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Review

Adipose tissue, angiogenesis and angio-MIR under physiological and pathological conditions

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ABSTRACT

Angiogenesis is a crucial process for the maintenance of normal tissue physiology and it is involved in tissue remodeling and regeneration. This process is essential for adipose tissue maintenance. The adipose tissue is composed by different cell types including stromal vascular cells as well as adipose stem cells (ASCs). In particular, ASCs are multipotent somatic stem cells that are able to differentiate and secrete several growth factors; they are recently emerging as a new cell reservoir for novel therapies and strategies in many diseases. Several studies suggest that ASCs have peculiar properties and participate in different disease-related processes such as angiogenesis. Furthermore, pathological expansion of adipose tissue brings to hypoxia, a major condition of unhealthy angiogenesis.

Recent evidences have shown that microRNAs (miRNAs) play a crucial role also on ASCs as they take part in stemness maintenance, proliferation, and differentiation. It has been suggested that some miRNAs (MIR126, MIR31, MIR221 MIR222, MIR17-92 cluster, MIR30, MIR100 and MIR486) are directly involved in the angiogenic process by controlling multiple genes involved in this pathway. With the present review, we aim at providing an updated summary of the importance of adipose tissue under physiological and pathological conditions and of its relationship with neovascularization process. In particular, we report an overview of the most important miRNAs involved in angiogenesis focusing on ASCs. Hopefully the data presented will bring benefit in developing new therapeutic strategies.

1. Introduction

Adipose tissue is a relevant metabolic and endocrine organ involved in various physiological functions, from heat production to hormone secretion. Adipose tissue is constantly evolving during lifetime and its composition changes from childhood to adulthood. It contains several cell types such as stromal vascular cells, which includes also adipose stem cells and endothelial progenitor cells. Adipose Stem Cells (ASCs) are today a promising and attractive solution for regenerative medicine due to their multipotent property (Leto Barone et al., 2013). ASCs are characterized by a high plasticity and can differentiate both towards mesenchymal cells such as adipocytes, osteocytes, chondrocytes, endothelial cells, and towards ectodermal and endodermal cells such as skin epithelial, digestive tract, liver, and lungs cells (Orbay et al., 2012). They can be isolated, expanded and induced to differentiate towards the above-mentioned lines under specific culture conditions. Recent studies have shown that, in many cell mechanisms, such as stemness maintenance, proliferation and differentiation of ASCs, miRNAs play a pivot regulatory role through the modulation of several receptors or ligands involved in these processes (Di Stefano et al., 2018). MicroRNAs are small non-coding RNA (18–20 base pairs) that negatively regulate gene expression by inhibiting mRNA translation. Numerous studies have focused on the identification of a specific miRNA signature in ASCs during maintenance, remodeling and mesenchymal differentiation including endothelial vessel formation. For this purpose, the term angio-miR was coined to identify specific classes

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of miRNAs that are involved in the regulation of endothelial cells differentiation during angiogenesis (Suárez et al., 2008; Wang and Olson, 2009).

Angiogenesis is a crucial process for the maintenance of normal tissue physiology and it is involved in tissue remodeling and regeneration (Viallard and Larrivée, 2017) through the formation of novel blood vessels network. The activation of angiogenesis is mediated by specific biochemical signals (Carmeliet, 2000). Generally, this process consists in multiple steps: stimulation and migration of endothelial cells (ECs), degradation of the surrounding capillary basal lamina, formation of capillary sprout and finally vessels maturation (Rajabi and Mousa, 2017). These events start in the embryonic stage and continue throughout life (Balaj et al., 2013). The process is finely regulated by several pro- and anti-angiogenic factors. The main pro-angiogenic factors involved in this process are the Vascular Endothelial Growth Factor (VEGF) family (VEGFA, VEGFB, VEGFC, VEGFD, VEGFE), and PGF1 and 2 (placental growth factor) (Mashreghi et al., 2017). VEGF binds to Vascular Endothelial Growth Factor Receptor 1/2 (VEGFR1 and VEGFR2), encouraging angiogenesis through the stimulation of endothelial cells to produce proteolytic enzymes, which destroy the perivascular extracellular matrix (ECM) and the basement membrane (Rajabi and Mousa, 2017). Endothelial cells proliferate and migrate into the perivascular area creating “primary sprouts”. The next phase leads to the formation of capillary loops, with the subsequent synthesis of a new basement membrane and blood vessels maturation (Koch and Distler, 2007). Even if angiogenesis is a physiological process, pathological angiogenesis is one of the crucial steps for some diseases correlated with adipose tissue such as obesity, diabetes, cardiovascular diseases, tumor growth and metastasis development (Carmeliet and Jain, 2011). Hypertrophic conditions of adipose cells cause a hypoxic state in the tissue that leads to several pathological mechanisms involved with chronic inflammation or insulin resistance (Divella et al., 2016).

This review describes the strict correlation between adipose tissue and angiogenic process, since there is a common ancestor. The angiogenic microenvironment is determinant for the disease development and adipose tissue is actively involved in this process. Furthermore, we provide an overview of the angiogenic process in several adipose-associated diseases, providing more detailed information on the main miRNAs directly involved (Fig. 1).

2. The link between adipose tissue and angiogenesis

2.1. Adipose tissue

Adipose tissue is mainly constituted by adipocytes and stromal vascular cells. The latter can give rise to different cell types such as preadipocytes, endothelial cells, pericytes, immunological cells (monocytes and macrophages) (Ahima and Flier, 2000). The vascular cells actively participate to growth homeostasis and adipose tissue development. Furthermore, in adipose tissue there is a reservoir of pluripotent stem cells that renews adipocytes progenitor or precursors (Zuk et al., 2002). Anatomically adipose tissues can be divided in two main different types, White Adipose Tissue (WAT) and Brown Adipose Tissue (BAT), which differ in both composition and function. White adipocytes are large cells containing a unilocular lipid droplet that occupies 90% of the total cell area. WAT is composed by precursor and mature adipocytes, but also by fibroblasts, macrophages and other cells that innervate and vascularize the connective tissue. WAT main function is to act as lipid deposit. Brown adipocytes are instead smaller than WAT cells and in BAT cells lipid droplets are disperse in the cytoplasm. The main physiological role of BAT is thermogenesis for heat production. Recently, a particular type of brown-like adipose tissue has been identified, which has intermediate characteristics between WAT and BAT. Adipocytes of BAT deposits in adults are called brown-like/beige: BRITE (Wu et al., 2012a). These adipocytes are enriched of uncoupling protein 1 (UCP1) in mitochondrial contents, essential for catalysing nonshivering thermogenesis (Heaton et al., 1978). These cells seem to derive from a reversible transdifferentiation phenomenon also called “beiging effect” in which the WAT, under specific stimuli (cold exposure (Luo et al., 2017c), reduced light exposure, age or physical exercise (Lee, 2018)), can be transformed in BAT to supply the thermogenic needs or vice versa to increase storage capacity (Smorlesi et al., 2012). Moreover, recently it has been described another adipocyte cell type called “pink adipocytes” present during the gestation and lactation period. They store lipids in the mammary gland and participate to secrete milk. After the lactation period, pink adipocytes disappear with involution of the mammary glands (Cinti, 2018).

2.2. Adipose microenvironment

Numerous signalling molecules compose the adipose microenvironment that can positively and negatively modulate angiogenic processes, homeostasis and vascular integrity regulating the interaction between vascular system and adipocytes. For example, while adiponectin negatively affects angiogenesis (Bråkenhielm et al., 2004b), leptin induces angiogenesis, vascular fenestration and vascular remodelling (Bouloumié et al., 1998; Cao et al., 2001). The vascular network performs the same functions both in WAT and BAT and its activity depends on the metabolic state of adipocytes. In a healthy person, vascular network of the adipose tissue has the role of transporting hormones and the vessels are rich in fenestrations allowing the passage of adipokines (Cao et al., 2001). Volume alteration of WAT (both expansion or shrinkage), depending on energy consumption, is needed for angiogenesis or vascular regression. For this reason, it has been demonstrated that there is a relationship between adipose and vascular tissue (Harwood, 2012). Adipose vasculature is also closely related with “beiging effect” through the production of angiogenic factors such as VEGF, interleukin 4 (IL4) or angiopoietin that are released from WAT to induce new blood vessels formation. In particular, Park et al., demonstrated that VEGFA overexpression in adipocytes increases the number of beige adipocytes and induces vascularization of subcutaneous fat. This process VEGFA-induced beiging is independent of interleukin-4 and to what happens during conventional cold-induced beiging. These processes are rapid, reversible and the cytokine exposure is essential for the effect. The results demonstrated that new vascularization precedes the UCP1 expression and the process are independent by adiponectin and IL4 expression but not from VEGFA (Park et al., 2017).

2.3. Adipose tissue vasculature

Blood vessels carry the nutrient and oxygen to adipocytes and also transport adipose precursor and stem cells to tissues districts (Gupta et al., 2012). Recent findings have demonstrated that vessel cells, under specific condition, can differentiate to preadipocytes and adipocytes (Gupta et al., 2012), and they probably possess mesenchymal characteristics (Corselli et al., 2010). Endothelial, pericytic and smooth-muscle-like cells express a specific pattern of molecules (PPARG, CD34, ACTA2 PDGFRB, NG2) that distinguish them from other cell types. It was demonstrated that endothelial cells and adipocytes have common progenitor cells (Planat-Benard et al., 2004). Over 40 years ago was first reported that immature adipocytes can be found nearby capillary, showing the close association between adipose progenitors and vascularization of adipose tissue (Napolitano, 1963) and the recent study of Gupta et al. is still supporting this evidence. Indeed, they demonstrated that a transcriptional regulator multi-zinc finger protein 423 (Zfp423) is expressed on preadipocytes with regulatory functions of some genes such as PPARG. Zfp423 regulates the adipocyte commitment in both adipose tissues (WAT and BAT) and interestingly its expression is reported only in adipose tissue-derived endothelial cell (Gupta et al., 2012).

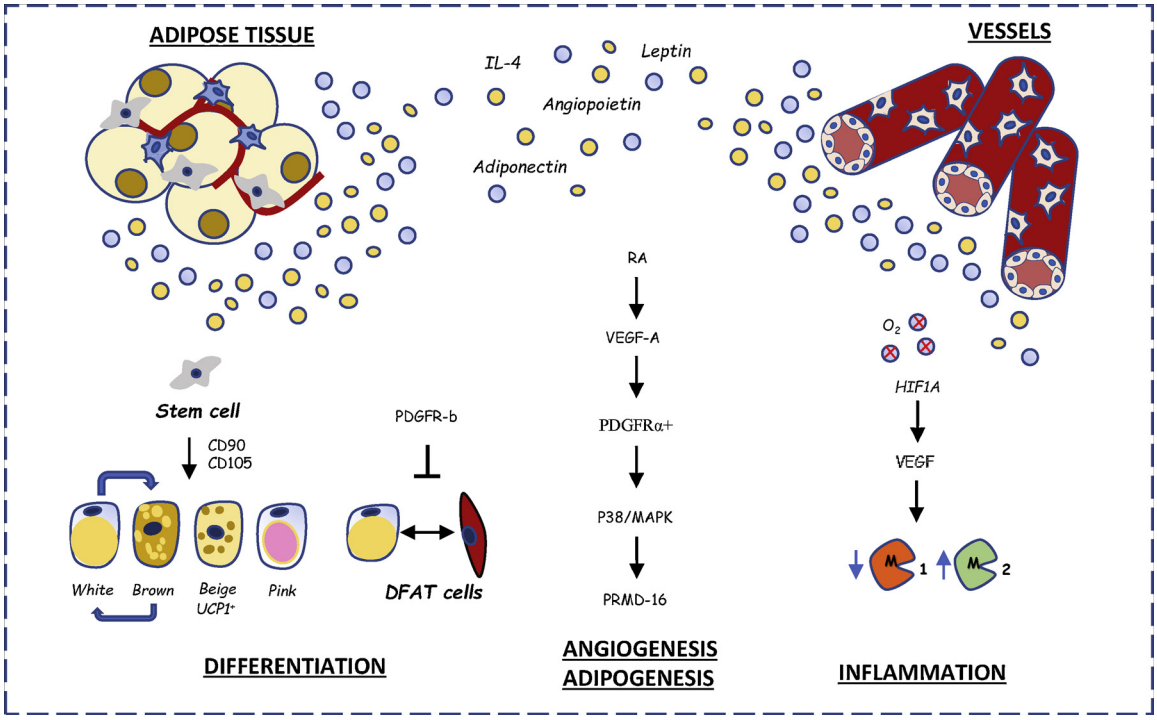

Fig. 1. The reservoir of mesenchymal stem cells renews the progenitor or precursors of adipocytes. Adipose stem cells became white (WAT), brown (BAT), beige (BRITE) and pink adipocytes. Adipocyte cells have the ability to de-differentiate into a particular cell lines so called De-differentiated FAT cells(DFAT), which in turn differentiate into all mesenchymal lineages, including endothelial cells, for example, activation of PDGFRB prevents differentiation towards WAT. Stromal vascular fraction and WAT, as endocrine tissue, secreting adipokines and paracrine factors, including adiponectin, leptin, angiopoietin and interleukin 4, that modulate both positively and negatively angiogenic processes, homeostasis and vascular integrity regulating the interaction between the vascular system and adipocytes. Retinoic acid (RA) increasing the number of vessels, stimulating the adipose progenitor cells in perivascular area through p38 MAP kinase phosphorylation and PRMD16 expression. In adipose tissue angiogenic microenvironment, the reduction of the partial pressure of the oxygen PO_2 follows an increase of HIF1A and in turn of VEGF, over-expression of the latter is closely associated with the accumulation of anti-inflammatory macrophages (M2) whereas it leads to the reduction of pro-inflammatory macrophages (M1).

