

MINI REVIEW

Mesenchymal stem cells in obesity: insights for translational applications

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Obesity is now a major public health problem worldwide. Lifestyle modification to reduce the characteristic excess body adiposity is important in the treatment of obesity, but effective therapeutic intervention is still needed to control what has become an obesity epidemic. Unfortunately, many anti-obesity drugs have been withdrawn from market due to adverse side effects. Bariatric surgery therefore remains the most effective therapy for severe cases, although such surgery is invasive and researchers continue to seek new control strategies for obesity. Mesenchymal stem cells (MSCs) are a major source of adipocyte generation, and studies have been conducted into the potential roles of MSCs in treating obesity. However, despite significant progress in stem cell research and its potential applications for obesity, adipogenesis is a highly complex process and the molecular mechanisms governing MSC adipogenesis remain ill defined. In particular, successful clinical application of MSCs will require extensive identification and characterization of the transcriptional regulators controlling MSC adipogenesis. Since obesity is associated with the incidence of multiple important comorbidities, an in-depth understanding of the relationship between MSC adipogenesis and the comorbidities of obesity is also necessary to evaluate the potential of effective and safe MSC-based therapies for obesity. In addition, brown adipogenesis is an attractive topic from the viewpoint of therapeutic innovation and future research into MSC-based brown adipogenesis could lead to a novel breakthrough. Ongoing stem cell studies and emerging research fields such as epigenetics are expected to elucidate the complicated mechanisms at play in MSC adipogenesis and develop novel MSC-based therapeutic options for obesity. This review discusses the current understanding of MSCs in adipogenesis and their potential clinical applications for obesity.

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Obesity is a complex medical condition characterized by the accumulation of excess body fat.¹ The rising incidence of obesity has become a major public health problem worldwide,^{2–4} particularly because obesity is a major risk factor for type 2 diabetes, dyslipidemia, hypertension, stroke, and cardiovascular disease.^{2,3} Obesity also increases the risk of certain types of cancer,^{5,6} and has a reported association with some psychiatric disorders.^{7,8} This robust increase in obesity and associated health complications has prompted multiple control strategies including lifestyle modifications, pharmacotherapy, and surgical approaches. However, lifestyle management has limited effects and many anti-obesity drugs have been withdrawn from market due to adverse side effects.² Thus, the most effective therapy for severe obesity is invasive, bariatric surgery, which has its own inherent risks

and remains contentious with respect to long-term efficacy and procedural safety.^{9–12}

Severe obesity is ascribed to both an increase in adipose cell size and increased adipocyte cell number.^{13–15} Recent evidence suggests that mesenchymal stem cells (MSCs) are a major source of adipocyte generation, with both MSCs in fat tissue and adipocyte progenitor cells originating from bone marrow implicated in adipogenesis in adult animals.^{16–19} MSCs are thus purported to play a vital role in obesity, and have received increasing attention as a new target for therapy. Since exercise suppresses obesity, Rubin *et al*²⁰ tested the hypothesis that low-magnitude mechanical signals would suppress adiposity, not by metabolizing existing adipose tissue, but instead by inhibiting the differentiation of MSCs into adipocytes. The authors demonstrated that 15 weeks of

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brief, daily exposure to high-frequency mechanical signals, induced at a magnitude well below that which would arise during walking, suppressed adipogenesis by 27% in C57BL/6 J mice.²⁰ They further reported that irradiated mice receiving bone marrow transplants from heterozygous green fluorescent protein (GFP)⁺ mice exhibited a 19% decrease in MSC to adipocyte differentiation after 6 weeks of the low-magnitude mechanical signals, indicating that the formation of new adipose tissue in their models was deterred by inhibiting MSC adipogenesis.²⁰ The authors thus suggested that obesity in humans could be prevented by controlling MSC adipogenesis.²⁰

The underlying pathophysiology of obesity remains ill defined and studies are ongoing into the potential roles of MSCs in managing obesity, and into their safety and efficacy in the clinical setting. This review discusses current understanding of the relationship between MSCs and obesity and its potential clinical implications.

