number was increased	Park et al., 2015
miR-1908	not identified	miR-1908 overexpression increased ASC proliferation	Yang et al., 2015
miR-302	not identified	Transfection of ASCs with miR-302 family members upregulated the mRNA expression levels of OCT4, NANOG, and SOX2 mRNAs	Taha et al., 2014
miR-302d	CDKN1A, CCL5	miR-302d induced proliferation and inhibited oxidant-induced cell death in ASCs	Kim et al., 2014
miR-363	E2F3	miR-363 inhibited mitotic clonal expansion and terminal differentiation of ASCs	Chen et al., 2014b
miR-137	CDC42	miR-137 inhibited the proliferation and adipogenic differentiation of hASCs by directly targeting CDC42	Shin et al., 2014
miR-210	PTPN2	miR-210 responded to ROS, and increased the proliferation and migration of ASCs NF-κB and Elk1 regulated miR-210 expression	Kim et al., 2013
miR-486-5p	SIRT1	miR-486-5p induced replicative senescence and inhibited proliferation of hASCs	Kim et al., 2012
miR-21	TGF receptor-β2	miR-21 regulated the proliferation and adipogenic differentiation of hASCs by acting through the SMAD3 pathway	Kim et al., 2009c
miR196a	HOXC8	miR-196a decreased hASC proliferation and osteogenic differentiation by targeting HOXC8	Kim et al., 2009d

lineages (Zuk et al., 2001, 2002). Therefore, this chapter summarizes miRNAs involved in various differentiation routes of ASCs.

microRNAs involved in adipocyte differentiation

ASCs originate from adipose tissue. They are the precursors of preadipocytes, which eventually differentiate into adipocytes. Adipogenic differentiation, including the increase in the size and number of adipocytes, induces adipose tissue expansion. Therefore, there is plentiful evidence of the involvement of miRNAs in the regulation of adipocyte differentiation of ASCs (Fig. 2, Table 2). For example, Tang et al. used the miRNA-chip technology to examine changes in miRNA expression, and found that miRNA-31, miR-125b-5p, and miRNA-326 were significantly deregulated in association with adipogenic differentiation (Tang et al., 2009). Chen et al. also performed a microarray analysis and found that miR-363 was downregulated during adipogenic differentiation, and its overexpression in ASCs was associated with the inhibition of the mitotic clonal expansion and terminal differentiation through the reduction of E2F3 expression (Chen et al., 2014a-d). miRNA-138 also played an inhibitory role during the adipogenic differentiation of ASCs by targeting EID-1 (Yang et al., 2011). Similarly, miR-22 inhibited ASC adipogenic differentiation through HDAC6 inhibition (Huang et al., 2012).

Peroxisome proliferator-activated receptor-γ (PPAR-γ) is present mainly in adipose tissue, and regulates fatty acid storage and glucose metabolism (Spiegelman et al., 1997; Spiegelman, 1998; Fajas et al., 2001). PPAR-γ acts as both a receptor and transcription factor, and genes activated by PPAR-γ stimulate lipid uptake and adipogenesis. Therefore, PPAR-γ is a key player in adipogenic differentiation, and miRNAs that target PPAR-γ to inhibit adipogenesis have been reported (Lee et al., 2011; Chen et al., 2015b). miR-130 and miR-540 impaired adipogenesis by acting on this transcription factor (Lee et al., 2011; Chen et al., 2015a,b). miR-27b also exhibited an inhibitory effect on adipogenic differentiation of hASCs that was mediated by the repression of PPAR-γ (Karbiener et al., 2009). Concomitantly, overexpression of miR-27a or miR-27b inhibited adipocyte differentiation by regulating PPAR-γ and PROHIBITIN expression (Karbiener et al., 2009; Kang et al., 2013).

Conversely, some miRNAs induce the adipogenic differentiation of ASCs. The expression of miR-21 was transiently increased after induction of adipogenic differentiation, and miR-21 induced adipogenesis (Kim et al., 2009c). miR-21 was a negative mediator of TGF-β signaling in hASCs as it impaired SMAD3

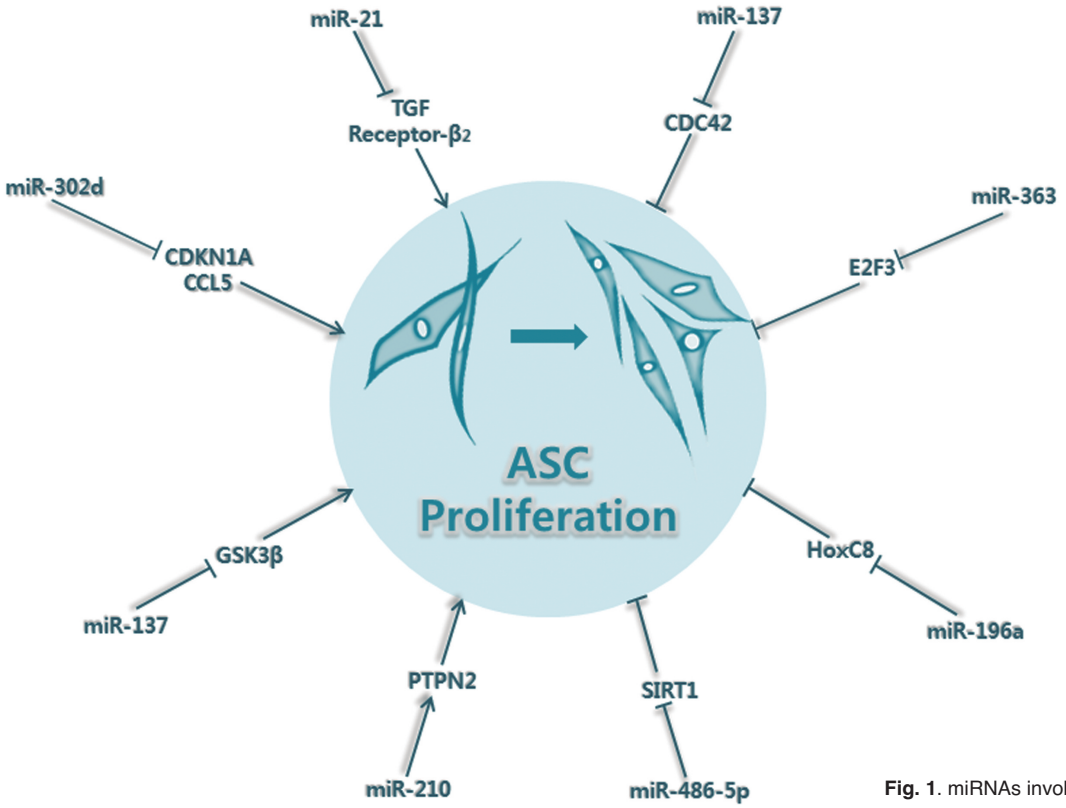

Fig. 1. miRNAs involved in the proliferation ASCs.

