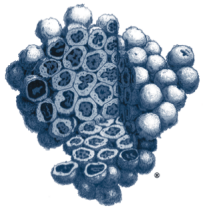

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Concise Review: Adipose Tissue-Derived Stromal Cells—Basic and Clinical Implications for Novel Cell-Based Therapies

Andreas Schäffler and Christa Büchler

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Concise Review: Adipose Tissue-Derived Stromal Cells—Basic and Clinical Implications for Novel Cell-Based Therapies

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Key Words. Adipocyte • Adipose tissue • Mesenchymal stem cell • Tissue engineering • Cell therapy

ABSTRACT

Compared with bone marrow-derived mesenchymal stem cells, adipose tissue-derived stromal cells (ADSC) do have an equal potential to differentiate into cells and tissues of mesodermal origin, such as adipocytes, cartilage, bone, and skeletal muscle. However, the easy and repeatable access to subcutaneous adipose tissue and the simple isolation procedures provide a clear advantage. Since extensive reviews focusing exclusively on ADSC are rare, it is the aim of this review to describe the preparation and isolation procedures for ADSC, to summarize the molecular characterization of ADSC, to describe the differentiation capacity of ADSC, and to discuss the mechanisms and future role of ADSC in cell therapy and tissue engineering. An initial effort has also been made to differentiate ADSC into hepatocytes, endocrine pancreatic cells, neurons, cardiomyocytes, hepatocytes, and endothelial/vascular cells. Whereas the lineage-specific differentiation into cells of mesodermal origin is well understood on a molecular basis, the molecular key events and transcription factors that initially allocate the ADSC to a lineage-specific differentiation are almost completely unknown. Decoding these molecular mechanisms is a prerequisite for developing novel cell therapies. STEM CELLS *2007;25:818–827*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Multipotent human and mouse MSC have the ability [1, 2] to differentiate into lineages of mesodermal tissues, such as skeletal muscle, bone, tendons, cartilage, and fat, under appropriate culturing conditions using specific hormones or growth factors [3–5]. MSC can routinely be isolated from several organs, such as fetal liver, umbilical cord blood, and bone marrow [6, 7]. The adipose tissue is a highly complex tissue and consists of mature adipocytes, preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, resident monocytes/macrophages [8, 9], and lymphocytes [10]. The stromal-vascular cell fraction (SVF) of the adipose tissue has come more and more into the focus of stem cell research [11, 12], since this tissue compartment provides a rich source [13, 14] of pluripotent adipose tissue-derived stromal cells.

There is a confusing inconsistency in the literature when using terms describing multipotent precursor cells from adipose tissue stroma, such as processed lipoaspirate (PLA) cells, adipose tissue-derived stromal cells (ADSC), preadipocytes, adipose stroma vascular cell fraction, and others. The term SVF corresponds to ADSC and describes cells obtained immediately after collagenase digestion. The critical point is the absence of a detailed molecular and cellular characterization of multipotent stem cells within the adipose stroma. Accordingly, the term ADSC will be used throughout this review. However, considerable effort has been made to characterize cellular and molecular properties of ADSC. This is a critical point in the field, and to date, there is currently no review available interpreting the complex data on ADSC or adipose tissue-derived multipotential precursor cells. Recently, Rodriguez et al. [15] described the isolation and culture of adipose tissue-derived stem cells with multipotent differentiation capacity at the single cell level. These cells maintain their characteristics with long-term passaging and develop the unique features of human adipocytes. We decided to use the term ADSC in this review as a compromise and only for cells that were (a) passaged several times, (b) shown to exert multipotential differentiation capacity, and/or (c) molecularly characterized by using a multipanel of mesenchymal differentiation markers according to Table 1.

The simple surgical procedure, the easy and repeatable access to the subcutaneous adipose tissue, and the uncomplicated enzyme-based isolation procedures make this tissue source for MSC most attractive for researchers and clinicians of nearly all medicinal subspecializations [12, 16] (Table 2). Therefore, ADSC do represent an alternative source of autologous adult stem cells that can be obtained repeatedly in large quantities under local anesthesia with a minimum of patient discomfort. Most importantly, a comparative analysis of MSC obtained from bone marrow, adipose tissue, and umbilical cord clearly showed that ADSC were not different regarding morphology, immune phenotype, success rate of isolating MSC, colony frequency, and differentiation capacity [7, 17].