In angiogenic microenvironment, the mature adipocyte cells can de-differentiated into a particular cell lines, the so-called De-differentiated FAT cells (DFAT), able to differentiate into all mesenchymal lineages, including endothelial cells (Cao, 2013). Initially, this particular population does not show the typical endothelial and pericitic expression pattern (CD31, CD146, CD34) which distinguish them from other progenitor cells; DFATs are probably precursor cells that can differentiate in various stromal cell lines (Cao, 2013). Endothelial and adipocytes cells interact with each other through several pathways involved also in proliferation and differentiation processes. For example, activation of PDGFRB is involved in proliferation of pericytes but inhibits their differentiation toward WAT adipocytes (Olson and Soriano, 2011). One study has shown that differentiation from pre-adipocytes to mature adipocytes is linked to high expression levels of angiogenic factors that induces robust angiogenic responses (Castellot et al., 1982). In early stages of embryonic development, the first sketches of adipose tissue have a perivascular origin, this event underlines important spatial and temporal relationships between adipogenesis and angiogenesis (Cao, 2007; Crandall et al., 1997).

3. The link between adipose tissue and angiogenesis process in diseases development

3.1. Obesity

Obesity is a major public health problem worldwide as it determines an increased risk of developing other relevant pathologies such as metabolic and cardiovascular diseases, diabetes, and cancer. The main aspects that contribute to the development of obesity are both social (wrong diet and sedentary lifestyle) and genetic factors. Obesity is a condition in which adipocytes increase in size and fatty acid content, causing an increase in intercapillary distance, reduced blood perfusion and a decrease in oxygen tension. It has been shown that tissue hypoxia, together with the induction of an angiogenesis process, stimulates a chronic inflammation process (Ye et al., 2007). Under hypoxic conditions, white and brown adipocytes differentially activate the expression of several genes. For example, HIF1A involvement is essential for gene expression activation in WAT, but not in BAT (Pino et al., 2012). In this context, VEGF expression reduces both tissue hypoxia, local fibrotic, inflammatory processes and protects against obesity and metabolic dysfunctions. Furthermore, Elias et al. have also demonstrated that in adipose tissue, the over-expression of VEGF is closely associated with the accumulation of anti-inflammatory macrophages (M2) whereas it leads to the reduction of pro-inflammatory macrophages (M1) (Elias et al., 2012). In obese subjects it has been observed that vascular network and angiogenesis modulate the sensitivity of adipocytes to insulin. Obese mice fed with high-fat diet (HFD) treated with an angiogenic inhibitor TNP-470 (a synthetic analogue of the fumagillin), showed a reduced vascular density corresponding to better insulin sensitivity (White et al., 2012; Bråkenhielm et al., 2004b). This inhibitor can also prevent the development of type II diabetes (T2D). However, there are discordant opinions on the functional role of angiogenesis in obesity. Indeed, despite some studies show that angiogenic inhibitors reduce body mass index (Bråkenhielm et al., 2004a; Rupnick et al., 2002), others report that VEGF overexpression hinders the expansion of adipose tissue (Wang et al., 2017). An active angiogenesis is the basis of browning or beiging process in which the adipocytes become more active metabolically and consume more energy. Wang et al showed that retinoic acid (RA), an active metabolite of Vitamin A, enhances the expression of VEGFA, increasing the number of vessels in WAT. The

ectopic stimulation of both factors increases the PDGFRA + adipose progenitor cells in the perivascular area, promoting their differentiation into beige adipocytes through p38/MAPK phosphorylation and subsequent PRMD16 expression (Wang et al., 2017).

3.2. Cardiovascular disease

Despite advances in medical treatment, cardiovascular diseases (CVDs) are still major causes of adult death. To date, many degenerative cardiovascular system diseases, such as ischemic heart disease and chronic limb ischemia, are subject of interest in regenerative medicine field as stem cells could be a promising treatment option due to their regenerative properties. The therapeutic contribution of ASCs to tissue repair includes two main strategies: the direct differentiation into cardiomyocytes, smooth muscle (SMCs) and endothelial (ECs) cells in order to restore the injured cells and the paracrine action promoting a new vessel formation (Meyer et al., 2009). Neovascularization is the gold standard of stem cell-based therapies, leading a physical incorporation of transplanted cells into the lesions (Kawamoto et al., 2003).

ASCs transplantation could represent one of the most effective method for the treatment of myocardial infarction. Strem et al demonstrated for the first time in mouse model that autologous ASCs directly injected into the left ventricular cavity start to express specific cardiac markers such as Myosin heavy chains (MYH), NK2 homeobox 5 (NKX2-5) and Troponin I1 (TNNI1) after 14 days from inoculation (Strem et al., 2005).

Peripheral artery disease (PAD) is another important vascular disease in which the use of mesenchymal stem cells can be advantageous. It is usually caused by atherosclerotic occlusion, which can lead to critical limb ischemia, tissue injuries and eventual limb loss (Raval and Losordo, 2013; Orbay et al., 2013). Critical limb ischemia is the most serious complication of PAD, followed by a series of problems, pain in ambulation and unhealed ulcers in severe cases. To date, one of the most effective methods for PAD treatment is therapeutic angiogenesis, which aims to promote neovascularization starting from pre-existing vessels (Ransohoff and Wu, 2012). Also, in this case ASCs could represent a therapeutic option; indeed, ASCs cultured in conditioned medium secrete cytokines and growth factors that could improve neovascularization. Rehman et al found that ASCs secrete VEGF up to 5 times more if they are cultured under hypoxic conditions (Rehman et al., 2004). Additionally, the conditioned supernatant of ASCs cultured under hypoxic conditions increased the growth of endothelial cells and reduced their apoptosis (Nakagami et al., 2005). In response to TGFB, the intramuscular injection of human ASCs could significantly improve neovascularization and blood perfusion in hindlimb ischemia (Park et al., 2013). Studies have shown how in a damaged tissue the microenvironment can allow the ASCs differentiation to SMCs. In particular, prostaglandin F2α (PGF2α) secreted by IL1B-activated macrophages are able to promote the differentiation of ASCs into SMCs in a hindlimb ischemia model (Lee et al., 2012). In the same model, it has also been shown that angiogenic factors produced by mesenchymal stem cells (bFGF, VEGF, TGFB, PDGF, angiopoietin-1, placental growth factor (PGF), IL6, and monocyte chemotactic protein 1 (MCP1), managed to facilitate vascular tissue regeneration (Kwon et al., 2014). Recently, spheroids from human adipose-derived stem cells (hASCs) represents a new population with improved angiogenic capabilities, in fact when they are implanted into mouse ischemic hindlimbs, the secretion of angiogenic and antiapoptotic factors is enhanced. Suk Ho Bhang et al also demonstrated that the expression of HGF, VEGF, and FGF2 is increased in ASCs cultured as spheroids compared to cells in monolayer, leading to inhibition of cell apoptosis (Bhang et al., 2011).

3.3. Diabetes

Diabetes is a common chronic metabolic disease that could lead to several complications and death. There are many factors related to diabetes, such as sedentary lifestyles, high consumption of foods rich in fats and sugars, overweight and obese condition especially in the young generation. This complex scenario is due to multiple elements: environmental, genetic and social factors (Hamdy et al., 2018). The most common therapy for diabetes is insulin treatment, in order to maintain stable levels of glycaemia (Paek et al., 2014). Despite insulin therapeutic progress, the life expectancy of diabetic patient is lower than healthy person. For this reason, research has moved forward to find alternative therapies. Recent studies in stem cell isolation and differentiation methodologies have led to production of cell lines with the ability to synthesize, package, and secrete insulin in response to glucose. In particular, ASCs have the capability to differentiate into insulin-producing cells (Nakao et al., 2010). Alternative insulin therapy in diabetic patients is the engraftment of transplanted islets, although there may be possible complications, including inflammation and apoptosis process, and ASCs can be used to strengthen treatment success. Indeed, as ASCs show angiogenic and anti-apoptotic properties, they could improve engraftment of transplanted islets through the secretion of trophic factor that enhanced vascularization and suppress inflammation (Kim et al., 2007).

A study conducted by Brissova et al. has investigated the morphology and functionality of pancreatic β-cell microvascularization following genetic modifications through the Cre-Lox system. In particular, they have created transgenic mice for VEGFA gene. Through the Cre-Lox system, the VEGFA gene was specifically removed in pancreatic β-cells. Transgenic Mice (RIP-Cre: Vegf fl / fl) showed a significant reduction in insulin production relative to the wild-type and moreover, by means of electronic microscopic investigations, the ultra-capillary micro-vascular structure of the β-islet in transgenic mice exhibited an important reduction in endothelial fenestration (Brissova et al., 2006). There are different pathways involved in ASCs behaviour, such as Notch, Wnt-βcatenin and FGF, associated with cell survival, proliferation, pluripotency and the lineage determination of stem cells. The main effect of these pathways in type 2 diabetes was determined by Ferrer Lorente study. They demonstrated for the first time that diabetes causes a decrease of ASCs pool from undifferentiated state versus adipogenic commitment as well as an impairment to form a capillary network (Ferrer-Lorente et al., 2014).

3.4. Cancer

Several articles suggest that ASCs show cancer-promoting properties and participate in different processes including angiogenesis and tumor microenvironment modifications. The tumor angiogenesis begins when a tumour extends massively and cannot be sustained by diffusion of oxygen and nutrients. The hypoxic environment stimulates tumour cells to increase the expression of genes responsible for the synthesis of pro-angiogenic proteins (Folkman, 2002), (Hanahan and Folkman, 1996). ASCs seem to be involved in promoting tumour expansion through stimulation of new vascularization and participating in the tumorigenic activity through the formation of inflammatory conditions (Bielli et al., 2014). As previously mentioned, WAT is the major component of adipose tissue and it that can expand through hypertrophic and hyperplastic state of white adipocytes maintaining a healthy condition with minimal inflammation. Conversely, pathological expansion of adipose tissue brings to hypoxia and production of reactive oxygen radicals (ROX) (Trayhurn, 2014). It is well known that in healthy cells, oxygen tension is a key regulator of angiogenesis and ECs and SMCs have various oxygen-sensing mechanisms, including oxygen-sensitive NADPH oxidases, endothelial nitric oxide synthase (eNOS) and hemeoxygenases. In order to grow or metastasize, tumor needs oxygen and nutrients that are provided by blood vessels. All these events lead to inflammation that is the main risk factor for cancer development. Several studies suggest that dysfunctional adipocytes are able to deregulate angiogenic process and therefore they are involved in cell

Table 1
Overview of miRNAs correlated with angiogenesis and adipose tissue related diseases.