ADIPOGENESIS FROM MSCs

MSCs are multipotent cells that can differentiate into a variety of cells of the mesodermal lineage including adipocytes,²¹ and transdifferentiate into specialized cells of the endodermal and ectodermal lineages.²² MSCs were also attributed with immunomodulatory properties that could potentially benefit patients who exhibit adverse immune responses from graft-versus-host and autoimmune diseases.^{23–25} These multipotent capacities of MSCs and their therapeutic potential has prompted many clinical and preclinical studies of MSC-based therapy for various diseases, with encouraging results reported thus far.^{26,27}

On the other hand, the roles of MSCs in obesity remain ill defined, and further understanding of the mechanisms, regulation and outcomes of adipogenesis is crucial for the development of MSC-based treatments for obesity. Although adipogenesis is a multistep process involving many cellular intermediates, for practical purposes it can be divided into two major phases (Figure 1).^{28–32} During the determination phase, multipotent MSCs differentiate into preadipocytes committed to the adipogenic lineage.^{28–32} Following determination, fibroblastic preadipocytes then become spherical mature adipocytes in the terminal differentiation phase.^{28–32} As a result, adipocytes can synthesize and transport lipids, secrete adipocyte-specific proteins, and express the machinery necessary for insulin sensitivity.^{28–32}

The mechanisms governing MSC adipogenesis are quite complex, with the major signaling pathways converging to regulate a range of transcription factors such as peroxisome proliferator-activated receptor-gamma (PPAR-gamma) and several members of the CCAAT/enhancer-binding proteins (C/EBPs) (Figure 1).^{30–34} PPAR-gamma is a nuclear hormone receptor and key regulator of adipogenesis,^{35,36} including *in vitro* adipogenesis from embryonic stem (ES) cells and embryonic fibroblasts.^{37,38} Specifically, Rosen *et al*³⁸ created chimeric mice derived from wild-type ES cells and cells with a

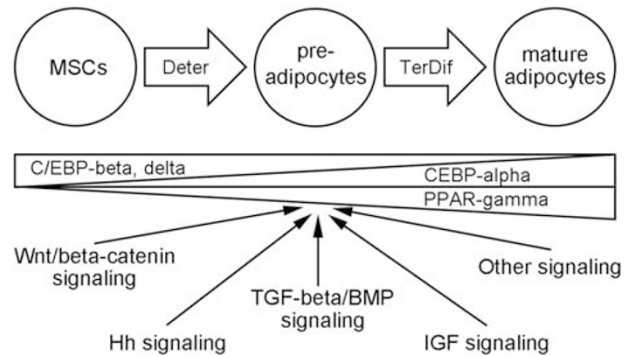


Figure 1 Schematic view of the regulatory mechanisms governing MSC adipogenesis. Adipogenesis can be practically divided into two major phases: the determination phase and the terminal differentiation phase. The former defines the process from multipotent MSCs to preadipocytes, whereas the latter governs how preadipocytes develop into mature adipocytes. The mechanisms underlying MSC adipogenesis are quite complex, with the major signaling pathways converging to regulate a range of transcription factors such as PPAR-gamma and several members of the C/EBP family. PPAR-gamma is considered the master regulator of adipogenesis. C/EBP-beta and C/EBP-delta are recognized to play an important role during the early stage of adipogenesis, whereas CEBP-alpha assumes a key role later in the differentiation process. PPAR-gamma and C/EBP-alpha also operate in a coordinated manner to stimulate adipogenesis. A number of signaling pathways control MSC adipogenesis; however, the full scope of transcriptional control and signaling pathways governing MSC adipogenesis has not been clarified. BMP, bone morphogenetic protein; C/EBP, CCAAT/enhancer-binding protein; Deter, determination phase; Hh, Hedgehog; IGF, insulin-like growth factor; MSCs, mesenchymal stem cells; PPAR, peroxisome proliferator-activated receptor; TerDif, terminal differentiation phase; TGF, transforming growth factor.