Table 2. miRNAs and adipogenic differentiation.

miRNA	Target	Function	Reference
miR-34a	not identified	miR-34a inhibited adipogenesis and osteogenesis of ASCs	Park et al., 2015
miR-140	NEAT1	miR-140 knockout in ASCs dramatically decreased adipogenic differentiation	Gernapudi et al., 2015
miR-125a-3p, miR-483-5p	RhoA, ERK1	miR-125a-3p and miR-483-5p coordinately promoted adipogenesis by suppressing the RhoA/ROCK1/ERK1/2 pathway	Chen et al., 2015a
miR-148a	WNT1	miR-148a is a human and murine obesity biomarker miR-148a promoted adipocyte differentiation	Shi et al., 2015
miR-204-5p	DVL3	miR-204-5p promoted adipogenesis by controlling DVL3 expression and subsequently inhibiting the activation of the WNT/β-catenin signaling pathway	He et al., 2015
miR-540	PPAR-γ	miR-540 is negative regulator of adipogenic differentiation	Chen et al., 2015b
miR-1908	not identified	miR-1908 inhibited adipogenic differentiation	Yang et al., 2015
miR-146b	KLF7	miR-146b overexpression promoted adipogenic differentiation	Chen et al., 2014c
miR-137	CDC42	miR-137 inhibited adipogenic differentiation of hASCs	Shin et al., 2014
miR-27	PROHIBITIN	Overexpression of miR-27a or miR-27b inhibited PROHIBITIN expression and adipocyte differentiation	Kang et al., 2013
miR-27b	PPARγ	miR-27b abundance decreased during adipogenesis miR-27b inhibited PPARγ and C/EBPα	Karbiener et al., 2009
miR-363	E2F3	miR-363 is a novel regulator of adipogenesis miR-363 inhibited mitotic clonal expansion and terminal differentiation of ASCs	Chen et al., 2014b
miR-17-5p, miR-106a	BMP2	miR-17-5p and miR-106a have dual functions; both promoted adipogenesis and inhibited osteogenesis	Li et al., 2013
miR-22	HDAC6	Upregulation of miR-22 inhibited adipogenic differentiation	Huang et al., 2012
miR-30c	PAI-1, ALK2	miR-30c expression was increased during adipogenesis; miR-30c overexpression induced the expression of adipocyte marker genes and triglyceride accumulation	Karbiener et al., 2011
miR-30	RUNX2	Expression of miR-30 family members was up-regulated during adipogenic differentiation miR-30a and miR-30d stimulated adipogenesis	Zaragosi et al., 2011
miR-130	PPARγ	Overexpression of miR-130 impaired adipogenesis and inhibition of miR-130 enhanced adipogenesis	Lee et al., 2011
miR-138	EID-1	miR-138 inhibits adipogenic differentiation of hASCs	Yang et al., 2011
miR-21	TGF receptor -β2	miR-21 expression was transiently increased after induction of adipogenic differentiation miR-21 induced adipogenesis	Kim et al., 2009c
miR-31, miR-326	not identified	miR-31 and miR-326 were downregulated in the process of ASC adipogenic differentiation	Tang et al., 2009

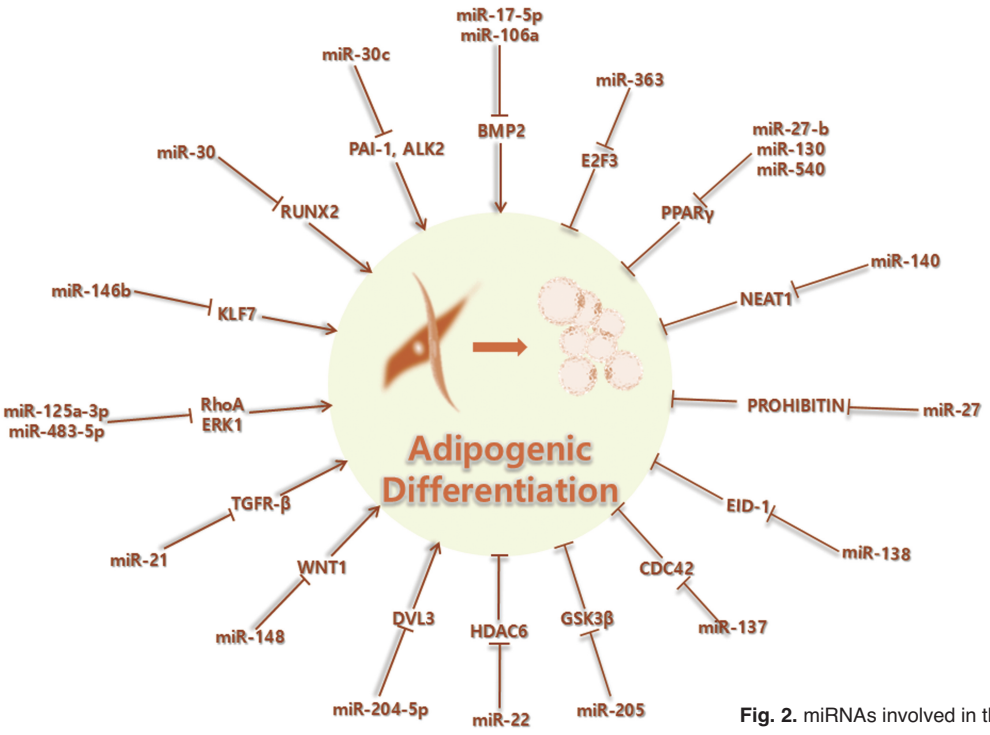

Fig. 2. miRNAs involved in the adipogenic differentiation of ASCs.

phosphorylation. miR-30a and miR-30d functionally stimulated adipogenesis by downregulating the transcription factor RUNX2, a major regulator of osteogenesis (Zaragosi et al., 2011). miR-30c expression was also increased during adipogenesis and its overexpression induced the expression of adipocyte marker genes and triglyceride accumulation through PAI-1 and ALK2 repression (Karbiener et al., 2011). miR-17-5p and miR-106a have dual functions, as they promoted adipogenesis as well as inhibited osteogenesis through BMP2 signaling in ASCs (Li et al., 2013). miR-125a-3p and miR-483-5p coordinately promoted adipogenesis by suppressing the RhoA/ROCK1/ERK1/2 pathway (Chen et al., 2015a,b). Similarly, miR-146b overexpression promoted adipogenic differentiation by inhibiting KLF7 (Chen et al., 2014a-c).