miRNAs	*Targets*	*Roles*
MIR126	VEGF, SPRED1, PIK3R2, VCAM1	Angiogenesis and vascular integrity maintenance/ Cellular proliferation/ Vascular inflammation/ Positive predictive marker for cardiovascular events
MIR31	RASA1, LATS2, PPPR2A, VEGF, HIF1A	Oncomir/ Angiogenesis regulation/ Tumor suppressor/ Biomarker obesity
MIR17-92 cluster	THBS1, CTGF, BIM, p21, JAK1	OncomiR/ Tumor angiogenesis/ Regulator growth blood vessels/ Tumor suppressor/ ASCs paracrine factor
MIR221/MIR222	TIMP2, TIMP3, PTEN, c-Kit, ERα, GLUT4	Angiomir/ Oncomir/ Vessel and tumor growth/ Cell proliferation/ Insuline resistance
MIR21	TGFB, TIMP3, BCL2, PTEN, SMAD7	Oncomir/ Apoptosis/ Invasion/ inflammation/ Angiogenesis/ Tissue neovascularization/ Insulin resistance deterioration
MIR100	TWIST1/SNAIL/ZEB1/SMARCA5/ HOXA1/mTOR	Tumor suppressor/ Cell proliferation/ Vascularization/ EMT/ Anti-angiogenic factor
MIR486	PTEN/CLDN10/CITRON/FGF9/BCL2	Tumor suppressor/ Prognostic factor/ Suppresses transformation, migration and growth of tumor cells/ Regulation hematopoiesis/ Mesenchymal differentiation/ Hypoxia
MIR30	DLL4/PAI1/ ALK2	Mesenchymal differentiation/ Angiomir/ Cancer development/ Regulator growth blood vessels

proliferation in prostatic, ovarian, breast and colon cancer (Bielli et al., 2014), (Zhang et al., 2010). ASCs derived from abdominal subcutaneous adipose tissue are able to induce breast cancer cell proliferation in vitro and more interestingly they improve breast cancer cells tumorigenicity in vivo. Indeed, after co-culturing with ASCs, breast cancer cells showed a modified gene expression profile, especially regarding the oestrogen receptor-alpha (ESR1). ASCs may stimulate tumor progression through the secretion of adipokines, such as adiponectin and leptin (Bertolini et al., 2012), In particular, the involvement of ASCs in inducing angiogenesis is due to migration of these cells toward tumor-conditioned media via the platelet-derived growth factor subunit B/ platelet-derived growth factor receptor beta (PDGFB/ PDGFRB) signalling pathway (Freese et al., 2015). Furthermore, in 2010 Razmkhah at al. demonstrated that ASCs stimulate the production of VEGF in breast cancer (Razmkhah et al., 2010). Indeed in ASCs isolated from breast cancer patients the relative quantification of VEGF, IGF1, HGF and IL8 is 2 times higher compared to controls. These findings demonstrated that breast cancer growth and progression could be supported by the presence of resident ASCs inside the scaffold of breast cancer tissue that favour the expression of tumor promoting factor. Colon rectal cancer (CRC) is characterized by several genetic mutations such as APC, TP53, PI3K, KRAS, BRAF, PTEN, microsatellite and chromosomal instability, epigenetic alterations (locus-specific CpG island methylation, global DNA hypomethylation). Environmental factors such as diabetes, obesity and metabolic syndrome are involved in CRC onset, and this demonstrates the strict relationship between excessive body weight and CRC. Tumor local adipocytes exhibit a phenotype that determines the secretion of specific factors directly involved in cell cycle activation and regulation, in angiogenesis process and also in inflammation regulation. Furthermore, cancer cells secrete some inflammatory cytokines (TNFA, IL6) that allow the activation of guest cells of the tumor microenvironment (TME) forming a paracrine/ autocrine loop. The evolution of tumor and its progression is determined by direct communication between resident adipocytes and colon cancer cells. To better understand this behaviour, it will be necessary to analyze the transcriptomic and the metabolomics of local colon cancer adipocytes (Tabuso et al., 2017).

3.5. Angio-miR produced by ASCs

Several biological processes in ASCs are controlled by small non-coding RNAs, so-called microRNAs (miRNAs). In particular, angio-miR constitutes a specific class of miRNAs that actively contributes to angiogenesis regulation and vessels remodelling. Many articles described that miRNAs can be transported from ASCs to other cells through vesicles (Chen et al., 2010). In particular, extracellular vesicles (EVs) are divided by size in apoptotic bodies (1–5 mm), exosomes (50 to 100 nm) and microvesicles (100 nm to 1 mm), which are secreted by almost every cell types including dendritic cells (Liu et al., 2016), epithelial cells (Borges et al., 2013) and mesenchymal stem cells (Shabbir et al., 2015). They play a fundamental role in cellular communication, releasing active molecules (mRNAs and miRNAs) in the physiological and pathological microenvironment. MiRNAs can play pro- or anti-angiogenic role during new vascular formation (Wang and Olson, 2009). In tissue environment, endothelial cells have a high plasticity and can be influenced by various factors, such as oxygen tension and biomechanical factors (velocity and flow patterns) derived from circulating blood. It has been demonstrated that endothelial cells modify their behaviour according to culture conditions and they are subjected to cellular aging in *in vitro* model. Therefore, culture conditions affect the endothelial miRNA profile or “endomiRNAs”. In fact, Kuosmanen showed that freshly isolated endothelial cells from umbilical cords have a distinct miRNA profile compared to cultured cells in different stages (Kuosmanen et al., 2017). Noteworthy several specific miRNAs are responsible of controlling the angiogenic process, many of them have been studied related to carcinogenesis but they are also involved in adipose tissue-related diseases (Table 1).

3.6. MIR126

MicroRNA 126 plays an essential role in both angiogenesis and vascular integrity maintenance (Wang et al., 2008). The most important targets of MIR126 are VEGF, SPRED1, VCAM1 and PIK3R2 that play a fundamental role in endothelial cell signalling or vascular function (Togliatto et al., 2016). In particular, SPRED1 inhibits the activation of MAP kinase pathways, while PIK3R2 negatively regulates the activity of PI3 kinase. These factors determine downstream changes in the phosphorylation status of ERK and AKT that are involved in cellular proliferation. Furthermore, a functional role of MIR126 is to modulate the expression of adhesion molecules and to control vascular inflammation influencing the leukocyte adhesion to endothelial cells through VCAM1 expression (Harris et al., 2008). Normally, stimulation of endothelial cells with VEGF determines a reorganization of cytoskeleton, but this rearrangement is defective in MIR126 knockdown cells (Fish et al., 2008). Cells with reduced levels of MIR126 were less responsive to VEGF and other growth factors, such as PDGFA, -B, -C and –D (Cao et al., 2002; Li et al., 2003). Similarly, EphrinB2, that inhibits MAP kinase signalling (Kim et al., 2002), is upregulated in both human and zebrafish endothelial cells with decreased MIR126 expression.

Togliatto et al. demonstrated that MIR126, VEGF and MMP2 are expressed in low concentration in extracellular vesicles (EVs) secreted by ASCs from obese patients compared to ASCs from normal patients. EVs from obese patients are biologically inactive for new vessel formation, but the number and size of EVs don’t change in obese and non-obese subjects (Togliatto et al., 2016). It has been also demonstrated that exosomes secreted by MIR126 overexpressing ASCs are able to prevent myocardial damage by protecting myocardial cells from apoptosis, inflammation, fibrosis, and increased angiogenesis (Luo

et al., 2017b). Moreover, increased level of circulating MIR126 was found in patients with hypertension and cardiac ischemia (Ren et al., 2013; Kontaraki et al., 2014). Interestingly plasma concentration of MIR126 is significantly associated with plasma levels of cardiac troponin I, thus indicating hearth injury. The levels of circulating MIR126 can, therefore, be used as a marker to identify people that show a higher risk of future cardiovascular events (Khanaghaei et al., 2016).

EV's containing MIR126 seems also to play a crucial role in diabetic patients; indeed, MIR126 expression is decreased in the EV's isolated from uncontrolled diabetes patients. However, there was no significant difference in MIR126 expression between EVs from healthy controls and well-controlled diabetes patients (Wu et al., 2016).

3.7. MIR31

MIR31 is considered both an oncomir and a tumor suppressor that plays a crucial role in several tumour types. In colonrectal cancer, MIR31 stimulates cells growth, playing, as an oncomir, an important role in RAS activity through the inhibition of RASA1 translation (Sun et al., 2013). In mouse and human lung cancers, MIR31 is over-expressed and seems to act oncomir mainly on 2 targets, large tumor suppressor 2 (LATS2) and PP2A regulatory subunit B alpha isoform (PPP2R2A) (Liu et al., 2010). In the Schmittgen study, instead, MIR31 acts as tumor suppressor, breast cancer patients with higher MIR31 expression, had prolonged survival (Schmittgen, 2010). MIR31 is also involved in angiogenesis regulation through VEGF pathway. VEGF is present at high levels in tissues in which the angiogenesis is active such as cancer and, in this context, it stimulates the production of MIR31. This regulation involves the activation of ERK and AKT signalling, driving to inhibition of TNFSF15 (TNF superfamily member 15) expression. TNFSF15a is an inhibitor of vascularization and, in contrast to VEGF, is upregulated in normal tissues whereas diminishes in angiogenic and cancer tissues, through a mechanism regulated by miRNAs (Deng et al., 2017). In fact, in ovarian cancer, TNFSF15 inhibits tumor growth and causes the arrest of endothelial cell proliferation (Deng et al., 2012) likewise MIR31 is correlated with chemio-resistance in ovarian cancer cell line. Mitamura et al demonstrated that up-regulation of MET downregulates MIR31 expression, causing drugs resistance to paclitaxel in ovarian cancer (Mitamura et al., 2013).

Recently, Tsai et al. suggested that MIR31 and MIR21 induce the migration and invasiveness of endothelial cells in the Kaposi's sarcoma associated herpesvirus (KSHV) by inducing matrix metalloproteinase expression (MMP1, MMP2 and MMP9). In particular, the M-Type K15 protein regulates miRNA expression determining cancer cell migration and invasion (Tsai et al., 2009; Qian et al., 2007).

Furthermore, it was demonstrated that MIR31 is abundant in microvesicles when ASCs are cultured in a preconditioned endothelial differentiation medium. In particular, MIR31 in the microvesicles contributes to the induction of angiogenesis by targeting factor-inhibiting HIF-1 (FIH1) in vascular endothelial cells (Kang et al., 2016).

In diabetic patients, endothelial dysfunction results in vascular disease, in this process endothelial progenitor cells (EPCs) play a crucial role, as they are responsible for functional preservation of the vascular endothelium. Indeed, the amount of circulating EPCs in diabetic patients is reduced resulting in vascular dysfunctions (Boehncke and Boehncke, 2011). MIR31 seems to be involved in the regulation of diabetic EPC function via the interaction with Satb2. An over-expression of MIR31 has been reported in diabetic EPCs and this up-regulation seems to be due to elevated glucose level. Therefore the reduced amount of EPCs in diabetic patients could be due to MIR31 up-regulation, which is triggered by glucose-elevated levels (Lian et al., 2018). These findings show that MIR31 may be a target to prevent vascular disease in diabetic patients.

MIR31 is also a relevant regulator in adipose tissue and it is involved in metabolic disease, such as obesity. MIR31 gene is located in a very prominent obesity quantitative trait loci (QTL) and it is reported that its expression is significantly higher in visceral adipose tissue of obese patients than healthy subjects (Gottmann et al., 2018). Moreover MIR31 was found to be upregulated in plasma samples of overweight or obese pre-pubertal children in a European cohort of subjects, and it could represent a novel biomarker of obesity and metabolic dysfunction (Iacomino et al., 2016).

3.8. MIR17-92 cluster

The MIR-17-92A1 cluster, also known as oncomir-1, was among the first miRNA that were linked to tumor angiogenesis. The cluster represents a prototypical polycistronic miRNA gene, encoding 6 mature miRNAs: MIR17, MIR18 A, MIR19 A, MIR20 A, MIR19B1, and MIR92A1; all processed from a single primary transcript, which are tightly grouped within an 800 base-pair region of human chromosome (Mendell, 2008) and is transcriptionally regulated by c-Myc (Dews et al., 2006). While the anti-angiogenic role of MIR92A1 is widely demonstrated, the others members probably have a pro-angiogenic action suppressing antiangiogenic molecules such as thrombospondin1 (TSP1) and connective tissue growth factor (CTGF) in tumor cells (Kuhnert and Kuo, 2010). However, there are contradictory studies that still do not define a clear role of the cluster in angiogenesis process. Doebele et al., for example, demonstrated anti-angiogenic properties of MIR17, MIR18 A, MIR19 A, and MIR20 A in endothelial cells, downregulating sprouting in ECs. The MIR17, MIR18 A over-expression inhibited the spheroid sprouting *in vitro* and matrigel plug neovascularization *in vivo* (Doebele et al., 2010). The main targets of MIR17 are BIM (a pro-apoptotic protein,) p21 (a cell-cycle inhibitor) and JAK1 (a tyrosine kinase, involved in apoptotis, cell cycle progression and migration of endothelial cells).