homozygous deletion of PPAR-gamma, and used this approach to grow normal tissues from a choice of cells with different genotypes.³⁸ By showing exclusion of PPAR-gamma null cells from adult adipose tissues, but not several other of the developed tissues, the authors concluded that PPAR-gamma is required for adipogenesis *in vivo*.³⁸

The C/EBPs are transcription factors characterized by a highly conserved, basic leucine zipper domain at the C terminus that facilitates homo- or heterodimerization and DNA binding.^{35,36,39,40} Six members of the family have been isolated and characterized thus far.⁴⁰ Of these, C/EBP-beta and C/EBP-delta are the first transcription factors induced during adipogenesis and play a vital role in directing the differentiation process (Figure 1).^{39,41} On the other hand, CEBP-alpha is induced to assume a key role later in the differentiation process (Figure 1).³⁶ The importance of C/EBPs in adipogenesis has been demonstrated in gain- and loss-of-function studies with C/EBP-alpha considered to play the most prominent role,^{36,39,41} by operating with PPAR-gamma in a coordinated manner to stimulate adipogenesis.³⁵ Importantly, PPAR-gamma can promote adipogenesis in C/EBP-alpha-deficient cells;⁴² however, C/EBP-alpha does not function similarly in the absence of PPAR-gamma.⁴³ In

addition to PPAR-gamma and C/EBP-alpha, other transcription factors interact at various stages of adipocyte differentiation. For example, gene expression profiles using microarray and quantitative reverse transcription polymerase chain reaction analyses of mRNAs isolated from adipocyte differentiation cultures suggested the importance of many transcriptional proteins and receptors for adipogenesis.^{39,44,45}

In terms of signaling pathways important for adipogenesis, Wnt/beta-catenin signaling is one of the most important and well studied.^{46,47} Activation of Wnt/beta-catenin signaling inhibits adipogenesis,⁴⁸⁻⁵⁰ whereas blocking endogenous Wnt signaling promotes adipogenic differentiation,^{49,51} suggesting that Wnts act as a brake for adipogenesis. Wnt10b is suggested to be the most important endogenous regulator of adipogenesis,⁴⁹ with transgenic mice overexpressing Wnt10b from the adipocyte-specific FABP4 promoter (FABP4-Wnt10b mice) showing reduced adiposity and resistance to diet-induced obesity.⁵⁰ In addition, Wright *et al*⁵² reported that expressing Wnt10b in the adipose tissue of *ob/ob* mice using a FABP4-Wnt10b transgene reduced adiposity in these mice. Those authors further demonstrated that Wnt10b protected against genetic obesity in mice due to ectopic expression of agouti (*A^y*).⁵²

Recent evidence suggests that Hedgehog (Hh) signaling plays an important role in MSC adipogenesis, with activation of this signaling impairing the differentiation process.^{53,54} Fontaine *et al*⁵⁵ also showed that Hh signaling decreases during adipocyte differentiation from adipose-derived human MSCs, and that activation of Hh signaling alters adipocyte morphology as well as insulin sensitivity, but inhibition of this signaling was not sufficient to trigger adipogenesis.⁵⁵ In other studies, Suh *et al*⁵⁴ showed that inhibiting Hh signaling increased adipogenic differentiation in 3T3-L1 murine preadipocytes, whereas Cousin *et al*⁵⁶ found that decreasing Hh signaling was not sufficient to trigger adipogenesis in the same cell type. Thus, while the anti-adipogenic role of Hh signaling seems to be established, the effect of inhibiting this signaling on adipogenesis remains controversial.⁵⁷