WNT proteins are secreted glycoproteins that act as signaling molecules and have important effects in stem cell biology and adipogenic differentiation (Bennett et al., 2002; Keats et al., 2014; Song et al., 2014). Activation of WNT/β-catenin signaling promotes differentiation of ASCs into myocytes and osteocytes while suppressing their commitment to the adipocyte lineage and terminal differentiation (Keats et al., 2014; Song et al., 2014; Shen et al., 2016). The involvement of miRNAs in the regulation of the WNT pathway has been reported. For example, miR-148a is a biomarker of obesity and it promoted adipocyte differentiation through WNT1 inhibition (Shi et al., 2015). Similarly, miR-204-5p promoted adipogenesis by controlling the expression of a WNT antagonist DVL3, and subsequently inhibiting the activation of the WNT/β-catenin signaling pathway (He et al., 2015).

miRNAs involved in osteogenic differentiation

ASCs are capable of differentiating into an osteogenic lineage. BMP signaling and its downstream mediators SMAD1 and RUNX2 play a key role during osteogenic differentiation. Thus, upon their downregulation, some osteoblast marker genes such as alkaline phosphatase, osteocalcin, osteopontin, and type I collagen became dysregulated. Multiple miRNAs have been found to regulate osteogenic differentiation of ASCs through their binding to the mRNAs of osteogenic transcription factors and blocking their translation (Fig. 3, Table 3). Zhang et al. investigated the miRNA expression profile during osteogenic differentiation of ASCs (Zhang et al., 2012b). They found that four miRNAs (miR-17, miR-20a, miR-20b, and miR-106a) were up-regulated and four miRNAs (miR-31, miR-125a-5p, miR-125b, and miR-193a) were down-regulated during ostogenic differentiation.

Overexpression of miR-100 inhibited osteogenic differentiation of hASCs through BMPR2 inhibition (Zeng et al., 2012). miR-26 expression increased during

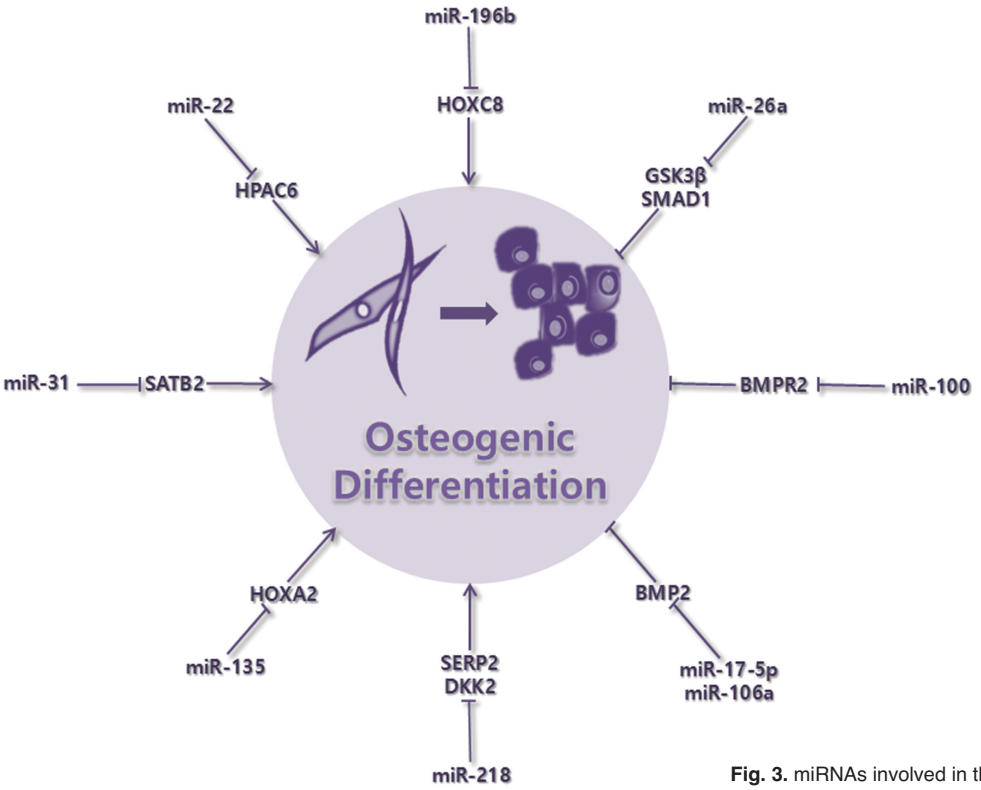

Fig. 3. miRNAs involved in the osteogenic differentiation of ASCs.

osteogenic differentiation of hASCs, but miR-26 inhibited the osteogenic differentiation of ASCs through the SMAD1 transcription factor, an important downstream mediator of BMP signaling (Luzi et al., 2008, 2012). miRNA-26a also modulated the late osteoblast differentiation of ASCs, and its expression was regulated by MEM1. However, reports on the role of miR-26a during osteogenesis are controversial (Su et al., 2015; Wang et al., 2015). Overexpression of miR-26a promoted hASC osteogenesis, and osteogenesis was repressed by miR-26a knockdown (Su et al., 2015). miR-26a directly targeted the 3'UTR of GSK3β, suppressing the expression of the GSK3β protein. In addition, miR 26a transfection significantly improved the osteogenic potency of ASCs when grown on a hydroxyapatite scaffold (Wang et al., 2015).