MIR92 A is considered a critical regulator of normal and pathological growth of blood vessels; it is expressed during the trans-differentiation from mesenchymal (adipose or bone marrow) to endothelial cells, in ischemic tissues (Bonauer et al., 2009; Iaconetti et al., 2012). Overexpression of MIR92 A in adipose stem cells suppresses ASCs ability to stimulate tube formation by HUVECs through down-regulation of its target, fibronectin receptor integrin subunit α5 (ITGA5) and a mitogen-activated protein kinase 4 (MEK4). Furthermore, this up-regulation also suppresses angiogenic growth factors production, such as HGF, secreted by MSCs. In particular, it was demonstrated that if the amount of HGF was restored with exogenous ones in endothelial differentiation medium (EDM)-preconditioned ASCs, the capacity to form vessels is re-established (Kalinina et al., 2015). While MIR92A1 inhibition induced a high increase of both phosphorylated ERK1/2 and JNK in ECs leading to cell proliferation and serum response factor (SRF) levels, a downstream mediator of VEGF signalling through MEK–ERK activation in ECs (Iaconetti et al., 2012).

The MIR17-92 cluster is also involved in cardiac development and its modifications seems to influence the genesis of several cardiovascular disease, such as cardiomyopathy, arrhythmias, heart failure and cardiac hypertrophy (Gu et al., 2017; van Almen et al., 2011; Danielson et al., 2013). In myocardial infarction (MI) MIR19A1 is able to stimulate the proliferation of cardiomyocytes through the regulation of PTEN and therefore it can represent a therapeutic target to protect heart from the ischemic injury caused by MI (Chen et al., 2013). MIR92A1 is normally highly expressed in endothelial cells; nevertheless, under ischemic conditions MIR92A1 over-expression in those cells inhibit angiogenesis in a mouse model (Bonauer et al., 2009). Also, in patients with coronary artery disease MIR17 and MIR92A1 exhibited higher levels compared with endothelial cells from healthy controls (Tijsen et al., 2012; Fichtlscherer et al., 2010).

3.9. MIR221/MIR222

microRNA 221 and microRNA222 are two flanking miRNAs that belong to the same family and are essential for vascular biology. This

miRNA cluster regulates endothelial cells (ECs) development and differentiation by inhibiting their proangiogenic roles (Chistiakov et al., 2015). They are also involved in tumor growth (Poliseno et al., 2006). In glioblastoma and prostate cancer, MIR221/222 are suggested to act as oncomiRs (le Sage et al., 2007), the overexpression of the cluster correlate with poor prognosis as well decreased $p27^{Kip1}$ levels (a cell cycle inhibitor and tumor suppressor) in cancer patients (le Sage et al., 2007; Galardi et al., 2007). Furthermore, in glioblastoma the over-expression of MIR221/222 determines a down-regulation of anti-angiogenic factor such as TIMP2, an inhibitor of metalloproteinases. This mechanism at the end promotes cell proliferation, angiogenesis and metastasis, by regulating the activity of metallopeptidases (MMPs) (Yang et al., 2015). Similarly in non-small cell lung cancer and hepatocellular carcinoma, the cluster acts by down-regulating TIMP3 and PTEN expression, causing cellular migration by AKT and ERK phosphorylation and invasion by MMP3 and MMP9 activity (Garofalo et al., 2009). C-Kit is another direct target of MIR221/MIR222 cluster in physiological and pathologic condition. C-Kit is a receptor for stem cell factor (SCF), a growth factor involved in angiogenesis. The transfection of HUVEC with MIR221 and MIR222 determines a down-regulation of C-Kit expression leading to the deregulation of cell migration and tubes formation capacity (Poliseno et al., 2006).

Nicoli et al supported an important role for MIR221 in endothelial tip cell proliferation and migration, in particular, they found that MIR221 is an essential for angiogenesis positive regulation in embryonic zebrafish by influencing cells sprouting through the down-regulation of two targets, CDKN1b and PIK3R1. The first is a cyclin dependent kinase inhibitor and the second is a p85-alpha regulatory subunit of the phosphoinositide-3-kinase (PI3K) complex. Over-expressions of the two targets reduced intersegmental vessel length and endothelial cell numbers *in vitro* zebrafish model (Nicoli et al., 2012). Other studies, instead, support their anti-angiogenic role, MIR221 and MIR222 have been shown to inhibit endothelial cell migration, proliferation and angiogenesis by targeting C-Kit (Urbich et al., 2008). An upregulation of MIR221/MIR222 in arterial ECs appears to be proatherogenic since these miRNAs downregulate endothelial nitric oxide synthase (eNOS) and inhibit angiogenesis essential for vascular repair (Rippe et al., 2012). Moreover, these miRNAs are down-regulated during adipogenesis and are associated with BMI in human adipose tissue samples (Ortega et al., 2010). MIR221/MIR222 cluster deregulation seems also to be involved in metabolic disease (Deiuliis, 2016). In particular MIR222 negatively regulate adipocyte insulin sensitivity in human by repressing ERα and GLUT4 expression (Shi et al., 2014); accordingly, its level is higher in murine models of diabetes (Chartoumpekis et al., 2012; Herrera et al., 2010) and in the omental adipose tissue of women with gestational diabetes (Shi et al., 2014). MIR221 is also involved in insulin resistance by down-regulating adiponectin expression. In obese patients, plasma levels of MIR221 are decreased (Ortega et al., 2013), while in non-obese females with metabolic syndrome it is increased (Wang et al., 2013).

3.10. MIR21

microRNA 21 is one of the most widely studied angiogenic miRNAs in several tumors, among which breast, colon and prostate cancers (Hicklin and Ellis, 2005; Krichevsky and Gabriely, 2009). Several studies suggest that MIR21 plays an important role in mediating angiogenesis, tissue neovascularization and then it is considered an oncogene involved in apoptosis, necrosis and invasion. The deregulation of MIR21 is also involved in other human pathologies where inflammation and cell proliferation play a critical role (Zhao et al., 2013; Heusschen et al., 2010).

Liu et al. showed that MIR21 down-regulation could decrease the expression of VEGF in prostate tumor, blocking tumor angiogenesis. The upregulation of the HIF1A and VEGF expression is controlled by AKT and ERK1/2 pathways (Liu et al., 2011). Shin et al demonstrated that inhibition of MIR21 or TGFB treatment increased the levels of VEGF and interleukin-6 in human adipose stem cells (hASCs) and increased tumor growth and angiogenic action of hASC in a hind limb ischemia model (Shin et al., 2012). Tumor growth decreased with the inhibition of transforming growth factor beta receptor II (TGFBR2) likewise MIR21 overexpression, while increased with co-transplantation with siSTAT3-hADSCs, indicating a modulation of TGFB signaling by MIR21.

The tissue inhibitor of matrix metalloproteinase 3 (TIMP3) is targeted by MIR21. Hu et al. demonstrated that MIR-21 causes the reduction of TIMP3 promoting the matrix metalloproteinase-2 (MMP2) and matrix metalloproteinase-9 (MMP9) expression in spinal cord injury *in vivo* and in ischemic/reperfusion HUVEC condition *in vitro* (Hu et al., 2016). MIR21 is also widely studied in retinal microvascular cells that require a healthy angiogenic process and where a deregulation of this process leads to retinopathy, for example in diabetes (Guduric-Fuchs et al., 2012). The studies demonstrated the pro-angiogenic activities of MIR21 *in vitro* and *in vivo* systems. Gutsaeva et al. showed that over-expression of MIR21 promotes tube formation of human retinal endothelial cells (HREC) under hypoxia condition, with an initial up-regulation of STAT3 and a final downregulation of TIMP3 (Gutsaeva et al., 2017). Finally, it was demonstrated also that knock-down of MIR21 had a distinct negative effect on neointimal lesion formation, thus suggesting again a pro-angiogenic effect of MIR21 by regulating PTEN and BCL2 levels (Ji et al., 2007). The angiogenic potential of ASCs seem also to be related to MIR21. Indeed, it has been shown that MIR21 is packed inside EVs secreted by ASC and it is able to regulate the angiogenic process.

In obese patients MIR21 expression in omental and subcutaneous adipose tissue did not show significant difference, however when the patients were subdivided according to the presence of diabetes it was reported and MIR21 is overexpressed in adipose tissue from patients with diabetes. Therefore, MIR21 seems to be linked to insulin-resistance deterioration within its pathophysiologic progression from obesity to diabetes (Guglielmi et al., 2017). Moreover, in diabetic patients treated with metformin, an anti-diabetic drug commonly used, MIR21 was shown to be strikingly downregulated by metformin in a time- and dose-dependent manner by directly targeting the 3′-UTR of PTEN and SMAD7 (Luo et al., 2017a).

3.11. MIR100

microRNA 100 was described in several tumor (oral squamous, nasopharynx and breast carcinoma). It plays a role as tumor suppressor but it is also correlated with cell proliferation and vascularization. In breast cancer, MIR100 could represent a possible biomarker for cancer diagnosis and prognosis, because it is downregulated in all subtypes of breast cancer (Chen et al., 2014). The authors described for the first time that the expression of MIR100 can induce epithelial-mesenchymal transition (EMT) controlling EMT genes such as TWIST, SNAIL and ZEB1. Mechanistically, MIR100 induces EMT by targeting SMARCA5, an epigenetic regulator of E-cadherin, and inhibits tumorigenesis, migration and invasion by targeting HOXA1 and his downstream targets MET, SMO (smoothened) and SEMA3c (semaphoring 3c) (Chen et al., 2014). MIR100 over-expression may sensitize tumor cells to radio- or chemotherapy, while its down-regulation may have a negative prognostic role. In particular, it was demonstrated that MIR100 can suppress *in vitro* angiogenesis through modulation of pro-angiogenic factors including VEGF (Grundmann et al., 2011). Pakravan et al demonstrated that MIR100 present in BM-MSC derived exosomes caused the down-regulation of VEGF in breast cancer cells, via mTOR pathway (Pakravan et al., 2017). mTOR plays a key role in proliferation and angiogenesis process (Humar et al., 2002), by positively control HIF1A expression that implies VEGF transcription (Pakravan et al., 2017). Through a paracrine mechanism, exosomal MIR100 may alter the function of breast cancer cells acting as an anti-angiogenic factor during

tumorigenesis. The antiangiogenic behaviour of MIR100 was also demonstrated in association with endothelial and vascular smooth muscle cells. The authors described the expression of MIR100 in these cells, demonstrating that its over-expression, during ischemia, decrease proliferation, tube formation, and sprouting activity of endothelial cells. Moreover, it acts as endogenous repressor of mTOR: a serine/threonine protein kinase mammalian target of rapamycin; requiring after hypoxia process for angiogenesis and endothelial cell proliferation. Inhibition of MIR100 could be a novel approach for the modulation of blood vessel growth (Grundmann et al., 2011). In obese normoglycemic subjects circulating MIR100 was significantly lower compared to obese subjects with diabetes (Pek et al., 2016).

3.12. MIR486

microRNA 486 is a tumor-suppressor that is often down-regulated in multiple cancers such as myeloid leukemia, gastric adenocarcinoma and lung cancer, but its function in cancer is still not fully understood (Li et al., 2018). A recent study showed that this miRNA suppresses the transformation, migration and growth of tumor cells in lung cancer. MIR486 expression in lung cancer cell line is significantly reduced compared with normal cell and this behaviour is due to ANK1 expression. ANK1 is a gene that encodes for the adapter protein ankyrin-1. MIR486 is positioned in the intron region of ANK1 (Shaham et al., 2015). In lung cancer patients, MIR486 and ANK1 are co-expressed and epigenetically repressed through hypermethylation of ANK1 promoter. Ren et al. focused on prognostic value of MIR486-5p expression in cancers of the digestive tract and they have demonstrated that in oesophageal and gastric tumors a reduced of unchanged MIR486-5p expression compared with surrounding normal tissues is associated with poor prognosis, whereas high MIR486-5p expression is associated with a better prognosis (Ren et al., 2016). This correlation is associated with microRNA 486 targets: tumor-suppressor as PTEN and invasion and cell proliferation genes as CLDN10 and CITRON. MIR486 is also able to inhibits FGF9, a family member of the fibroblast growth factor which is involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. In gastric cancer, high expression of MIR486 is correlated with shorter overall survival (OS) (Chen et al., 2015).