ADSC can easily be isolated from human adipose tissue [13, 18, 19], and they have the potential to differentiate into bone, cartilage, tendons, skeletal muscle, and fat when cultivated under lineage-specific conditions [6, 18–20]. Tissue engineering of these mesenchymal organs (Table 2) is of major interest in human diseases, such as inherited, traumatic, or degenerative bone, joint, and soft tissue defects (skeletal regeneration and cartilage repair). Plastic tissue reconstruction after tumor sur-

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Table 1. Molecular phenotype of adipose tissue-derived stromal cells

ATMSC-positive cellular markers and genes	ATMSC-negative cellular markers and genes
CD9	**CD11b**
CD10	**CD14**
CD13	**CD19**
CD29	CD31
CD44	**CD34**
CD49 (d)	**CD45**
CD49 (e)	**CD79α**
CD54	CD80
CD55	CD117
CD59	CD133
CD73	CD144
CD90	**HLA-DR**
CD105	**c-kit**
CD106	MyD88
CD146	STRO-1
CD166	Lin
HLA I	HLA II
Fibronectin	
Endomucin	
ASMA	
Vimentin	
Collagen-1	

The adipose tissue-derived stromal cell expression of surface markers and genes is summarized according to data derived from the literature [6, 16, 19, 20, 41, 42, 44]. Note that all the gene and surface marker expression profiles apply to in vitro-expanded cells, not primary cells. All attempts to establish both an exact phenotypical definition of mesenchymal stem cells and a clear discrimination between these cells and fibroblasts have been unsuccessful up to now. As a minimal prerequisite [42], mesenchymal stem cells must express CD105, CD73, and CD90 and lack the hematopoietic lineage markers c-kit, CD14, CD11b, CD34, CD45, CD79α, CD19, and HLA-DR (printed in boldface).
Abbreviations: ASMA, smooth muscle cell-specific alpha actin; ATMSC, adipose tissue-derived mesenchymal stem cells; HLA, human leukocyte antigen.

gery for breast cancer and other malignancies and reconstruction of muscle and adipose tissue defects after burn injury do represent additional needs for cell-based therapies. In addition, ADSC were demonstrated to have the potential for endothelial [21] and macrophage [22] differentiation. Moreover, an initial effort has been made regarding the differentiation of ADSC across the germ leaf-specific tissues into nonmesenchymal tissues ("cross-differentiation"), such as neurons or endocrine pancreatic cells.

The aims of this review are:

- To describe the isolation procedures for ADSC,
- To summarize the molecular characterization of ADSC,
- To describe the differentiation capacity of ADSC, and
- To discuss the mechanisms and future role of ADSC in mesenchymal tissue repair and tissue engineering.

It is not the aim of the present review to discuss the characteristics and differentiation processes of MSC derived from other commonly used tissues, such as bone marrow, umbilical cord blood, or fetal liver.

Preparation and Molecular Characterization of Adipose Tissue-Derived Mesenchymal Stem Cells

Fibroblast-like adipose tissue-derived mesenchymal stem cells (ATMSC) are morphologically similar to MSC obtained from other tissues during isolation and culturing [23]. Moreover, ADSC have the capacity to differentiate into cells of mesenchymal origin, such as adipocytes, myocytes, chondrocytes, and osteocytes [7, 13, 18–20, 24]. Factors such as donor age, type (white or brown adipose tissue), and localization (subcutaneous or visceral adipose tissue) of the adipose tissue, type of surgical procedure, culturing conditions, exposure to plastic, plating density, and media formulations might influence both proliferation rate and differentiation capacity of ADSC.

Neither the type of surgical procedure nor the anatomical site of the adipose tissue affects the total number of viable cells that can be obtained from the SVF [16, 25]. At least in the murine system, there is increasing evidence that both the cellular composition and the differentiation capacity of the SVF display heterogeneity according to the localization of the adipose tissue [11]. In humans, data supporting this observation are still lacking. However, since different anatomical localizations of fat tissues have their own metabolic characteristics, such as lipolytic activity, fatty acid composition, and gene expression profile, the source of subcutaneous adipose tissue grafts (abdominal-subcutaneous vs. peripheral-subcutaneous) might influence the long-term characteristics of the fat graft. In rabbits, the osteogenic potential of ADSC isolated from visceral adipose tissue was reported to be more effective compared with ADSC isolated from subcutaneous adipose tissue [26]. One case study [27] reported on the significant enlargement of a pedicle flap of skin/adipose tissue transferred from the subcutaneous abdominal region to the patient's dorsum of hand (autologous fat grafting). This enlargement occurred in parallel to the abdominal weight gain of the patient over time. Accordingly, transferred adipocytes might retain the properties of their site of