Certain miRNAs have been indicated as positive regulators in the osteogenic differentiation of ASCs (Kim et al., 2009a-d; Deng et al., 2013; Zhang et al., 2014). For example, Kim et al. reported that miR-196a was upregulated in osteogenic differentiation of ASCs and enhanced this process by targeting the HOXC8 gene (Kim et al., 2009a-d). Another positive regulator of osteogenic differentiation was miR-218. It was upregulated during osteogenic differentiation, and enhanced osteogenic differentiation by targeting SFRP2 and DKK2, both of which are antagonists of the WNT signaling pathway (Zhang et al., 2014). miR-31 transfection enhanced osteogenic differentiation through SATB2 inhibition, and miR-31-modified ASCs repaired rat critical-sized calvarial defects (Deng et al., 2013). miRNA-22 also acted positively on osteogenic differentiation, with enhanced alkaline phosphatase activity and increased levels of osteo-specific markers. Furthermore, miRNA-22 directly repressed HDAC6 to regulate the balance between osteogenic and adipogenic differentiation of ASCs (Huang et al., 2012). miR-135 was upregulated during osteogenesis and repressed HOXA2 expression through binding to the 3'UTR of the HOXA2 mRNA, and as such the miR-135/HOXA2/RUNX2 pathway contributed to the regulation of osteogenesis and bone regeneration (Xie et al., 2016). Finally, co-transduction of miR-148b and BMP-2 also promoted the osteogenic differentiation of hASCs (Liao et al., 2014).

Chondrogenic, neuronal and hepatogenic differentiations

Although a significant proportion of the evidence reports on the chondrogenic differentiation of ASCs, studies describing an involvement of miRNAs in chondrogenesis are few (Fig. 4, Table 4). Eight miRNAs (miRNA-193b, miRNA-199a-3p/hsa-miRNA-199b-3p, miRNA-455-3p, miRNA-210, miRNA-381, miRNA-92a, miRNA-320c and miRNA-136) were identified as being upregulated, whereas another four miRNAs (miRNA-490-5p, miRNA-4287, miRNA-BART8* and miRNA-US25-1*) were downregulated during chondrogenic differentiation (Zhang et al., 2012a). Hou et al. also found that miR-193b expression changed significantly during chondrogenesis of ASCs and that it inhibited early chondrogenesis by targeting TGF-β2 and TGFR-β3 (Hou et al., 2015a). They also found that the expression of miR-92a was elevated during the chondrogenic differentiation of hASCs, and that it enhanced the expression levels of col9a2 and aggrecan. A total of 279 genes were predicted as potential target genes of miR 92a (Hou et al., 2015b). miR-194 levels gradually decreased during the chondrogenic differentiation of hASCs (Xu et al., 2012). Down-regulation of miR-194 increased its direct target gene, SOX5, and resulted in enhanced chondrogenic differentiation of hASCs, whereas up-regulation decreased SOX5 and inhibited chondrogenesis.

Table 3. miRNAs and osteogenic differentiation.

miRNA	Target	Function	Reference
miR-135	HOXA2	miR-135 was upregulated during osteogenesis miR-135/HOXA2/RUNX2 pathway might contribute to the regulation osteogenesis and bone regeneration by ASCs	Xie et al., 2016
miR26a	GSK3β	miR-26a enhanced the osteogenic differentiation of MSCs, but inhibited the osteogenic differentiation of ASCs	Su et al., 2015
miR-26a	not identified	miR 26a transfection significantly improved the osteogenic potency of ASCs in a hydroxyapatite scaffold	Wang et al., 2015
miR-26a	SMAD1	MEM1 regulated the expression of miR-26	Luzi et al., 2012
miR-26a	SMAD1	miR-26 expression increased during osteogenic differentiation of hASCs miR-26 inhibited the osteogenic differentiation of ASCs	Luzi et al., 2008
miR-148b	not identified	Co-transduction of miR-148b and BMP-2 promoted the osteogenic differentiation of hASCs	Liao et al., 2014
miR-218	SFRP2, DKK2	miR-218 was upregulated during osteogenic differentiation and enhanced the osteogenic differentiation through the WNT/β-catenin signaling pathway	Zhang et al., 2014
miR-31	SATB2	miR-31 transfection enhanced osteogenic differentiation	Deng et al., 2013
miR-17-5p, miR-106a	BMP2	miR-17-5p and miR-106a have dual functions and they promoted adipogenesis and inhibited osteogenesis	Li et al., 2013
miR-100	BMPR2	Overexpression of miR-100 inhibited osteogenic differentiation of hASCs	Zeng et al., 2012
miR-22	HDAC6	Upregulation of miR-22 promoted osteogenic differentiation	Huang et al., 2012
miR196b	HOXC8	miR-196a enhanced osteogenic differentiation of ASCs	Kim et al., 2009d

ASCs can also differentiate into neuron-like cells and such differentiation is mediated by IGF-1 signaling (Ning et al., 2008). Both IBMX and IGF-I upregulated the neuronal differentiation and miR-133b expression in ASCs (Ning et al., 2009). Overexpression of miR-133b downregulated PITX3 and IGF-1 receptor (IGFR-1), as miR-133b targets the 3'UTR of IGFR-1 (Ning et al., 2009). IGF-1 signaling and miR-133b co-regulate potential neural differentiation of ASCs through a feedback mechanism, in which IGF-1 upregulates miR-

Table 4. miRNAs and other differentiation pathways.

miRNA	Target	Function	Reference
miR-92a	not identified	Expression of miR-92a was elevated during the chondrogenic differentiation of hASCs miR-92a enhanced the expression of col9a2 and aggrecan	Hou et al., 2015b
miR-193b	TGF-β2, TGFR-β3	miR-193b inhibited early chondrogenesis	Hou et al., 2015a
miR-194	SOX5	miR-194 levels gradually decreased during the chondrogenic differentiation of hASCs downregulation of miR-194 enhanced chondrogenic differentiation	Xu et al., 2012
miR-124	Sp1	miR-124 suppressed Sp1 expression, which in turn increased the neuronal differentiation of ASCs	Mondanizadeh et al., 2015
miR-133b	IGFR-1	IBMX and IGF-1 upregulated miR-133b expression during neurogenesis	Ning et al., 2009
Let-7f	not identified	Let-7f negatively regulated the hepatic differentiation of ASCs	Davoodian et al., 2014b
Let-7b	HNF4a, HNF6	Transient inhibition of Let-7b activated the hepatic differentiation of ASCs	Alizadeh et al., 2015
miR-122	not identified	miR-122 was upregulated during hepatic differntiation and its overexpression increased expression of several hepatocyte markers such as ALB, AFP, CK18, CK19, HNF4a	Davoodian et al., 2014a