The transforming growth factor (TGF)-beta superfamily exhibits various actions in many cell types including MSCs and adipocytes, with TGF-beta signaling via the bone morphogenetic protein (BMP) ligands shown to regulate adipogenesis.^{58,59} Ignatz *et al*⁶⁰ further showed that TGF-beta inhibits adipocyte differentiation of 3T3-L1 cells, while Zhou *et al*⁶¹ demonstrated that cooperative TGF-beta/Wnt signaling could inhibit adipogenesis in human MSCs. TGF-beta seems to have an anti-adipogenic effects on MSCs, although the effects of BMPs on adipogenesis differ among different cell types and BMP ligands.⁶² For example, BMP2 inhibited adipogenesis in the human marrow stromal cell line hMS,⁶³ but promoted adipogenesis in the murine mesenchymal stem cell line C3H10T1/2.^{64,65} Further, BMP7 promotes adipogenesis in human MSCs⁶⁶ while BMP4 plays that role in C3H10T1/2 cells.⁶⁷ Further studies are needed to determine the mechanisms by which TGF-beta/BMP signaling affects

adipogenesis and the implication for such findings in different cell types.

Finally, insulin-like growth factor (IGF) signaling is also important for adipogenesis. IGF-1 is considered to stimulate adipogenesis,⁶⁸ and Holzenberger *et al*⁶⁹ demonstrated a strong growth deficit in the fat tissue of mice generated to have very low IGF-1 receptor levels. However, the precise roles and mechanisms of IGF signaling in MSC adipogenesis are still unclear.

The full scope of transcriptional control and signaling pathways governing MSC adipogenesis has not been well defined, and the realization of MSC-based therapeutic strategies for obesity relies on ongoing and future studies to further elucidate these mechanisms.

MSCs AND THE COMORBIDITIES OF OBESITY

Obesity is associated with multiple comorbidities. One of the strongest associations is with type 2 diabetes,^{70,71} suggesting an important link between this prevalent disease and MSC adipogenesis. While such a link raises the potential of controlling MSC adipogenesis for new disease prevention strategies, there is still much to be learned before we fully understand the role of MSCs in development of type 2 diabetes. Besides the control of adipogenesis, several studies have examined the potential roles of MSCs in treating diabetes (Table 1). For example, MSCs can differentiate into insulin-producing cells, suggesting MSCs as a source of transplantation material in the treatment of diabetes,⁷²⁻⁷⁴ while other studies demonstrated the angiogenic and anti-inflammatory potential of MSCs in supporting islet transplantation.^{75,76} Furthermore, Dave *et al*⁷⁷ reported a clinical trial showing that co-infusion of *in vitro*-generated insulin-producing cells differentiated from autologous adipose-derived MSCs and bone marrow-derived hematopoietic stem cells into the portal circulation, thymus, and

Table 1 Example studies of the relationship between MSC and obesity comorbidities

Comorbidity	Findings	References
DM	MSC as a source of transplantation material	72-74
	MSC supports islet transplantation	75,76
	MSC treatment improves HbA1c	77
DL	MSC infusion improves DL	78
	LXR-alpha inhibits MSC adipogenesis	90
HTN	Pts with HTN exhibit increased circulating MSCs	95
	Ang II inhibits MSC adipogenesis	98
	AT ₂ R inhibits MSC adipogenesis	98,100

Abbreviations: Ang II, angiotensin II; AT₂R, angiotensin AT₂ receptor; DL, dyslipidemia; DM, diabetes mellitus; HbA1c, glycosylated hemoglobin; HTN, hypertension; LXR, liver X receptor; MSC, mesenchymal stem cell; Pts, patients.

subcutaneous tissue increased serum C-peptide levels and improved glycosylated hemoglobin levels. Significant progress has thus been made in the field of MSC and diabetes, and further research is warranted to realize the clinical potential of MSC adipogenesis in this area.