Table 2. Clinical implications of tissue engineering in relation to cell-specific differentiation programs of adipose tissue-derived stromal cells

Type of differentiation	Clinical implications
Adipogenic	Breast soft tissue reconstruction after tumor surgery for breast cancer, breast asymmetry, and soft tissue and subdermal defects after trauma, surgery, or burn injury
Chondrogenic	Cartilage repair in joint and disc defects, plastic reconstruction of ear and nose defects
Osteogenic	Skeletal regeneration of inherited and tumor- or trauma-induced bone defects
Myogenic	Tissue reconstruction after trauma and surgery, dystrophic muscle disorders
Cardiomyogenic	Heart muscle regeneration, functional improvement after myocardial infarction, heart failure
Vascular/endothelial	Neovascularization, ischemic diseases
Neurogenic	Brain injury, stroke, peripheral nerve injury
Pancreatic/endocrine	Insulin-secreting cells, type 1 diabetes mellitus
Hepatic	Chronic liver failure, hepatic regeneration, hepatocyte transplantation
Hematopoietic	GVHD, bone marrow support

The lineage-specific differentiation of adipose tissue-derived stromal cells might offer future perspectives in organ-specific tissue engineering and tissue reconstruction. Note that many of these theoretical applications (e.g. cardiomyocytes or neuronal cells) are far from clinical use. Moreover, safety issues concerning the clinical applications might resemble those when using bone marrow-derived mesenchymal stem cell but have not yet been investigated extensively in the human system.
Abbreviation: GVHD, graft-versus-host disease.

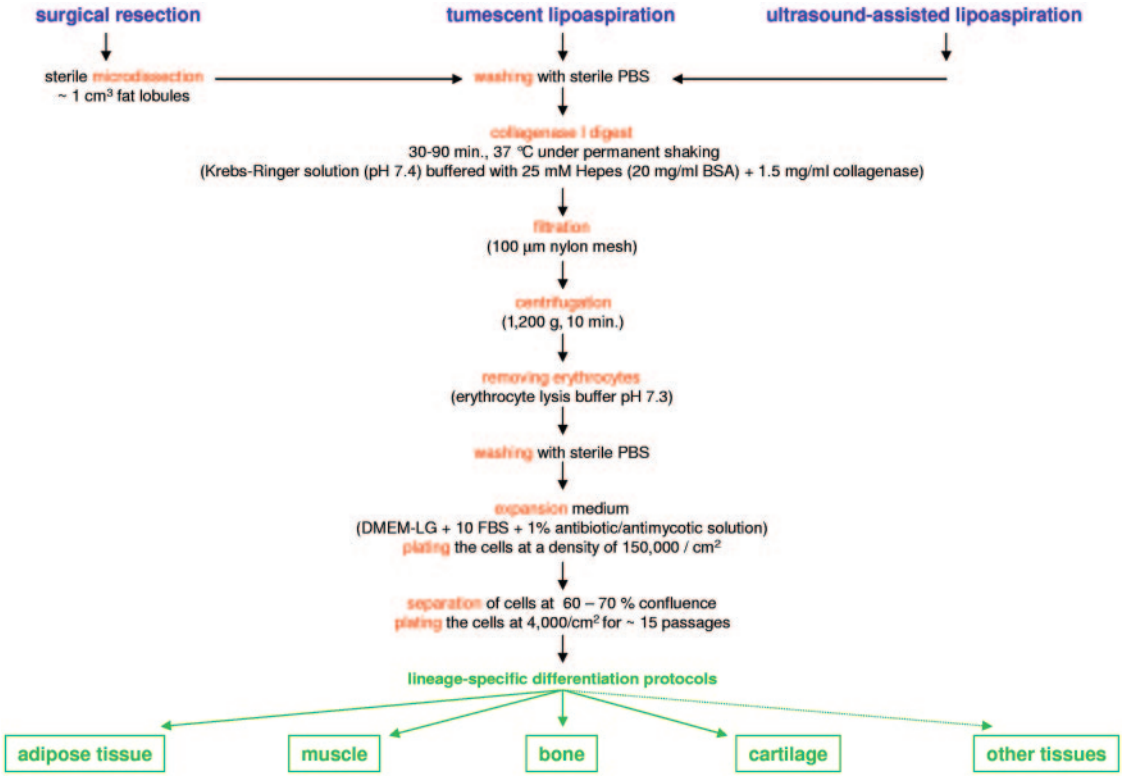

Figure 1. Preparation procedure of adipose tissue-derived stromal cells. Adipose tissue can be easily obtained by surgical resection, tumescent lipoaspiration, or ultrasound-assisted lipoaspiration. The principal steps of mesenchymal stem cell preparation and culturing are depicted. Exact protocols can be obtained from the literature. Expanded stromal cells can be used for several lineage-specific differentiation protocols as a basis for tissue engineering. Note that this procedure is depicted for the illustration of the basic steps and thus cannot be generalized. Abbreviations: BSA, bovine serum albumin; DMEM-LG, Dulbecco's modified Eagle's medium, low glucose concentration; FBS, fetal bovine serum; min., minutes; PBS, phosphate-buffered saline.