Several evidences demonstrate that MIR486 is also involved in regulation of hematopoiesis and it participates to osteogenic and adipogenic differentiation of mesenchymal stem cells (MSCs) through Sirt1 gene (Shi et al., 2016). Shi et al. speculated that MIR486 could be regulated by hypoxia determining the secretion of angiogenic growth factors in BM-MSCs, in particular it was up-regulated, with HGF and VEGF, in time dependent manner after exposure to hypoxia *in vitro* model. In this condition, MIR486 was regulated through PTEN/AKT signalling. Hypoxia and MIR486 overexpression determine the down-regulation of PTEN and upregulation of pAKT (Shi et al., 2016).

MIR486 play also a relevant role in cardiomyocytes. Indeed, it is implied in the regulation of cardiomyocytes apoptosis, a common pathological manifestation that occurs in several heart diseases. In particular MIR486 regulate cardiomyocyte apoptosis via p53-mediated BCL2 associated mitochondrial apoptotic pathway (Sun et al., 2017).

3.13. MIR30

The components of the microRNA 30 family have the same sequence and are coded by 6 genes located on human chromosomes 1, 6 and 8. Members of the MIR30 family are implicated in mesenchymal differentiation (osteogenesis and adipogenesis) (Wu et al., 2012b), angiogenesis and cancer development (Lung, Breast, Thyroid etc.) (Braun et al., 2010). Especially, MIR30 is correlated with physiological and pathological epithelial-mesenchymal transition (EMT) and vice versa (MET). *In vitro* experiments demonstrated that the overexpression of MIR30B on non-tumorigenic murine hepatocyte cell line (AML12) inhibit the TGFB-induced EMT, blocking cells migration through the down-regulation of Snail1 and E-cadherin levels. The others members, MIR-30C, -30D and -30E are widely down-regulated from TGFB1-induced Snail1 upregulation in AML12 cells compared to MIR30 A, that it is slightly regulated in EMT process (Zhang et al., 2012).

MIR30 family regulates a vascular sprouting and vessels branching, through modulation of Delta-like 4 (DLL4) ligand. DLL4 is highly expressed in endothelial cell, primarily, during pathologic angiogenesis, but also during the correct development of intersegmental vessel IVS in Zebrafish (Bridge et al. 2012). Bridge et al. demonstrated that MIR30 overexpression, in particular MIR30B and MIR30C, in HUVECs cells promotes angiogenic sprouting. In this situation when DLL4 signalling or expression is inhibited by over expressing of MIR30B, there is an excessive sprouting from existing vessels (Bridge et al. 2012). MIR30C was also investigated during mesenchymal differentiation versus adipogenesis in obese conditions. Karbiener et al demonstrated that MIR30C expression was regulated during early adipocyte differentiation, through two direct targets such as PAI-1 (Plasminogen activator inhibitor-1) and ALK2 (activin receptor-like kinase-2) (Karbiener et al., 2011).

Furthermore, another pro-angiomiR is MIR30B. The overexpression of this miRNA enhanced the pro-angiogenic capacity of endothelial cells through the treatment with exosomes secreted from MSCs. MIR30B present in exosomes significantly increased the cumulative tube length and the formation of tubular structures (Gong et al. 2017).

4. Conclusion

The potential application of ASCs in regenerative therapies is both innovative and promising. ASCs possess highly plasticity and can differentiate primarily in many lineages such as adipocytes, osteocytes, chondrocytes, endothelial cells and others (Orbay et al., 2012). ASCs have been studied for a long time for their capability in medicine fields and several studies are focusing on the involvement of these cells in the development of different diseases. The physiological functions of ASCs are the replacement of connective tissues by differentiation into specific mesenchymal cells; nevertheless, it has been recently demonstrated that ASCs actively participate in the generation on new blood vessels. Indeed they seem to be involved in tumor expansion promotion through stimulation of new angiogenic network, carrying out a pro-tumor action due to an inflammatory microenvironment (Bielli et al., 2014). The excess of abdominal adipose tissue has been already associated with several pathological conditions. Indeed, obesity represents an important risk factor for stroke, hypertension, type II diabetes and different cancer types such as breast, colon and endometrial cancer. Regard this topic it is necessary to deepen the studies to clarify the impact of adiposity on cancer incidence. Many studies have demonstrated that angiogenic factors are able to stimulate the differentiation of ASCs from pre-adipocytes to mature cells. WAT, as endocrine tissue, is an active secretory tissue releasing adipokines and paracrine factors, signalling molecules that compose the adipose microenvironment and can modulate both positively and negatively angiogenic processes, homeostasis and vascular integrity, regulating the interaction between the vascular system and adipocytes. This process is known as angiogenic switch in which there is a mutual change between adipocytes and endothelial cells (Szöke and Brinchmann, 2012).

The main aim of our review is to understand the role of miRNAs in angiogenesis process and if they can modulate the ASCs response. There are still few studies that focus on this aspect. Several biological processes, such as maintaining stemness, proliferation, differentiation and angiogenesis of adipose stem cells are controlled by microRNAs. Our literature analysis showed that MIR126, MIR31, MIR92a, MIR221, MIR30, MIR100 and MIR486, secreted by ASCs, are directly involved in angiogenesis process by controlling pathways involved in this event. These important findings offer the possibility to speculate on exploiting these small nucleic acids in the therapeutic field, where miRNAs could

become pharmacological targets for the treatment of vascular diseases associated with obesity or cancer. Consequently, miRNAs could be used as prognostic biomarkers that support the angiogenesis process stimulated by ASCs in different types of cancer and adipose tissue related diseases.

These findings require further investigation to validate the miRNAs and their target genes to be used in clinical practice. Moreover, the therapeutic success could be dependent on in-depth mechanistic understanding of angiogenesis role in the modulation of adipose tissue expansion and regression.

Conflict of interest

The authors have no competing interests.

References

Ahima, R.S., Flier, J.S., 2000. Adipose tissue as an endocrine organ. Trends Endocrinol. Metab. 11, 327–332.

Balaj, S., King, A., Crombleholme, T.M., Keswani, S.G., 2013. The role of endothelial progenitor cells in postnatal vasculogenesis: implications for therapeutic neovascularization and wound healing. Adv. Wound Care (New Rochelle) 2, 283–295.

Bertolini, F., Lohsiriwat, V., Petit, J.Y., Kolonin, M.G., 2012. Adipose tissue cells, lipotransfer and cancer: a challenge for scientists, oncologists and surgeons. Biochim. Biophys Acta 1826, 209–214.

Bhang, S.H., Cho, S.W., La, W.G., Lee, T.J., Yang, H.S., Sun, A.Y., Baek, S.H., Rhie, J.W., Kim, B.S., 2011. Angiogenesis in ischemic tissue produced by spheroid grafting of human adipose-derived stromal cells. Biomaterials 32, 2734–2747.

Bielli, A., Scioli, M.G., Gentile, P., Agostinelli, S., Tarquini, C., Cervelli, V., Orlandi, A., 2014. Adult adipose-derived stem cells and breast cancer: a controversial relationship. Springerplus 3, 345.

Boehncke, W.H., Boehncke, S., 2011. Systemic antipsoriatic therapy may reverse endothelial dysfunction. Br. J. Dermatol. 164, 1397–1398 author reply 1398.

Bonauer, A., Carmona, G., Iwasaki, M., Mione, M., Koyanagi, M., Fischer, A., Burchfield, J., Fox, H., Doebele, C., Ohtani, K., Chavakis, E., Potente, M., Tjwa, M., Urbich, C., Zeiher, A.M., Dimmeler, S., 2009. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. Science 324, 1710–1713.

Borges, F.T., Melo, S.A., Özdemir, B.C., Kato, N., Revuelta, I., Miller, C.A., Gattone, V.H., LeBleu, V.S., Kalluri, R., 2013. TGF-β1-containing exosomes from injured epithelial cells activate fibroblasts to initiate tissue regenerative responses and fibrosis. J. Am. Soc. Nephrol. 24, 385–392.

Bouloumié, A., Drexler, H.C., Lafontan, M., Busse, R., 1998. Leptin, the product of Ob gene, promotes angiogenesis. Circ. Res. 83, 1059–1066.

Braun, J., Hoang-Vu, C., Dralle, H., Hüttelmaier, S., 2010. Downregulation of microRNAs directs the EMT and invasive potential of anaplastic thyroid carcinomas. Oncogene 29, 4237–4244.

Bridge, G., Monteiro, R., Henderson, S., Emuss, V., Lagos, D., Georgopoulou, D., Patient, R., Boshoff, C., 2012. The microRNA-30 family targets DLL4 to modulate endothelial cell behavior during angiogenesis. Blood 120, 5063–5072.

Brissova, M., Shostak, A., Shiota, M., Wiebe, P.O., Poffenberger, G., Kantz, J., Chen, Z., Carr, C., Jerome, W.G., Chen, J., Baldwin, H.S., Nicholson, W., Bader, D.M., Jetton, T., Gannon, M., Powers, A.C., 2006. Pancreatic islet production of vascular endothelial growth factor–a is essential for islet vascularization, revascularization, and function. Diabetes 55, 2974–2985.

Bråkenhielm, E., Cao, R., Gao, B., Angelin, B., Cannon, B., Parini, P., Cao, Y., 2004a. Angiogenesis inhibitor, TNP-470, prevents diet-induced and genetic obesity in mice. Circ. Res. 94, 1579–1588.

Bråkenhielm, E., Veitonmäki, N., Cao, R., Kihara, S., Matsuzawa, Y., Zhivotovsky, B., Funahashi, T., Cao, Y., 2004b. Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. Proc. Natl. Acad. Sci. U. S. A. 101, 2476–2481.

Cao, R., Brakenhielm, E., Wahlestedt, C., Thyberg, J., Cao, Y., 2001. Leptin induces vascular permeability and synergistically stimulates angiogenesis with FGF-2 and VEGF. Proc. Natl. Acad. Sci. U. S. A. 98, 6390–6395.

Cao, R., Bråkenhielm, E., Li, X., Pietras, K., Widenfalk, J., Ostman, A., Eriksson, U., Cao, Y., 2002. Angiogenesis stimulated by PDGF-CC, a novel member in the PDGF family, involves activation of PDGFR-alphaalpha and -alphabeta receptors. FASEB J. 16, 1575–1583.

Cao, Y., 2007. Angiogenesis modulates adipogenesis and obesity. J. Clin. Invest. 117, 2362–2368.

Cao, Y., 2013. Angiogenesis and vascular functions in modulation of obesity, adipose metabolism, and insulin sensitivity. Cell Metab. 18, 478–489.

Carmeliet, P., 2000. Mechanisms of angiogenesis and arteriogenesis. Nat. Med. 6, 389–395.

Carmeliet, P., Jain, R.K., 2011. Molecular mechanisms and clinical applications of angiogenesis. Nature 473, 298–307.

Castellot, J.J., Karnovsky, M.J., Spiegelman, B.M., 1982. Differentiation-dependent stimulation of neovascularization and endothelial cell chemotaxis by 3T3 adipocytes. Proc. Natl. Acad. Sci. U. S. A. 79, 5597–5601.

Chartoumpekis, D.V., Zaravinos, A., Ziros, P.G., Iskrenova, R.P., Psyrogiannis, A.I., Kyriazopoulou, V.E., Habeos, I.G., 2012. Differential expression of microRNAs in adipose tissue after long-term high-fat diet-induced obesity in mice. PLoS One 7, e34872.

Chen, D., Sun, Y., Yuan, Y., Han, Z., Zhang, P., Zhang, J., You, M.J., Teruya-Feldstein, J., Wang, M., Gupta, S., Hung, M.C., Liang, H., Ma, L., 2014. miR-100 induces epithelial-mesenchymal transition but suppresses tumorigenesis, migration and invasion. PLoS Genet. 10 e1004177.

Chen, H., Ren, C., Han, C., Wang, D., Chen, Y., Fu, D., 2015. Expression and prognostic value of miR-486-5p in patients with gastric adenocarcinoma. PLoS One 10 e0119384.

Chen, J., Huang, Z.P., Seok, H.Y., Ding, J., Kataoka, M., Zhang, Z., Hu, X., Wang, G., Lin, Z., Wang, S., Pu, W.T., Liao, R., Wang, D.Z., 2013. mir-17-92 cluster is required for and sufficient to induce cardiomyocyte proliferation in postnatal and adult hearts. Circ. Res. 112, 1557–1566.

Chen, T.S., Lai, R.C., Lee, M.M., Choo, A.B., Lee, C.N., Lim, S.K., 2010. Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs. Nucleic Acids Res. 38, 215–224.