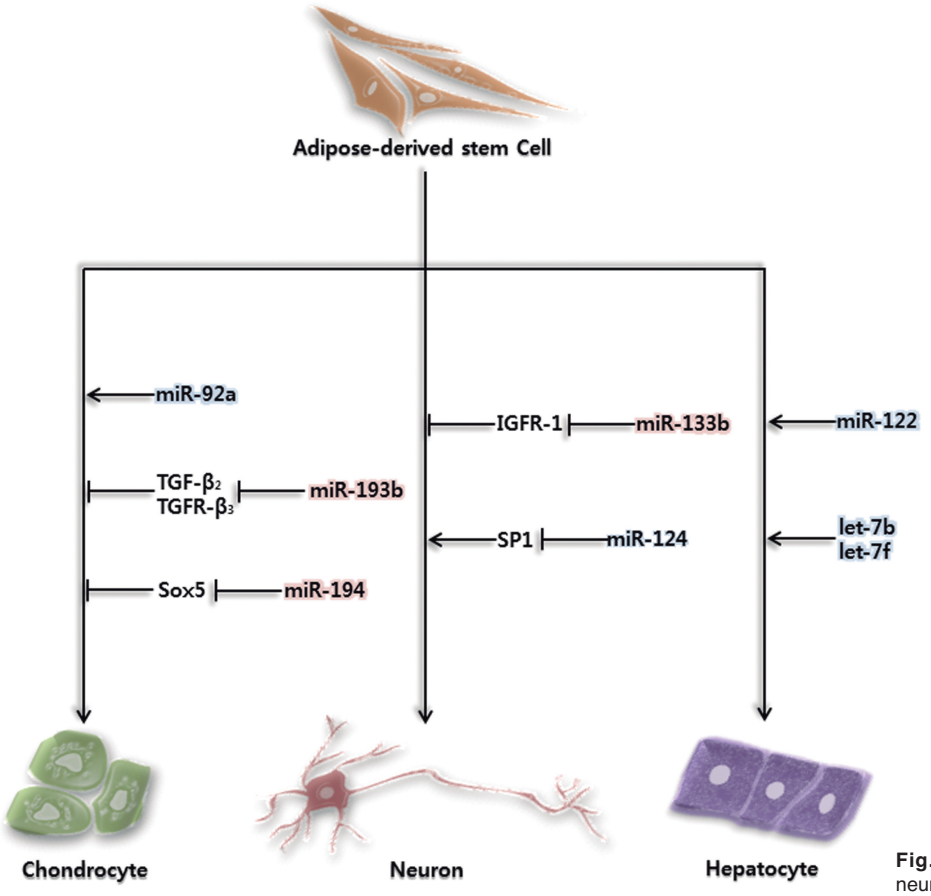

Fig. 4. miRNAs involved in the chondrogrnic, neuronal, and hepatogenic differentiation of ASCs.

133b and in turn miR-133b downregulates IGFR-1. In addition, miR-124 was expressed abundantly in neurons and increased the neuronal differentiation of ASCs, through its direct targeting of Sp1 3'UTR (Mondanizadeh et al., 2015).

miRNA involvement during hepatocyte differentiation has also been reported (Table 4). The let-7 miRNA family, and in particular let-7b, was reported to inhibit the hepatic commitment of hASCs. Inhibition of let-7b caused an upregulation of liver-enriched transcription factors (HNF4a and HNF6) and increased the expression of miR-122 (Alizadeh et al., 2015). Similarly, let-7f has been revealed as a negative regulator of hepatic differentiation, and inhibition of let-7f facilitated the induction of hASCs into hepatocyte-like cells (Davoodian et al., 2014a). miR-122 was upregulated during hepatic differentiation of hASCs, and stable miR-122 overexpression in hASCs resulted in an increased expression of hepatocyte markers such as ALB, AFP, CK18, CK19, and HNF4a (Davoodian et al., 2014a).

Conclusion

Our knowledge of the regulation of miRNAs in stem cells has expanded tremendously over the last few years. Therefore, miRNAs have become the emerging regulators in the control of stem cell self-renewal and differentiation. In this review, we focused on the biological activity of miRNAs in ASCs. Here, we describe evidence that specific miRNAs participate in ASCs proliferation and differentiation. For example, we used approximately forty references to make four tables and one figure to summarize the miRNAs that are involved in the regulation of ASCs. We have listed the miRNAs involved in the proliferation, and adipogenic and osteogenic differentiation of ASCs in separate tables. In addition, miRNAs involved in the chondrogenic, neurogenic and hepatogenic differentiation of ASCs are summarized in one table. As shown in the tables, miRNAs have diverse inhibiting and stimulating effects on the proliferation and differentiation of ASCs. Although some studies did not identify the target molecule, miRNAs have an important effect on the proliferation and differentiation of ASCs by regulating target mRNAs.

An understanding of the roles of miRNAs in the proliferation and differentiation of ASCs will greatly facilitate their application at a therapeutic level, where miRNAs can be used as direct pharmacological targets to treat ASC-associated diseases. In other words, ASCs could be genetically modified to alter their miRNA profile and regulate the expression of specific mRNAs, and such genetically modified ASCs could enhance the therapeutic potential of ASCs in the clinic. Therefore, a comprehensive understanding of the effects of miRNAs on the proliferation and differentiation of ASCs at both the molecular and physiological level is very important and valuable to enhance the regenerative potential of ASCs.

Acknowledgements. This research was supported by the National Research Foundation (NRF), funded by the Korean government (2014054836).

Disclosure of potential conflict of interest. The authors declare that they have no financial interests relevant to this manuscript.