origin. Future studies have to clarify whether different anatomical sources of ADSC (subcutaneous-peripheral, subcutaneous-abdominal, and visceral/omental) exhibit a different metabolic and cellular behavior after cell therapy.

The frequency of proliferating MSC and the population doubling time are dependent on the surgical procedure, with some advantages for resection and tumescent liposuction compared with ultrasound-assisted liposuction [16]. In one study comparing bone marrow-derived mesenchymal stem cells (BMMSC) and lipoaspirate-derived ADSC [28] from the same patient, no significant differences were observed regarding the yield of adherent stromal cells, growth kinetics, cell senescence, multilineage differentiation capacity, or gene transduction efficiency. Metabolic characteristics and fat cell viability seem not to differ when comparing standard liposuction with syringe aspiration, and no unique combination of preparation or harvesting techniques has appeared superior to date [25]. Although attachment and proliferation capacity are more pronounced in ADSC derived from younger donors compared with older donors, the differentiation capacity is maintained with aging [29]. Material obtained by lipoaspiration still contains viable cells [30] and can be used directly for mesenchymal stem cell preparation. Even cryopreservation of adipose tissue lipoaspirates is suitable for yielding a significant amount of processed cells for further differentiation [31]. PLA cells can easily be obtained by cosmetic liposuction and grown under standard tissue culture conditions. The multilineage differentiation capacity of PLA cells has already been proven [13]. Total adipose tissue obtained by surgery first has to be microdissected under sterile conditions to obtain small fat lobules (~0.5–1 cm^3). The basic steps and principles of ADSC preparation are depicted in Figure 1. However, it has to be considered that the isolation procedure can affect the cells. Not only can viability and differentiation capacity be affected but also different collagenase batches and centrifugation speeds can cause the isolation of different cell subsets. Thus, a detailed molecular characterization of the isolated cells has to be performed.

Isolated ADSC can be cryopreserved and expanded easily in vitro. Under the conditions commonly used, these cells develop a fibroblast-like morphology. The greatest number of adipocytes can be obtained from cultures plated at low density [20]. The adipogenic differentiation potential was more effective when cells were grown in Dulbecco's modified Eagle's medium (DMEM)/MCDB compared with α-modified Eagle's medium [20], whereas these culture media are similarly effective during osteogenic differentiation [20]. Both low plating density and the use of DMEM/MCDB media facilitate ADSC differentiation. Moreover, the media composition does strongly influence gene expression. Dicker et al. [19] investigated the effect of different cell culture media on ADSC gene expression and identified differential expression of 441 genes [20]. Even the contact to plastic and the time on plastic seem to have an influence on cell surface marker gene expression [32].

By using antioxidants, such as *N*-acetyl-L-cysteine and L-ascorbic acid-2-phosphate, and a low calcium concentration, growth rate and life span of ADSC can be increased [33]. ADSC have the same differentiation potential as described for BMMSC. However, some characteristics, such as the colony frequency and the maintenance of proliferating ability in culture, seem even to be superior in ADSC compared with BMMSC [20, 25]. The proliferation of ADSC can be stimulated by fibroblast growth factor 2 (FGF-2) via the FGF-receptor-2 [34], by sphin-

gosylphosphorylcholine via activation of c-jun N-terminal kinase (JNK) [35], by platelet-derived growth factor via activation of JNK [36], and by oncostatin M via activation of the microtubule-associated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) and the JAK3/STAT1 pathway [37]. In addition, ADSC do express an autocrine FGF-2 loop that maintains their self-renewal ability in vitro [38]. Since inhibition of MEK1 reduces the clonogenic potential of ADSC without affecting their differentiation potential, the ERK1/2 signaling pathway seems to be involved in the FGF-2-mediated self-renewal [38]. In addition, the longevity of human ADSC can be extended by overexpression of the catalytic subunit of the human telomerase gene [39]. ADSC are known to secrete potent growth factors, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and insulin-like growth factor 1 (IGF-1) [40]. Tumor necrosis factor-α can significantly increase the secretion of VEGF, HGF, and IGF-1 from ADSC by a p38 mitogen-activated protein kinase-dependent mechanism [40]. The increasing knowledge on the molecular mechanisms regulating ADSC proliferation might be useful for the improvement of isolation and culturing procedures.