Chistiakov, D.A., Sobenin, I.A., Orekhov, A.N., Bobryshev, Y.V., 2015. Human miR-221/222 in physiological and atherosclerotic vascular remodeling. Biomed Res. Int. 2015, 354517.

Cinti, S., 2018. Pink adipocytes. Trends Endocrinol. Metab. 29, 651–666.

Corselli, M., Chen, C.W., Crisan, M., Lazzari, L., Péault, B., 2010. Perivascular ancestors of adult multipotent stem cells. Arterioscler. Thromb. Vasc. Biol. 30, 1104–1109.

Crandall, D.L., Hausman, G.J., Kral, J.G., 1997. A review of the microcirculation of adipose tissue: anatomic, metabolic, and angiogenic perspectives. Microcirculation 4, 211–232.

Danielson, L.S., Park, D.S., Rotllan, N., Chamorro-Jorganes, A., Guijarro, M.V., Fernandez-Hernando, C., Fishman, G.I., Phoon, C.K., Hernando, E., 2013. Cardiovascular dysregulation of miR-17-92 causes a lethal hypertrophic cardiomyopathy and arrhythmogenesis. FASEB J. 27, 1460–1467.

Deiuliis, J.A., 2016. MicroRNAs as regulators of metabolic disease: pathophysiologic significance and emerging role as biomarkers and therapeutics. Int. J. Obes. (Lond.) 40, 88–101.

Deng, H.T., Liu, H.L., Zhai, B.B., Zhang, K., Xu, G.C., Peng, X.M., Zhang, Q.Z., Li, L.Y., 2017. Vascular endothelial growth factor suppresses TNFSF15 production in endothelial cells by stimulating miR-31 and miR-20a expression via activation of Akt and Erk signals. FEBS Open Biol. 7, 108–117.

Deng, W., Gu, X., Lu, Y., Gu, C., Zheng, Y., Zhang, Z., Chen, L., Yao, Z., Li, L.Y., 2012. Down-modulation of TNFSF15 in ovarian cancer by VEGF and MCP-1 is a pre-requisite for tumor neovascularization. Angiogenesis 15, 71–85.

Dews, M., Homayouni, A., Yu, D., Murphy, D., Sevignani, C., Wentzel, E., Furth, E.E., Lee, W.M., Enders, G.H., Mendell, J.T., Thomas-Tikhonenko, A., 2006. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. Nat. Genet. 38, 1060–1065.

Di Stefano, A.B., Grisafi, F., Castiglia, M., Perez, A., Montesano, L., Gulino, A., Toia, F., Fanale, D., Russo, A., Moschella, F., Leto Barone, A.A., Cordova, A., 2018. Spheroids from adipose-derived stem cells exhibit an miRNA profile of highly undifferentiated cells. J. Cell. Physiol. 233 (11), 8778–8789.

Divella, R., De Luca, R., Abbate, I., Naglieri, E., Daniele, A., 2016. Obesity and cancer: the role of adipose tissue and adipo-cytokines-induced chronic inflammation. J. Cancer 7, 2346–2359.

Doebele, C., Bonauer, A., Fischer, A., Scholz, A., Reiss, Y., Urbich, C., Hofmann, W.K., Zeiher, A.M., Dimmeler, S., 2010. Members of the microRNA-17-92 cluster exhibit a cell-intrinsic antiangiogenic function in endothelial cells. Blood 115, 4944–4950.

Elias, I., Franckhauser, S., Ferré, T., Vilà, L., Tafuro, S., Muñoz, S., Roca, C., Ramos, D., Pujol, A., Riu, E., Ruberte, J., Bosch, F., 2012. Adipose tissue overexpression of vascular endothelial growth factor protects against diet-induced obesity and insulin resistance. Diabetes 61, 1801–1813.

Ferrer-Lorente, R., Bejar, M.T., Tous, M., Vilahur, G., Badimon, L., 2014. Systems biology approach to identify alterations in the stem cell reservoir of subcutaneous adipose tissue in a rat model of diabetes: effects on differentiation potential and function. Diabetologia 57, 246–256.

Fichtlscherer, S., De Rosa, S., Fox, H., Schwietz, T., Fischer, A., Liebetrau, C., Weber, M., Hamm, C.W., Röxe, T., Müller-Ardogan, M., Bonauer, A., Zeiher, A.M., Dimmeler, S., 2010. Circulating microRNAs in patients with coronary artery disease. Circ. Res. 107, 677–684.

Fish, J.E., Santoro, M.M., Morton, S.U., Yu, S., Yeh, R.F., Wythe, J.D., Ivey, K.N., Bruneau, B.G., Stainier, D.Y., Srivastava, D., 2008. miR-126 regulates angiogenic signaling and vascular integrity. Dev. Cell 15, 272–284.

Folkman, J., 2002. Role of angiogenesis in tumor growth and metastasis. Semin Oncol 29, 15–18.

Freese, K.E., Kokai, L., Edwards, R.P., Philips, B.J., Sheikh, M.A., Kelley, J., Comerci, J., Marra, K.G., Rubin, J.P., Linkov, F., 2015. Adipose-derived stems cells and their role in human cancer development, growth, progression, and metastasis: a systematic review. Cancer Res. 75, 1161–1168.

Galardi, S., Mercatelli, N., Giorda, E., Massalini, S., Frajese, G.V., Ciafrè, S.A., Farace, M.G., 2007. miR-221 and miR-222 expression affects the proliferation potential of human prostate carcinoma cell lines by targeting p27Kip1. J. Biol. Chem. 282, 23716–23724.

Garofalo, M., Di Leva, G., Romano, G., Nuovo, G., Suh, S.S., Ngankeu, A., Taccioli, C., Pichiorri, F., Alder, H., Secchiero, P., Gasparini, P., Gonelli, A., Costinean, S., Acunzo, M., Condorelli, G., Croce, C.M., 2009. miR-221&222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. Cancer Cell 16, 498–509.

Gong, M., Yu, B., Wang, J., Wang, Y., Liu, M., Paul, C., Millard, R.W., Xiao, D.S., Ashraf, M., Xu, M., 2017. Mesenchymal stem cells release exosomes that transfer miRNAs to