References

Alizadeh E., Akbarzadeh A., Eslaminejad M.B., Barzegar A., Hashemzadeh S., Nejati-Koshki K. and Zarghami N. (2015). Up regulation of liver-enriched transcription factors hnf4a and hnf6 and liver-specific microrna (mir-122) by inhibition of let-7b in mesenchymal stem cells. Chem. Biol. Drug Design 85, 268-279.

Bennett C.N., Ross S.E., Longo K.A., Bajnok L., Hemati N., Johnson K.W., Harrison S.D. and MacDougald O.A. (2002). Regulation of wnt signaling during adipogenesis. J. Biol. Chem. 277, 30998-31004.

Chen T.S. and Lim S.K. (2013). Measurement of precursor mirna in exosomes from human esc-derived mesenchymal stem cells. Meth. Mol. Biol. 1024, 69-86.

Chen J., Deng S., Zhang S., Chen Z., Wu S., Cai X., Yang X., Guo B. and Peng Q. (2014a). The role of mirnas in the differentiation of adipose-derived stem cells. Curr. Stem Cell Res. Ther. 9, 268-279.

Chen L., Cui J., Hou J., Long J., Li C. and Liu L. (2014b). A novel negative regulator of adipogenesis: Microrna-363. Stem Cells 32, 510-520.

Chen L., Dai Y.M., Ji C.B., Yang L., Shi C.M., Xu G.F., Pang L.X., Huang F.Y., Zhang C.M. and Guo X.R. (2014c). Mir-146b is a regulator of human visceral preadipocyte proliferation and differentiation and its expression is altered in human obesity. Mol. Cell. Endocrinol. 393, 65-74.

Chen K., He H., Xie Y., Zhao L., Zhao S., Wan X., Yang W. and Mo Z. (2015a). Mir-125a-3p and mir-483-5p promote adipogenesis via suppressing the rhoa/rock1/erk1/2 pathway in multiple symmetric lipomatosis. Sci. Rep. 5, 11909.

Chen L., Chen Y., Zhang S., Ye L., Cui J., Sun Q., Li K., Wu H. and Liu L. (2015b). Mir-540 as a novel adipogenic inhibitor impairs adipogenesis via suppression of ppargamma. J. Cell. Biochem. 116, 969-976.

Davoodian N., Lotfi A.S., Soleimani M. and Mowla S.J. (2014a). Microrna-122 overexpression promotes hepatic differentiation of human adipose tissue-derived stem cells. J. Cell. Biochem. 115, 1582-1593.

Davoodian N., Lotfi A.S., Soleimani M., Mola S.J. and Arjmand S. (2014b). Let-7f microrna negatively regulates hepatic differentiation of human adipose tissue-derived stem cells. J. Physiol. Biochem. 70, 781-789.

Deng Y., Zhou H., Zou D., Xie Q., Bi X., Gu P. and Fan X. (2013). The role of mir-31-modified adipose tissue-derived stem cells in repairing rat critical-sized calvarial defects. Biomaterials 34, 6717-6728.

Fajas L., Debril M.B. and Auwerx J. (2001). PPAR gamma: An essential role in metabolic control. Nutrition, metabolism, and cardiovascular diseases. Nutr. Metab. Cardiovasc. Dis. 11, 64-69.

Gernapudi R., Wolfson B., Zhang Y., Yao Y., Yang P., Asahara H. and Zhou Q. (2015). Microrna 140 promotes expression of long noncoding rna neat1 in adipogenesis. Mol. Cell. Biol. 36, 30-38.

Gimble J.M., Katz A.J. and Bunnell B.A. (2007). Adipose-derived stem cells for regenerative medicine. Circ. Res. 100, 1249-1260.

Gimble J.M., Bunnell B.A., Chiu E.S. and Guilak F. (2011). Concise review: Adipose-derived stromal vascular fraction cells and stem cells: Let's not get lost in translation. Stem Cells 29, 749-754.

He H., Chen K., Wang F., Zhao L., Wan X., Wang L. and Mo Z. (2015). Mir-204-5p promotes the adipogenic differentiation of human adipose-derived mesenchymal stem cells by modulating dvl3 expression and suppressing wnt/beta-catenin signaling. Int. J. Mol. Med. 35, 1587-1595.

Helwak A., Kudla G., Dudnakova T. and Tollervey D. (2013). Mapping the human mirna interactome by clash reveals frequent noncanonical binding. Cell 153, 654-665.

Hou C., Yang Z., Kang Y., Zhang Z., Fu M., He A., Zhang Z. and Liao W. (2015a). Mir-193b regulates early chondrogenesis by inhibiting the tgf-beta2 signaling pathway. FEBS lett. 589, 1040-1047.

Hou C., Zhang Z., Zhang Z., Wu P., Zhao X., Fu M., Sheng P., Kang Y. and Liao W. (2015b). Presence and function of microrna-92a in chondrogenic atdc5 and adipose-derived mesenchymal stem cells. Mol. Med. Rep. 12, 4877-4886.

Huang S., Wang S., Bian C., Yang Z., Zhou H., Zeng Y., Li H., Han Q. and Zhao R.C. (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.

Kang T., Lu W., Xu W., Anderson L., Bacanamwo M., Thompson W., Chen Y.E. and Liu D. (2013). Microrna-27 (mir-27) targets prohibitin and impairs adipocyte differentiation and mitochondrial function in human adipose-derived stem cells. J. Biol. Chem. 288, 34394-34402.

Karbiener M., Neuhold C., Opriessnig P., Prokesch A., Bogner-Strauss J.G. and Scheideler M. (2011). Microrna-30c promotes human adipocyte differentiation and co-represses pai-1 and alk2. RNA Biol. 8, 850-860.

Karbiener M., Fischer C., Nowitsch S., Opriessnig P., Papak C., Ailhaud G., Dani C., Amri E.Z. and Scheideler M. (2009). Microrna mir-27b impairs human adipocyte differentiation and targets ppargamma. Biochem. Biophys. Res. Commun. 390, 247-251.

Keats E.C., Dominguez J.M. 2nd, Grant M.B. and Khan Z.A. (2014). Switch from canonical to noncanonical wnt signaling mediates high glucose-induced adipogenesis. Stem Cells 32, 1649-1660.