Dyslipidemia is also a comorbidity of obesity, and while a recent infusion of adipose-derived MSCs improved dyslipidemia in obese mice (Table 1),⁷⁸ the relationship between MSCs and dyslipidemia remains largely unknown. Regarding cholesterol/lipid homeostasis, liver X receptor (LXR)-alpha deserves special consideration. LXR-alpha is an oxysterol-regulated nuclear hormone receptor with a central role in cholesterol and lipid homeostasis.^{79–84} Importantly, Gao *et al*⁸⁵ reported that the chronic activation of LXR by its agonist blocked the development of high-fat diet-induced obesity in mice, suggesting LXR as a potential target for obesity prevention. Accumulating evidence also suggests a direct role for LXR-alpha in adipose tissue, with increased expression of LXR-alpha demonstrated during adipogenesis and high expression levels of LXR-alpha and many LXR-alpha target genes found in adipocytes.^{45,86–89} Although the precise relationship between LXR-alpha and MSC biology remains undefined, we recently reported the role of LXR-alpha in MSC adipogenesis using murine MSCs isolated from the bone marrow of wild type (WT) and LXR-null mice, as well as cell lines stably overexpressing GFP-LXR-alpha or GFP alone, generated from WT murine MSCs by retroviral infection.⁹⁰ Deletion of LXR accelerated adipogenesis in murine MSCs, as determined by lipid droplet accumulation and adipogenesis-related gene expression, while overexpression of LXR-alpha inhibited adipogenesis.⁹⁰ Because Wnt/beta-catenin signaling is crucial for suppressing adipogenesis, we further examined the effects of deleting LXR or overexpressing LXR-alpha on Wnt expression and cellular beta-catenin levels in the murine MSCs. We found a decrease in both pathway markers in the absence of LXR and an increase in the cells overexpressing LXR-alpha.⁹⁰ Our data thus showed that LXR-alpha has an inhibitory effect on adipogenesis of murine MSCs with Wnt/beta-catenin signaling (Table 1),⁹⁰ although the clinical relevance of this suppressive effect remains to be explored. Interestingly, Beaven *et al*⁹¹ reported increased total body adiposity in LXR-null mice bred onto a leptin-deficient *ob/ob* background compared with that in WT *ob/ob* mice. On the other hand, Kalaany *et al*⁹² reported that LXR-null mice were resistant to diet-induced obesity due to increased energy expenditure. Adipose tissue-specific LXR-null mice have not yet been generated, and hence, the precise roles of LXR in adipose tissue *in vivo* remain unknown. Further studies are necessary to elucidate the roles of LXR in adipogenesis and clinical obesity.

Another important comorbidity of obesity is hypertension.^{93,94} Marketou *et al*⁹⁵ reported increased numbers of circulating MSCs in patients with hypertension compared to normotensive patients (Table 1); however, little is known about the relationship between MSC and obesity-associated

hypertension. In this context, the renin-angiotensin system (RAS) is of note. The RAS is an endocrine system and considered central in the development of hypertension.⁹⁶ In addition to the circulating RAS, the importance of local RAS at the tissue level is recognized.^{96,97} Previously, we demonstrated that human bone marrow-derived MSCs contain a local RAS and that endogenous angiotensin II (Ang II) production is increased in human MSCs undergoing adipogenic differentiation through increased local renin expression.⁹⁸ We also showed that Ang II inhibits adipogenesis of human MSCs associated with an increase in angiotensin AT₂ receptors,⁹⁸ and that pharmacological blockade of these receptors promotes adipogenesis (Table 1)⁹⁸ while inhibiting the osteogenesis of human MSCs.⁹⁹ Recently, we further characterized how the AT₂ receptor (AT₂R) affects the differentiation of MSCs to adipocytes using a genetic model.¹⁰⁰ Murine MSCs were isolated from the bone marrow of AT₂R-null mice and wild-type littermates. Compared with MSCs isolated from littermate mice, MSCs from AT₂R-null mice exhibited significantly increased adipogenesis, as determined by lipid droplet accumulation and adipogenesis-related gene expression.¹⁰⁰ The AT₂R-null group also showed significantly decreased Wnt10b expression accompanied by decreased beta-catenin levels (*vs* littermate).¹⁰⁰ Our results thus revealed that AT₂R inhibits adipogenic differentiation in murine MSCs via Wnt10b/beta-catenin signaling (Table 1).¹⁰⁰ These findings suggest an association between MSC adipogenesis and hypertension, based on the central role that the RAS plays in the pathophysiology of hypertension, although such a relationship seems to be quite complex. We also reported that murine MSCs could develop into renin-producing granular cells under the activation of LXR-alpha.¹⁰¹ However, because renin is the first enzymatic and rate-limiting step of the RAS, and LXR-alpha is considered central to the pathophysiology of dyslipidemia, the link between RAS and LXR-alpha in MSCs¹⁰² further complicates the relationship between MSC adipogenesis and the comorbidities of obesity. Further concentrated research on the relationship between MSC adipogenesis and the comorbidities of obesity is needed to evaluate the potential of effective and safe MSC-based therapies for obesity.