endothelial cells and promote angiogenesis. Oncotarget 8, 45200–45212.
Gottmann, P., Ouni, M., Saussenthaler, S., Roos, J., Stirm, L., Jähnert, M., Kamitz, A., Hallahan, N., Jonas, W., Fritsche, A., Häring, H.U., Staiger, H., Blüher, M., Fischer-Posovszky, P., Vogel, H., Schürmann, A., 2018. A computational biology approach of a genome-wide screen connected miRNAs to obesity and type 2 diabetes. Mol. Metab. 11, 145–159.
Grundmann, S., Hans, F.P., Kinniry, S., Heinke, J., Helbing, T., Bluhm, F., Sluijter, J.P., Hoefer, I., Pasterkamp, G., Bode, C., Moser, M., 2011. MicroRNA-100 regulates neovascularization by suppression of mammalian target of rapamycin in endothelial and vascular smooth muscle cells. Circulation 123, 999–1009.
Gu, H., Liu, Z., Zhou, L., 2017. Roles of miR-17-92 cluster in cardiovascular development and common diseases. Biomed. Res. Int. 2017, 9102909.
Guduric-Fuchs, J., O'Connor, A., Cullen, A., Harwood, L., Medina, R.J., O'Neill, C.L., Stitt, A.W., Curtis, T.M., Simpson, D.A., 2012. Deep sequencing reveals predominant expression of miR-21 amongst the small non-coding RNAs in retinal microvascular endothelial cells. J. Cell. Biochem. 113, 2098–2111.
Guglielmi, V., D'Adamo, M., Menghini, R., Cardellini, M., Gentileschi, P., Federici, M., Sbraccia, P., 2017. MicroRNA 21 is up-regulated in adipose tissue of obese diabetic subjects. Nutr. Healthy Aging 4, 141–145.
Gupta, R.K., Mepani, R.J., Kleiner, S., Lo, J.C., Khandekar, M.J., Cohen, P., Frontini, A., Bhowmick, D.C., Ye, L., Cinti, S., Spiegelman, B.M., 2012. Zfp423 expression identifies committed preadipocytes and localizes to adipose endothelial and perivascular cells. Cell Metab. 15, 230–239.
Gutsaeva, D.R., Thounaojam, M., Rajpurohit, S., Powell, F.L., Martin, P.M., Goei, S., Duncan, M., Bartoli, M., 2017. STAT3-mediated activation of. Oncotarget 8, 103568–103580.
Hamdy, O., Ashrafzadeh, S., Mottalib, A., 2018. Weight management in patients with type 2 diabetes: a multidisciplinary real-world approach. Curr. Diab. Rep. 18, 66.
Hanahan, D., Folkman, J., 1996. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 86, 353–364.
Harris, T.A., Yamakuchi, M., Ferlito, M., Mendell, J.T., Lowenstein, C.J., 2008. MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. Proc. Natl. Acad. Sci. U. S. A. 105, 1516–1521.
Harwood, H.J., 2012. The adipocyte as an endocrine organ in the regulation of metabolic homeostasis. Neuropharmacology 63, 57–75.
Heaton, G.M., Wagenvoord, R.J., Kemp, A., Nicholls, D.G., 1978. Brown-adipose-tissue mitochondria: photoaffinity labelling of the regulatory site of energy dissipation. Eur. J. Biochem. 82, 515–521.
Herrera, B.M., Lockstone, H.E., Taylor, J.M., Ria, M., Barrett, A., Collins, S., Kaisaki, P., Argoud, K., Fernandez, C., Travers, M.E., Grew, J.P., Randall, J.C., Gloyn, A.L., Gauguier, D., McCarthy, M.I., Lindgren, C.M., 2010. Global microRNA expression profiles in insulin target tissues in a spontaneous rat model of type 2 diabetes. Diabetologia 53, 1099–1109.
Heusschen, R., van Gink, M., Griffioen, A.W., Thijssen, V.L., 2010. MicroRNAs in the tumor endothelium: novel controls on the angioregulatory switchboard. Biochim. Biophys. Acta 1805, 87–96.
Hicklin, D.J., Ellis, L.M., 2005. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. J. Clin. Oncol. 23, 1011–1027.
Hu, J., Ni, S., Cao, Y., Zhang, T., Wu, T., Yin, X., Lang, Y., Lu, H., 2016. The angiogenic effect of microRNA-21 targeting TIMP3 through the regulation of MMP2 and MMP9. PLoS One 11 e0149537.
Humar, R., Kiefer, F.N., Berns, H., Resink, T.J., Battegay, E.J., 2002. Hypoxia enhances vascular cell proliferation and angiogenesis in vitro via rapamycin (mTOR)-dependent signaling. FASEB J. 16, 771–780.
Iacomino, G., Russo, P., Stillitano, I., Lauria, F., Marena, P., Ahrens, W., De Luca, P., Siani, A., 2016. Circulating microRNAs are deregulated in overweight/obese children: preliminary results of the I.FAmily study. Genes Nutr. 11, 7.
Iaconetti, C., Polimeni, A., Sorrentino, S., Sabatino, J., Pironti, G., Esposito, G., Curcio, A., Indolfi, C., 2012. Inhibition of miR-92a increases endothelial proliferation and migration in vitro as well as reduces neointimal proliferation in vivo after vascular injury. Basic Res. Cardiol. 107, 296.
Ji, R., Cheng, Y., Yue, J., Yang, J., Liu, X., Chen, H., Dean, D.B., Zhang, C., 2007. MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. Circ. Res. 100, 1579–1588.
Kalinina, N., Klink, G., Glukhanyuk, E., Lopatina, T., Efimenko, A., Akopyan, Z., Tkachuk, V., 2015. miR-92a regulates angiogenic activity of adipose-derived mesenchymal stromal cells. Exp. Cell Res. 339, 61–66.
Kang, T., Jones, T.M., Naddell, C., Bacanamwo, M., Calvert, J.W., Thompson, W.E., Bond, V.C., Chen, Y.E., Liu, D., 2016. Adipose-derived stem cells induce angiogenesis via microvesicle transport of miRNA-31. Stem Cells Transl. Med. 5, 440–450.
Karbiener, M., Neuhold, C., Opriessnig, P., Prokesch, A., Bogner-Strauss, J.G., Scheideler, M., 2011. MicroRNA-30c promotes human adipocyte differentiation and co-represses PAI-1 and ALK2. RNA Biol. 8, 850–860.
Kawamoto, A., Tkebuchava, T., Yamaguchi, J., Nishimura, H., Yoon, Y.S., Milliken, C., Uchida, S., Masuo, O., Iwaguro, H., Ma, H., Hanley, A., Silver, M., Kearney, M., Losordo, D.W., Isner, J.M., Asahara, T., 2003. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. Circulation 107, 461–468.
Khanaghaei, M., Tourkianvalashani, F., Hekmatimoghaddam, S., Ghasemi, N., Rahaie, M., Khorramshahi, V., Sheikhpour, A., Heydari, Z., Pourrajab, F., 2016. Circulating miR-126 and miR-499 reflect progression of cardiovascular disease; correlations with uric acid and ejection fraction. Heart Int. 11, e1–e9.
Kim, I., Ryu, Y.S., Kwak, H.J., Ahn, S.Y., Oh, J.L., Yancopoulos, G.D., Gale, N.W., Koh, G.Y., 2002. EphB ligand, ephrinB2, suppresses the VEGF- and angiopoietin 1-induced Ras/mitogen-activated protein kinase pathway in venous endothelial cells. FASEB J. 16, 1126–1128.
Kim, Y., Kim, H., Cho, H., Bae, Y., Suh, K., Jung, J., 2007. Direct comparison of human mesenchymal stem cells derived from adipose tissues and bone marrow in mediating neovascularization in response to vascular ischemia. Cell. Physiol. Biochem. 20, 867–876.
Koch, A.E., Distler, O., 2007. Vasculopathy and disordered angiogenesis in selected rheumatic diseases: rheumatoid arthritis and systemic sclerosis. Arthritis Res. Ther. 9 (Suppl 2), S3.
Kontaraki, J.E., Marketou, M.E., Zacharis, E.A., Parthenakis, F.I., Vardas, P.E., 2014. MicroRNA-9 and microRNA-126 expression levels in patients with essential hypertension: potential markers of target-organ damage. J. Am. Soc. Hypertens. 8, 368–375.
Krichevsky, A.M., Gabriely, G., 2009. miR-21: a small multi-faceted RNA. J. Cell. Mol. Med. 13, 39–53.
Kuhnert, F., Kuo, C.J., 2010. miR-17-92 angiogenesis micromanagement. Blood 115, 4631–4633.
Kuosmanen, S.M., Kansanen, E., Sihvola, V., Levonen, A.L., 2017. MicroRNA profiling reveals distinct profiles for tissue-derived and cultured endothelial cells. Sci. Rep. 7, 10943.
Kwon, H.M., Hur, S.M., Park, K.Y., Kim, C.K., Kim, Y.M., Kim, H.S., Shin, H.C., Won, M.H., Ha, K.S., Kwon, Y.G., Lee, D.H., 2014. Multiple paracrine factors secreted by mesenchymal stem cells contribute to angiogenesis. Vasc. Pharmacol. 63, 19–28.
le Sage, C., Nagel, R., Egan, D.A., Schrier, M., Mesman, E., Mangiola, A., Anile, C., Maira, G., Mercatelli, N., Ciafrè, S.A., Farace, M.G., Agami, R., 2007. Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. EMBO J. 26, 3699–3708.
Lee, H.J., 2018. Exercise training regulates angiogenic gene expression in white adipose tissue. J. Exerc. Rehabil. 14, 16–23.
Lee, M.J., Kim, M.Y., Heo, S.C., Kwon, Y.W., Kim, Y.M., Do, E.K., Park, J.H., Lee, J.S., Han, J., Kim, J.H., 2012. Macrophages regulate smooth muscle differentiation of mesenchymal stem cells via a prostaglandin $F_2\alpha$-mediated paracrine mechanism. Arterioscler. Thromb. Vasc. Biol. 32, 2733–2740.
Leto Barone, A.A., Giunta, G., Toia, F., Cordova, A., Moschella, F., 2013. Adipose-derived stem cells: true or false? A different point of view. J Craniofac. Surg 24, 1072.
Li, C., Zheng, X., Li, W., Bai, F., Lyu, J., Meng, Q.H., 2018. Serum miR-486-5p as a diagnostic marker in cervical cancer: with investigation of potential mechanisms. BMC Cancer 18, 61.
Li, H., Fredriksson, L., Li, X., Eriksson, U., 2003. PDGF-D is a potent transforming and angiogenic growth factor. Oncogene 22, 1501–1510.
Lian, W., Hu, X., Shi, R., Han, S., Cao, C., Wang, K., Li, M., 2018. MiR-31 regulates the function of diabetic endothelial progenitor cells by targeting Satb2. Acta Biochim. Biophys. Sin. (Shanghai) 50, 336–344.
Liu, L.Z., Li, C., Chen, Q., Jing, Y., Carpenter, R., Jiang, Y., Kung, H.F., Lai, L., Jiang, B.H., 2011. MiR-21 induced angiogenesis through AKT and ERK activation and HIF-1α expression. PLoS One 6, e19139.
Liu, T., Zhang, X., Gao, S., Jing, F., Yang, Y., Du, L., Zheng, G., Li, P., Li, C., Wang, C., 2016. Exosomal long noncoding RNA CRNDE-h as a novel serum-based biomarker for diagnosis and prognosis of colorectal cancer. Oncotarget 7, 85551–85563.
Liu, X., Sempere, L.F., Ouyang, H., Memoli, V.A., Andrew, A.S., Luo, Y., Demidenko, E., Korc, M., Shi, W., Preis, M., Dragnev, K.H., Li, H., Direnzo, J., Bak, M., Freemantle, S.J., Kauppinen, S., Dmitrovsky, E., 2010. MicroRNA-31 functions as an oncogenic microRNA in mouse and human lung cancer cells by repressing specific tumor suppressors. J. Clin. Invest. 120, 1298–1309.
Luo, M., Tan, X., Mu, L., Luo, Y., Li, R., Deng, X., Chen, N., Ren, M., Li, Y., Wang, L., Wu, J., Wan, Q., 2017a. MiRNA-21 mediates the antiangiogenic activity of metformin through targeting PTEN and SMAD7 expression and PI3K/AKT pathway. Sci. Rep. 7, 43427.
Luo, Q., Guo, D., Liu, G., Chen, G., Hang, M., Jin, M., 2017b. Exosomes from MiR-126-overexpressing adscs are therapeutic in relieving acute myocardial ischaemic injury. Cell. Physiol. Biochem. 44, 2105–2116.
Luo, X., Jia, R., Luo, X.Q., Wang, G., Zhang, Q.L., Qiao, H., Wang, N., Yan, J.Q., 2017c. Cold exposure differentially stimulates angiogenesis in BAT and WAT of mice: implication in adrenergic activation. Cell. Physiol. Biochem. 42, 974–986.
Mashreghi, M., Azarpara, H., Bazaz, M.R., Jafari, A., Masoudifar, A., Mirzaei, H., Jaafari, M.R., 2017. Angiogenesis biomarkers and their targeting ligands as potential targets for tumor angiogenesis. J. Cell. Physiol.
Mendell, J.T., 2008. miRiad roles for the miR-17-92 cluster in development and disease. Cell 133, 217–222.
Meyer, G.P., Wollert, K.C., Lotz, J., Pirr, J., Rager, U., Lippolt, P., Hahn, A., Fichtner, S., Schaefer, A., Arseniev, L., Ganser, A., Drexler, H., 2009. Intracoronary bone marrow cell transfer after myocardial infarction: 5-year follow-up from the randomized-controlled BOOST trial. Eur. Heart J. 30, 2978–2984.
Mitamura, T., Watari, H., Wang, L., Kanno, H., Hassan, M.K., Miyazaki, M., Katoh, Y., Kimura, T., Tanino, M., Nishihara, H., Tanaka, S., Sakuragi, N., 2013. Downregulation of miRNA-31 induces taxane resistance in ovarian cancer cells through increase of receptor tyrosine kinase MET. Oncogenesis 2, e40.
Nakagami, H., Maeda, K., Morishita, R., Iguchi, S., Nishikawa, T., Takami, Y., Kikuchi, Y., Saito, Y., Tamai, K., Ogihara, T., Kaneda, Y., 2005. Novel autologous cell therapy in ischemic limb disease through growth factor secretion by cultured adipose tissue-derived stromal cells. Arterioscler. Thromb. Vasc. Biol. 25, 2542–2547.
Nakao, N., Nakayama, T., Yahata, T., Muguruma, Y., Saito, S., Miyata, Y., Yamamoto, K., Naoe, T., 2010. Adipose tissue-derived mesenchymal stem cells facilitate hematopoiesis in vitro and in vivo: advantages over bone marrow-derived mesenchymal stem cells. Am. J. Pathol. 177, 547–554.
Napolitano, L., 1963. The differentiation of white adipose cells. an electron microscope study. J. Cell Biol. 18, 663–679.
Nicoli, S., Knyphausen, C.P., Zhu, L.J., Lakshmanan, A., Lawson, N.D., 2012. miR-221 is