Kim W.S., Park B.S., Sung J.H., Yang J.M., Park S.B., Kwak S.J. and Park J.S. (2007). Wound healing effect of adipose-derived stem cells: A critical role of secretory factors on human dermal fibroblasts. J. Dermatol. Sci. 48, 15-24.

Kim V.N., Han J. and Siomi M.C. (2009a). Biogenesis of small rnas in animals. Nat. Rev. Mol. Cell Biol. 10, 126-139.

Kim W.S., Park B.S. and Sung J.H. (2009b). The wound-healing and antioxidant effects of adipose-derived stem cells. Exp. Opin. Biol. Ther. 9, 879-887.

Kim Y.J., Hwang S.J., Bae Y.C. and Jung J.S. (2009c). Mir-21 regulates adipogenic differentiation through the modulation of tgf-beta signaling in mesenchymal stem cells derived from human adipose tissue. Stem Cells 27, 3093-3102.

Kim Y.J., Bae S.W., Yu S.S., Bae Y.C. and Jung J.S. (2009d). Mir-196a regulates proliferation and osteogenic differentiation in mesenchymal stem cells derived from human adipose tissue. J. Bone Min. Res. 24, 816-825.

Kim Y.J., Hwang S.H., Lee S.Y., Shin K.K., Cho H.H., Bae Y.C. and Jung J.S. (2012). Mir-486-5p induces replicative senescence of human adipose tissue-derived mesenchymal stem cells and its expression is controlled by high glucose. Stem Cells Dev. 21, 1749-1760.

Kim J.H., Park S.G., Song S.Y., Kim J.K. and Sung J.H. (2013). Reactive oxygen species-responsive mir-210 regulates proliferation and migration of adipose-derived stem cells via ptpn2. Cell Death Dis. 4, e588.

Kim J.Y., Shin K.K., Lee A.L., Kim Y.S., Park H.J., Park Y.K., Bae Y.C. and Jung J.S. (2014). Microrna-302 induces proliferation and inhibits oxidant-induced cell death in human adipose tissue-derived mesenchymal stem cells. Cell Death Dis. 5, e1385.

Lakshmipathy U., Davila J. and Hart R.P. (2010). Mirna in pluripotent stem cells. Regen. Med. 5, 545-555.

Lee R.C., Feinbaum R.L. and Ambros V. (1993). The c. Elegans heterochronic gene lin-4 encodes small rnas with antisense complementarity to lin-14. Cell 75, 843-854.

Lee E.K., Lee M.J., Abdelmohsen K., Kim W., Kim M.M., Srikantan S., Martindale J.L., Hutchison E.R., Kim H.H., Marasa B.S., Selimyan R., Egan J.M., Smith S.R., Fried S.K. and Gorospe M. (2011). Mir-130 suppresses adipogenesis by inhibiting peroxisome proliferator-activated receptor gamma expression. Mol. Cell. Biol. 31, 626-638.

Lewis B.P., Burge C.B. and Bartel D.P. (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microrna targets. Cell 120, 15-20.

Li H., Li T., Wang S., Wei J., Fan J., Li J., Han Q., Liao L., Shao C. and Zhao R.C. (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.

Liao Y.H., Chang Y.H., Sung L.Y., Li K.C., Yeh C.L., Yen T.C., Hwang S.M., Lin K.J. and Hu Y.C. (2014). Osteogenic differentiation of adipose-derived stem cells and calvarial defect repair using baculovirus-mediated co-expression of bmp-2 and mir-148b. Biomaterials 35, 4901-4910.

Luzi E., Marini F., Sala S.C., Tognarini I., Galli G. and Brandi M.L. (2008). Osteogenic differentiation of human adipose tissue-derived stem cells is modulated by the mir-26a targeting of the smad1 transcription factor. J. Bone Min. Res. 23, 287-295.

Luzi E., Marini F., Tognarini I., Galli G., Falchetti A. and Brandi M.L. (2012). The regulatory network menin-microrna 26a as a possible target for rna-based therapy of bone diseases. Nucleic Acid Ther. 22, 103-108.

Martin E.C., Qureshi A.T., Dasa V., Freitas M.A., Gimble J.M. and Davis T.A. (2016). MicroRNA regulation of stem cell differentiation and diseases of the bone and adipose tissue: Perspectives on miRNA biogenesis and cellular transcriptome. Biochimie 124, 98-111.

Mondanizadeh M., Arefian E., Mosayebi G., Saidijam M., Khansarinejad B. and Hashemi S.M. (2015). MicroRNA-124 regulates neuronal differentiation of mesenchymal stem cells by targeting sp1 mRNA. J. Cell Biochem. 116, 943-953.

Ning H., Lin G., Fandel T., Banie L., Lue T.F. and Lin C.S. (2008). Insulin growth factor signaling mediates neuron-like differentiation of adipose-tissue-derived stem cells. Differentiation 76, 488-494.

Ning H., Huang Y.C., Banie L., Hung S., Lin G., Li L.C., Lue T.F. and Lin C.S. (2009). MicroRNA regulation of neuron-like differentiation of adipose tissue-derived stem cells. Differentiation 78, 253-259.

Park B.S., Jang K.A., Sung J.H., Park J.S., Kwon Y.H., Kim K.J. and Kim W.S. (2008). Adipose-derived stem cells and their secretory factors as a promising therapy for skin aging. Dermatol. Surg. 34, 1323-1326.

Park H., Park H., Pak H.J., Yang D.Y., Kim Y.H., Choi W.J., Park S.J., Cho J.A. and Lee K.W. (2015). Mir-34a inhibits differentiation of human adipose tissue-derived stem cells by regulating cell cycle and senescence induction. Differentiation 90, 91-100.

Schoolmeesters A., Eklund T., Leake D., Vermeulen A., Smith Q., Force Aldred S. and Fedorov Y. (2009). Functional profiling reveals critical role for mirna in differentiation of human mesenchymal stem cells. PloS One 4, e5605.

Shen J., James A.W., Zhang X., Pang S., Zara J.N., Asatrian G., Chiang M., Lee M., Khadarian K., Nguyen A., Lee K.S., Siu R.K., Tetradis S., Ting K. and Soo C. (2016). Novel wnt regulator nel-like molecule-1 antagonizes adipogenesis and augments osteogenesis induced by bone morphogenetic protein 2. Am. J. Pathol. 186, 419-434.