EPIGENETICS IN THE RELATIONSHIP BETWEEN MSC ADIPOGENESIS AND OBESITY

There has been increasing interest in the role epigenetics plays in obesity development.^{3,103} Epigenetic mechanisms affect gene function without modifying the DNA sequence.¹⁰³ In addition to the classical epigenetic mechanisms including DNA methylation and histone modification, microRNAs (miRNAs) comprising species of short noncoding RNA that regulate gene expression post-transcriptionally, have emerged as important epigenetic players.¹⁰⁴ For example, Chen *et al*¹⁰⁵ reported that the level of miR-146b increased during adipogenesis from human MSCs to mature adipocytes, and further showed the adipogenesis promotion by gain- and loss-

of-function studies. In addition, Ahn *et al*¹⁰⁶ demonstrated that *in vivo* knockdown of miR-146b by a locked nucleic acid miR-146b antagomir significantly reduced fat volume and body weight in obese mice. These data suggest that miR-146b could be a potential target for treating obesity. Li *et al*¹⁰⁷ also showed the promotion of adipogenesis by miR-17-5p and miR-106a in human adipose-derived MSCs by gain- and loss-of-function studies, while Shi *et al*¹⁰⁸ suggested miR-148a as a potential biomarker of obesity, by showing increased levels of this miRNA in adipose tissue of obese people and mice fed a high-fat diet. Gain- and loss-of-function studies by the latter group also showed that miR-148a accelerated adipogenesis in human adipose-derived MSCs, and that this miRNA acted by suppressing its target gene, Wnt1, suggesting miR-148a promotes MSC adipogenesis through the repression of Wnt1 signaling.¹⁰⁸ The same group recently reported miRNA microarray data showing differential expression in mature adipocytes compared with human adipose-derived MSCs that suggested potential therapeutic targets for controlling obesity.¹⁰⁹

Regarding the classical epigenetic mechanisms, Noer *et al*¹¹⁰ examined the DNA methylation status of adipogenic and non-adipogenic genes in human adipose-derived MSCs, and found that the adipogenic gene promoters were hypomethylated in MSCs, whereas non-adipogenic promoters were methylated. However, DNA methylation reflected neither transcriptional status nor potential for gene expression upon differentiation, suggesting that DNA methylation might not be the sole determinant of adipogenic potential and could constitute a molecular signature of MSCs.¹¹⁰ On the other hand, Zhao *et al*¹¹¹ investigated whether DNA methylation and histone acetylation at the C/EBP- α promoter are involved in MSC adipogenesis using bone marrow-derived MSCs from the glucocorticoid-induced osteoporosis (GIO) mouse model, in which adipogenic potential exceeds osteogenic potential. These authors observed DNA hypomethylation, high PPAR- γ binding, low histone deacetylase binding, and histone hyperacetylation in the -1286 bp/-1065 bp region of the C/EBP- α promoter in MSCs from GIO mice compared with normal mice, suggesting DNA methylation and histone acetylation status on the C/EBP- α promoter as markers of adipogenic potential in MSCs.¹¹¹ Furthermore, Hemming *et al*¹¹² demonstrated the vital role of histone methylation and demethylation by Enhancer of Zeste homology 2 and lysine demethylase 6A in adipogenesis of human bone marrow-derived MSCs using overexpression, siRNA knockdown, and enzymatic inhibition.