required for endothelial tip cell behaviors during vascular development. Dev. Cell 22, 418–429.
Olson, L.E., Soriano, P., 2011. PDGFRβ signaling regulates mural cell plasticity and inhibits fat development. Dev. Cell 20, 815–826.
Orbay, H., Tobita, M., Mizuno, H., 2012. Mesenchymal stem cells isolated from adipose and other tissues: basic biological properties and clinical applications. Stem Cells Int. 2012, 461718.
Orbay, H., Zhang, Y., Hong, H., Hacker, T.A., Valdovinos, H.F., Zagzebski, J.A., Theuer, C.P., Barnhart, T.E., Cai, W., 2013. Positron emission tomography imaging of angiogenesis in a murine hindlimb ischemia model with 64Cu-labeled TRC105. Mol. Pharm. 10, 2749–2756.
Ortega, F.J., Mercader, J.M., Catalán, V., Moreno-Navarrete, J.M., Pueyo, N., Sabater, M., Gómez-Ambrosi, J., Anglada, R., Fernández-Formoso, J.A., Ricart, W., Frühbeck, G., Fernández-Real, J.M., 2013. Targeting the circulating microRNA signature of obesity. Clin. Chem. 59, 781–792.
Ortega, F.J., Moreno-Navarrete, J.M., Pardo, G., Sabater, M., Hummel, M., Ferrer, A., Rodriguez-Hermosa, J.I., Ruiz, B., Ricart, W., Peral, B., Fernández-Real, J.M., 2010. MiRNA expression profile of human subcutaneous adipose and during adipocyte differentiation. PLoS One 5, e9022.
Paek, H.J., Kim, C., Williams, S.K., 2014. Adipose stem cell-based regenerative medicine for reversal of diabetic hyperglycemia. World J. Diabetes 5, 235–243.
Pakravan, K., Babashah, S., Sadeghizadeh, M., Mowla, S.J., Mossahebi-Mohammadi, M., Ataei, F., Dana, N., Javan, M., 2017. MicroRNA-100 shuttled by mesenchymal stem cell-derived exosomes suppresses in vitro angiogenesis through modulating the mTOR/HIF-1α/VEGF signaling axis in breast cancer cells. Cell. Oncol. Dordr. 40, 457–470.
Park, J., Kim, M., Sun, K., An, Y.A., Gu, X., Scherer, P.E., 2017. VEGF-a-expressing adipose tissue shows rapid beiging and enhanced survival after transplantation and confers IL-4-independent metabolic improvements. Diabetes 66, 1479–1490.
Park, W.S., Heo, S.C., Jeon, E.S., Hong, D.H., Son, Y.K., Ko, J.H., Kim, H.K., Lee, S.Y., Kim, J.H., Han, J., 2013. Functional expression of smooth muscle-specific ion channels in TGF-β(1)-treated human adipose-derived mesenchymal stem cells. Am. J. Physiol. Cell Physiol. 305, C377–91.
Pek, S.L., Sum, C.F., Lin, M.X., Cheng, A.K., Wong, M.T., Lim, S.C., Tavintharan, S., 2016. Circulating and visceral adipose miR-100 is down-regulated in patients with obesity and Type 2 diabetes. Mol. Cell. Endocrinol. 427, 112–123.
Pino, E., Wang, H., McDonald, M.E., Qiang, L., Farmer, S.R., 2012. Roles for peroxisome proliferator-activated receptor γ (PPARγ) and PPARγ coactivators 1α and 1β in regulating response of white and brown adipocytes to hypoxia. J. Biol. Chem. 287, 18351–18358.
Planat-Benard, V., Silvestre, J.S., Cousin, B., André, M., Nibbelink, M., Tamarat, R., Clergue, M., Manneville, C., Saillan-Barreau, C., Duriez, M., Tedgui, A., Levy, B., Pénicaud, L., Casteilla, L., 2004. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. Circulation 109, 656–663.
Poliseno, L., Tuccoli, A., Mariani, L., Evangelista, M., Citti, L., Woods, K., Mercatanti, A., Hammond, S., Rainaldi, G., 2006. MicroRNAs modulate the angiogenic properties of HUVECs. Blood 108, 3068–3071.
Qian, L.W., Xie, J., Ye, F., Gao, S.J., 2007. Kaposi's sarcoma-associated herpesvirus infection promotes invasion of primary human umbilical vein endothelial cells by inducing matrix metalloproteinases. J. Virol. 81, 7001–7010.
Rajabi, M., Mousa, S.A., 2017. The role of angiogenesis in cancer treatment. Biomedicines 5.
Ransohoff, J.D., Wu, J.C., 2012. Imaging stem cell therapy for the treatment of peripheral arterial disease. Curr. Vasc. Pharmacol. 10, 361–373.
Raval, Z., Losordo, D.W., 2013. Cell therapy of peripheral arterial disease: from experimental findings to clinical trials. Circ. Res. 112, 1288–1302.
Razmkhah, M., Jaberipour, M., Hosseini, A., Safaei, A., Khalatbari, B., Ghaderi, A., 2010. Expression profile of IL-8 and growth factors in breast cancer cells and adipose-derived stem cells (ASCs) isolated from breast carcinoma. Cell Immunol 265, 80–85.
Rehman, J., Traktuev, D., Li, J., Merfeld-Clauss, S., Temm-Grove, C.J., Bovenkerk, J.E., Pell, C.L., Johnstone, B.H., Considine, R.V., March, K.L., 2004. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation 109, 1292–1298.
Ren, C., Chen, H., Han, C., Fu, D., Zhou, L., Jin, G., Wang, F., Wang, D., Chen, Y., Ma, L., Zheng, X., Han, D., 2016. miR-486-5p expression pattern in esophageal squamous cell carcinoma, gastric cancer and its prognostic value. Oncotarget 7, 15840–15853.
Ren, J., Zhang, J., Xu, N., Han, G., Geng, Q., Song, J., Li, S., Zhao, J., Chen, H., 2013. Signature of circulating microRNAs as potential biomarkers in vulnerable coronary artery disease. PLoS One 8 e80738.
Rippe, C., Blimline, M., Magerko, K.A., Lawson, B.R., LaRocca, T.J., Donato, A.J., Seals, D.R., 2012. MicroRNA changes in human arterial endothelial cells with senescence: relation to apoptosis, eNOS and inflammation. Exp. Gerontol. 47, 45–51.
Rupnick, M.A., Panigrahy, D., Zhang, C.Y., Dallabrida, S.M., Lowell, B.B., Langer, R., Folkman, M.J., 2002. Adipose tissue mass can be regulated through the vasculature. Proc. Natl. Acad. Sci. U. S. A. 99, 10730–10735.
Schmittgen, T.D., 2010. miR-31: a master regulator of metastasis? Future Oncol. 6, 17–20.
Shabbir, A., Cox, A., Rodriguez-Menocal, L., Salgado, M., Van Badiavas, E., 2015. Mesenchymal stem cell exosomes induce proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis in vitro. Stem Cells Dev. 24, 1635–1647.
Shaham, L., Vendramini, E., Ge, Y., Goren, Y., Birger, Y., Tijssen, M.R., McNulty, M., Geron, I., Schwartzman, O., Goldberg, L., Chou, S.T., Pitman, H., Weiss, M.J., Michaeli, S., Sredni, B., Göttgens, B., Crispino, J.D., Taub, J.W., Izraeli, S., 2015. MicroRNA-486-5p is an erythroid oncomiR of the myeloid leukemias of Down syndrome. Blood 125, 1292–1301.
Shi, X.F., Wang, H., Xiao, F.J., Yin, Y., Xu, Q.Q., Ge, R.L., Wang, L.S., 2016. MiRNA-486 regulates angiogenic activity and survival of mesenchymal stem cells under hypoxia through modulating Akt signal. Biochem. Biophys. Res. Commun. 470, 670–677.
Shi, Z., Zhao, C., Guo, X., Ding, H., Cui, Y., Shen, R., Liu, J., 2014. Differential expression of microRNAs in omental adipose tissue from gestational diabetes mellitus subjects reveals miR-222 as a regulator of ERα expression in estrogen-induced insulin resistance. Endocrinology 155, 1982–1990.
Shin, K.K., Lee, A.L., Kim, J.Y., Lee, S.Y., Bae, Y.C., Jung, J.S., 2012. miR-21 modulates tumor outgrowth induced by human adipose tissue-derived mesenchymal stem cells in vivo. Biochem. Biophys. Res. Commun. 422, 633–638.
Smorlesi, A., Frontini, A., Giordano, A., Cinti, S., 2012. The adipose organ: white-brown adipocyte plasticity and metabolic inflammation. Obes. Rev. 13 (Suppl 2), 83–96.
Strem, B.M., Zhu, M., Alfonso, Z., Daniels, E.J., Schreiber, R., Beygui, R., Begyui, R., MacLellan, W.R., Maclellan, W.R., Hedrick, M.H., Fraser, J.K., 2005. Expression of cardiomyocytic markers on adipose tissue-derived cells in a murine model of acute myocardial injury. Cytotherapy 7, 282–291.
Sun, D., Yu, F., Ma, Y., Zhao, R., Chen, X., Zhu, J., Zhang, C.Y., Chen, J., Zhang, J., 2013. MicroRNA-31 activates the RAS pathway and functions as an oncogenic MicroRNA in human colorectal cancer by repressing RAS p21 GTPase activating protein 1 (RASA1). J. Biol. Chem. 288, 9508–9518.
Sun, Y., Su, Q., Li, L., Wang, X., Lu, Y., Liang, J., 2017. MiR-486 regulates cardiomyocyte apoptosis by p53-mediated BCL-2 associated mitochondrial apoptotic pathway. BMC Cardiovasc. Disord. 17, 119.
Suárez, Y., Fernández-Hernando, C., Yu, J., Gerber, S.A., Harrison, K.D., Pober, J.S., Iruela-Arispe, M.L., Merkenschlager, M., Sessa, W.C., 2008. Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. Proc. Natl. Acad. Sci. U. S. A. 105, 14082–14087.
Szöke, K., Brinchmann, J.E., 2012. Concise review: therapeutic potential of adipose tissue-derived angiogenic cells. Stem Cells Transl. Med. 1, 658–667.
Tabuso, M., Homer-Vanniasinkam, S., Adya, R., Arasaradnam, R.P., 2017. Role of tissue microenvironment resident adipocytes in colon cancer. World J. Gastroenterol. 23, 5829–5835.
Tijsen, A.J., Pinto, Y.M., Creemers, E.E., 2012. Circulating microRNAs as diagnostic biomarkers for cardiovascular diseases. Am. J. Physiol. Heart Circ. Physiol. 303, H1085–95.
Togliatto, G., Dentelli, P., Gili, M., Gallo, S., Deregibus, C., Biglieri, E., Iavello, A., Santini, E., Rossi, C., Solini, A., Camussi, G., Brizzi, M.F., 2016. Obesity reduces the pro-angiogenic potential of adipose tissue stem cell-derived extracellular vesicles (EVs) by impairing miR-126 content: impact on clinical applications. Int. J. Obes. (Lond) 40, 102–111.
Trayhurn, P., 2014. Hypoxia and adipocyte physiology: implications for adipose tissue dysfunction in obesity. Annu. Rev. Nutr. 34, 207–236.
Tsai, Y.H., Wu, M.F., Wu, Y.H., Chang, S.J., Lin, S.F., Sharp, T.V., Wang, H.W., 2009. The M type K15 protein of Kaposi's sarcoma-associated herpesvirus regulates microRNA expression via its SH2-binding motif to induce cell migration and invasion. J. Virol. 83, 622–632.
Urbich, C., Kuehbacher, A., Dimmeler, S., 2008. Role of microRNAs in vascular diseases, inflammation, and angiogenesis. Cardiovasc. Res. 79, 581–588.
van Almen, G.C., Verhesen, W., van Leeuwen, R.E., van de Vrie, M., Eurlings, C., Schellings, M.W., Swinnen, M., Cleutjens, J.P., van Zandvoort, M.A., Heymans, S., Schroen, B., 2011. MicroRNA-18 and microRNA-19 regulate CTGF and TSP-1 expression in age-related heart failure. Aging Cell 10, 769–779.
Viallard, C., Larrivée, B., 2017. Tumor angiogenesis and vascular normalization: alternative therapeutic targets. Angiogenesis 20, 409–426.
Wang, B., Fu, X., Liang, X., Deavila, J.M., Wang, Z., Zhao, L., Tian, Q., Zhao, J., Gomez, N.A., Trombetta, S.C., Zhu, M.J., Du, M., 2017. Retinoic acid induces white adipose tissue browning by increasing adipose vascularity and inducing beige adipogenesis of PDGFRα. Cell Discov. 3, 17036.
Wang, S., Aurora, A.B., Johnson, B.A., Qi, X., McAnally, J., Hill, J.A., Richardson, J.A., Bassel-Duby, R., Olson, E.N., 2008. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev. Cell 15, 261–271.
Wang, S., Olson, E.N., 2009. AngiomiRs–key regulators of angiogenesis. Curr. Opin. Genet. Dev. 19, 205–211.
Wang, Y.T., Tsai, P.C., Liao, Y.C., Hsu, C.Y., Juo, S.H., 2013. Circulating microRNAs have a sex-specific association with metabolic syndrome. J. Biomed. Sci. 20, 72.
White, H.M., Acton, A.J., Considine, R.V., 2012. The angiogenic inhibitor TNP-470 decreases caloric intake and weight gain in high-fat fed mice. Obesity (Silver Spring) 20, 2003–2009.
Wu, J., Boström, P., Sparks, L.M., Ye, L., Choi, J.H., Giang, A.H., Khandekar, M., Virtanen, K.A., Nuutila, P., Schaart, G., Huang, K., Tu, H., van Marken Lichtenbelt, W.D., Hoeks, J., Enerbäck, S., Schrauwen, P., Spiegelman, B.M., 2012a. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150, 366–376.
Wu, K., Yang, Y., Zhong, Y., Ammar, H.M., Zhang, P., Guo, R., Liu, H., Cheng, C., Koroscil, T.M., Chen, Y., Liu, S., Bihl, J.C., 2016. The effects of microvesicles on endothelial progenitor cells are compromised in type 2 diabetic patients via downregulation of the miR-126/VEGFR2 pathway. Am. J. Physiol. Endocrinol. Metab. 310, E828–37.
Wu, T., Zhou, H., Hong, Y., Li, J., Jiang, X., Huang, H., 2012b. miR-30 family members negatively regulate osteoblast differentiation. J. Biol. Chem. 287, 7503–7511.
Yang, F., Wang, W., Zhou, C., Xi, W., Yuan, L., Chen, X., Li, Y., Yang, A., Zhang, J., Wang, T., 2015. MiR-221/222 promote human glioma cell invasion and angiogenesis by targeting TIMP2. Tumour Biol. 36, 3763–3773.
Ye, J., Gao, Z., Yin, J., He, Q., 2007. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. Am. J. Physiol. Endocrinol. Metab. 293, E1118–E1128.
Zhang, J., Zhang, H., Liu, J., Tu, X., Zang, Y., Zhu, J., Chen, J., Dong, L., 2012. miR-30 inhibits TGF-β1-induced epithelial-to-mesenchymal transition in hepatocyte by

targeting Snail1. Biochem. Biophys. Res. Commun. 417, 1100–1105.
Zhang, Y., Bellows, C.F., Kolonin, M.G., 2010. Adipose tissue-derived progenitor cells and cancer. World J. Stem Cells 2, 103–113.
Zhao, Y., Xu, Y., Luo, F., Xu, W., Wang, B., Pang, Y., Zhou, J., Wang, X., Liu, Q., 2013. Angiogenesis, mediated by miR-21, is involved arsenite-induced carcinogenesis. Toxicol. Lett. 223, 35–41.
Zuk, P.A., Zhu, M., Ashjian, P., De Ugarte, D.A., Huang, J.I., Mizuno, H., Alfonso, Z.C., Fraser, J.K., Benhaim, P., Hedrick, M.H., 2002. Human adipose tissue is a source of multipotent stem cells. Mol. Biol. Cell 13, 4279–4295.