Shi C., Zhang M., Tong M., Yang L., Pang L., Chen L., Xu G., Chi X., Hong Q., Ni Y., Ji C. and Guo X. (2015). Mir-148a is associated with obesity and modulates adipocyte differentiation of mesenchymal stem cells through wnt signaling. Sci. Rep. 5, 9930.

Shin K.K., Kim Y.S., Kim J.Y., Bae Y.C. and Jung J.S. (2014). Mir-137 controls proliferation and differentiation of human adipose tissue stromal cells. Cell. Physiol. Biochem. 33, 758-768.

Song K., Wang S., Mani M. and Mani A. (2014). Wnt signaling, de novo lipogenesis, adipogenesis and ectopic fat. Oncotarget 5, 11000-11003.

Spiegelman B.M. (1998). PPAR-gamma: Adipogenic regulator and thiazolidinedione receptor. Diabetes 47, 507-514.

Spiegelman B.M., Hu E., Kim J.B. and Brun R. (1997). PPAR gamma and the control of adipogenesis. Biochimie 79, 111-112.

Su X., Liao L., Shuai Y., Jing H., Liu S., Zhou H., Liu Y. and Jin Y. (2015). Mir-26a functions oppositely in osteogenic differentiation of bmscs and adscs depending on distinct activation and roles of wnt and bmp signaling pathway. Cell Death Dis. 6, e1851.

Taha M.F., Javeri A., Rohban S. and Mowla S.J. (2014). Upregulation of pluripotency markers in adipose tissue-derived stem cells by mir-302 and leukemia inhibitory factor. Biomed Res. Int. 2014, 941486.

Tang Y.F., Zhang Y., Li X.Y., Li C., Tian W. and Liu L. (2009). Expression of mir-31, mir-125b-5p, and mir-326 in the adipogenic differentiation process of adipose-derived stem cells. J. Integrative Biol. 13, 331-336.

Tay Y., Zhang J., Thomson A.M., Lim B. and Rigoutsos I. (2008a). MicroRNAs to nanog, oct4 and sox2 coding regions modulate embryonic stem cell differentiation. Nature 455, 1124-1128.

Tay Y.M., Tam W.L., Ang Y.S., Gaughwin P.M., Yang H., Wang W., Liu R., George J., Ng H.H., Perera R.J., Lufkin T., Rigoutsos I., Thomson A.M. and Lim B. (2008b). MicroRNA-134 modulates the differentiation of mouse embryonic stem cells, where it causes post-transcriptional attenuation of nanog and lrh1. Stem Cells 26, 17-29.

Wang Z., Zhang D., Hu Z., Cheng J., Zhuo C., Fang X. and Xing Y. (2015). MicroRNA-26a-modified adipose-derived stem cells incorporated with a porous hydroxyapatite scaffold improve the repair of bone defects. Mol. Med. Rep. 12, 3345-3350.

Wightman B., Ha I. and Ruvkun G. (1993). Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in c. Elegans. Cell 75, 855-862.

Winter J., Jung S., Keller S., Gregory R.I. and Diederichs S. (2009). Many roads to maturity: Microrna biogenesis pathways and their regulation. Nat. Cell Biol. 11, 228-234.

Xie Q., Wang Z., Zhou H., Yu Z., Huang Y., Sun H., Bi X., Wang Y., Shi W., Gu P. and Fan X. (2016). The role of mir-135-modified adipose-derived mesenchymal stem cells in bone regeneration. Biomaterials 75, 279-294.

Xu J., Kang Y., Liao W.M. and Yu L. (2012). Mir-194 regulates chondrogenic differentiation of human adipose-derived stem cells by targeting sox5. PloS One 7, e31861.

Yang Z., Bian C., Zhou H., Huang S., Wang S., Liao L. and Zhao R.C. (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.

Yang L., Shi C.M., Chen L., Pang L.X., Xu G.F., Gu N., Zhu L.J., Guo X.R., Ni Y.H. and Ji C.B. (2015). The biological effects of hsa-mir-1908 in human adipocytes. Mol. Biol. Rep. 42, 927-935.

Yoo J.K., Kim C.H., Jung H.Y., Lee D.R. and Kim J.K. (2014). Discovery and characterization of mirna during cellular senescence in bone marrow-derived human mesenchymal stem cells. Exp. Gerontol. 58, 139-145.

Zaragosi L.E., Wdziekonski B., Brigand K.L., Villageois P., Mari B., Waldmann R., Dani C. and Barbry P. (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.

Zeng Y., Qu X., Li H., Huang S., Wang S., Xu Q., Lin R., Han Q., Li J. and Zhao R.C. (2012). Microrna-100 regulates osteogenic differentiation of human adipose-derived mesenchymal stem cells by targeting bmpr2. FEBS lett. 586, 2375-2381.

Zhang Z., Kang Y., Zhang Z., Zhang H., Duan X., Liu J., Li X. and Liao W. (2012a). Expression of microRNAs during chondrogenesis of human adipose-derived stem cells. Osteoarthritis and cartilage / OARS, Osteoarthrit. Res. Soc. 20, 1638-1646.

Zhang Z.J., Zhang H., Kang Y., Sheng P.Y., Ma Y.C., Yang Z.B., Zhang Z.Q., Fu M., He A.S. and Liao W.M. (2012b). MiRNA expression profile during osteogenic differentiation of human adipose-derived stem cells. J. Cell. Biochem. 113, 888-898.

Zhang W.B., Zhong W.J. and Wang L. (2014). A signal-amplification circuit between mir-218 and wnt/beta-catenin signal promotes human adipose tissue-derived stem cells osteogenic differentiation. Bone 58, 59-66.

Zuk P.A., Zhu M., Mizuno H., Huang J., Futrell J.W., Katz A.J., Benhaim P., Lorenz H.P. and Hedrick M.H. (2001). Multilineage cells from human adipose tissue: Implications for cell-based therapies. Tissue Engin. 7, 211-228.

Zuk P.A., Zhu M., Ashjian P., De Ugarte D.A., Huang J.I., Mizuno H., Alfonso Z.C., Fraser J.K., Benhaim P. and Hedrick M.H. (2002). Human adipose tissue is a source of multipotent stem cells. Mol. Biol. Cell 13, 4279-4295.

Accepted July 4, 2016