Epigenetics is a rapidly evolving area of research, and there is still much to be learned before we fully understand the epigenetic mechanisms at play in MSC adipogenesis and obesity.¹¹³ Further studies are expected to open possibilities for a new level of therapeutic options for obesity.

COMPLEXITY OF MSC ADIPOGENESIS AS A POTENTIAL THERAPEUTIC TARGET FOR OBESITY

Obesity is a medical condition involving the excess accumulation of adipose tissue; however, simply inhibiting adipogenesis is not a practical strategy to manage obesity. Imbalance between energy intake and expenditure must also be acknowledged. Producing new adipocytes might prevent the increase in large insulin-resistant adipocytes. Therefore, requisite adipogenesis exists. On the other hand, Rieusset *et al*¹¹⁴ reported that decreasing PPAR- γ activity, either by treatment with a PPAR- γ -specific antagonist or by invalidation of one allele of the PPAR- γ gene, protected mice from high-fat diet-induced adipocyte hypertrophy and insulin resistance. It is thus possible that decreased PPAR- γ expression in adipose tissue improves insulin sensitivity, as demonstrated in PPAR- γ -deficient mice^{115,116} or by using a PPAR- γ -specific antagonist.¹¹⁴ Nevertheless, it remains contentious as to the effects of PPAR- γ activity, its agonist, and its antagonist on insulin sensitivity.^{114–118} Balanced adipogenesis based on energy intake and expenditure seems to be important.

A relationship between MSCs and ectopic lipid accumulation in insulin-responsive tissues is another complicated issue. For example, skeletal muscle is considered the main destination for insulin-stimulated glucose disposal.^{119,120} To this end, Jacob *et al*¹²¹ showed that intramyocellular lipid content was increased in insulin-resistant offspring of type 2 diabetic subjects compared with insulin-sensitive control subjects and suggested that increased intramyocellular lipid accumulation might contribute to the defective glucose uptake in the skeletal muscle of insulin-resistant subjects. In addition, ectopic lipid accumulation in liver is also associated with insulin resistance,^{122–124} and ectopic lipid accumulation in pancreas could contribute to beta-cell dysfunction and a negative association between pancreatic fat and insulin secretion.^{125–127} MSC adipogenesis in adipose tissue could have a role in decreasing ectopic lipid accumulation; however, MSCs also exist in skeletal muscle, liver, and pancreas, and MSC adipogenesis in such tissues further complicates this context.^{128–131} Uezumi *et al*¹³² identified mesenchymal progenitors in the muscle interstitium and demonstrated that only these cells among the muscle-derived cell populations exhibited efficient adipogenic differentiation both *in vitro* and *in vivo*, suggesting that these cells are responsible for ectopic fat cell formation in skeletal muscle. Although the relationship between MSCs and ectopic lipid accumulation remains largely unknown, this link is an area deserving more research.

Finally, brown and white adipocytes are also complicated though important topics (Figure 2). Adipose tissue is traditionally classified as either white adipose tissue (WAT) or brown adipose tissue (BAT).¹³³ WAT functions to store excess energy, whereas BAT is involved in thermogenesis and energy expenditure.¹³³ Because of this unique thermogenic capacity of BAT resulting from the expression of uncoupling

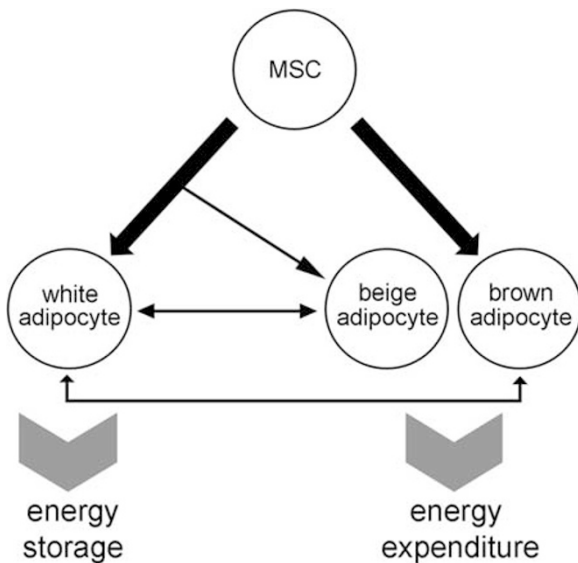


Figure 2 Schematic diagram of white, brown, and beige adipogenesis. Adipocytes differentiated from mesenchymal stem cells (MSCs) are traditionally classified as either white adipocytes or brown adipocytes. White adipocytes play a key role in energy storage, whereas brown adipocytes are specialized for energy expenditure. Of note, recent studies suggest that a third type of adipocytes, namely beige adipocytes, with similar functions to those of brown adipocytes and possibly transdifferentiated from mature white adipocytes or white precursors. Reversible transdifferentiation between white and beige adipocytes, and between white and brown adipocytes are also suggested.

protein 1 in the mitochondrial inner membrane,¹³⁴ BAT activation-regeneration in animal models were studied and reported to improve obesity and insulin resistance.^{135–139} More importantly, recent studies suggest that another type of brown adipocytes, called beige or bright adipocytes, with similar functions to those of brown adipocytes could be recruited in WAT upon environmental stimulation and/or pharmacologic treatment (Figure 2).^{134,140} Among these, García-Alonso *et al*^{141,142} reported prostaglandin E2 as a key regulator of white-to-brown adipogenesis, while Schulz *et al*¹⁴³ showed that Sca-1⁺ adipogenic progenitor cells from murine subcutaneous white fat were highly inducible to differentiate into brown-like adipocytes upon stimulation with BMP7. In addition, Rosenwald *et al*¹⁴⁴ demonstrated that cold-induced bright or beige adipocytes in mice were reversed into white adipocytes on warm adaptation and that those white adipocytes converted into bright adipocytes on additional cold stimulation. Reversible transdifferentiation between white and beige adipocytes, and between white and brown adipocytes are suggested.^{144–146} On the other hand, Wang *et al*¹⁴⁷ reported that cold exposure induced beige adipocytes as *de novo* adipogenesis in subcutaneous fat from mice. Taken together, these data indicate that beige adipogenesis combining MSCs in WAT is a promising research area of fat biology expected to identify new therapeutic strategies for obesity.

FUTURE DIRECTIONS AND CONCLUSION

In terms of future research, identification and thorough characterization of the transcriptional regulators that control MSC adipogenesis is a vital first step. To this end, current and future genome-wide studies are promising tools that could help to answer many of the remaining questions. Second, understanding how the epigenetics of MSC adipogenesis plays a role in obesity needs to progress. Epigenetics is a rapidly evolving area of research with recent miRNA expression profiling studies during MSC adipogenesis revealing several miRNAs with a specific expression pattern. The challenge for future studies is the gain key insights into the epigenetic regulatory mechanisms governing MSC adipogenesis with the potential to provide novel therapeutic targets. Third, the effects of MSC therapies on the comorbidities of obesity must be further studied. The relationship between MSC adipogenesis and such comorbidities remains obscure, and an in-depth understanding is necessary to properly evaluate the potential of effective and safe MSC-based therapeutic strategies for obesity. Finally, future research into the molecular control of brown or beige adipogenesis could lead to a novel breakthrough. Interventions such as controlling brown adipogenesis from MSCs and/or white-to-brown adipogenesis could form the basis of future therapeutic strategies. Importantly, obesity is a heterogeneous group of conditions with multiple causes including genetic and environmental factors. Identification and characterization of different obese phenotypes in further research including emerging fields such as epigenetics are important in the quest for individualized therapeutic strategies for patients with obesity. In the future, it is possible that MSCs may be used to control adipogenesis and/or the comorbidities of obesity individually as an anti-obesity treatment in clinical practice.

In conclusion, MSCs are a promising therapeutic option for obesity in the future and further studies will hopefully elucidate the mechanisms at play in the relationship between MSC adipogenesis and obesity, leading ultimately to novel MSC-based therapeutic options for patients.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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