Knowledge of the global gene and protein expression profile of ADSC is a prerequisite both for culturing and lineage-specific differentiation and thus for a highly effective cell therapy. Although the surface marker protein expression profile (determined by fluorescence-activated cell sorting) and the gene expression profile of ADSC (determined by microarray experiments) seem to be similar to that of BMMSC [23, 41], there are also some molecular differences. However, studies directly comparing the gene and protein expression profile between ADSC and BMMSC are rare [6, 20].

Wagner et al. [6] analyzed the global gene expression profile of human MSC derived from adipose tissue, bone marrow, and umbilical cord by microarray experiments. They compared the gene expression signature both among these tissues and with that obtained from normal fibroblasts. They found 25 genes (including *fibronectin, ECM2, glypican-4, ID1, NFIB, HOXA5,* and *HOXB6*) that were overlapping and upregulated in the MSC preparations compared with fibroblasts [6]. However, no phenotypic differences were found among the three stem cell preparations when using a panel of 22 surface antigens [6]. In contrast, several hundred expressed sequence tags were identified to be differentially expressed when comparing ADSC with BMMSC and umbilical cord-derived stem cells [6]. Lee et al. [20] reported 24 genes to be upregulated in ADSC compared with BMMSC, and they described the differential expression profile of eight surface marker proteins in these cells. According to their data [20], less than 1% of genes are estimated to be differentially expressed between ADSC and BMMSC.

Although BMMSC are phenotypically clearly described, the phenotypic characterization of ADSC still is in its infancy, and all attempts to establish an exact phenotypical definition of ATMSC and a clear discrimination between these cells and fibroblasts have been unsuccessful to date. Therefore, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has proposed, most recently, a minimal set of four criteria to define human MSC [42]:

1. MSC have to be plastic-adherent when maintained under standard culture conditions.
2. MSC must have the ability for osteogenic, adipogenic, and chondrogenic differentiation.
3. MSC must express CD73, CD90, and CD105 (Table 1).
4. MSC must lack expression of the hematopoietic lineage markers c-kit, CD14, CD11b, CD34, CD45, CD19, CD79α, and human leukocyte antigen (HLA)-DR (Table 1).

The known ADSC expression profile of surface markers and genes is summarized in Table 1 according to data derived from the literature [6, 16, 19, 20, 25, 41–45]. Although these expression data do support the hypothesis that ADSC and BMMSC have originated from identical precursor cells [20, 25], conclusive experimental evidence for this suggested identity is still lacking. Due to the intrinsic nature of adipose tissue-derived stromal cells [41], one has to be cautious when comparing mesenchymal stem cells with (multipotent) precursor cells isolated from adipose tissue stroma. When interpreting the expression data summarized in Table 2, it has to be considered that, for example, HLA-DR can be induced by interferon-γ. Similarly, CD34 expression can also be seen at least during the first passages. This problem cannot be satisfyingly solved at present, and more detailed molecular data are necessary before a real and multipotent adipose tissue-derived mesenchymal stem cell can be clearly characterized and distinguished from intrinsic, pluripotent, adipose tissue stromal cell precursors.

The lack of HLA-DR expression and the immunosuppressive properties of ADSC [46] make these cells suitable for allogenic transplantation procedures lacking the risk of tissue rejection. ADSC do not provoke in vitro alloreactivity of incompatible lymphocytes, and they suppress mixed lymphocyte reaction and lymphocyte proliferative response to mitogens [46]. These findings support the idea that ADSC share immunosuppressive properties with BM-MSC and therefore might represent an alternative source to BM-MSC.

Differentiation Capacity of Adipose Tissue-Derived Mesenchymal Stem Cells

Allocation and Differentiation

MSC have the ability to differentiate into mesodermal cells (Table 3), such as adipocytes, fibroblasts, myocytes, osteocytes, and cartilagocytes, processes named lineage-specific differentiation [2]. Among these cell types of mesodermal origin, the differentiation process can be switched, for example, by overexpression of lineage-specific transcription factors. Thus, overexpression of peroxisome proliferator-activated receptor γ (PPARγ) in fibroblasts or myocytes results in adipogenic differentiation. This characteristic process (trans-germ plasticity) is termed trans-differentiation. Surprisingly, ADSC do not only have the potential to differentiate into cells and organs of mesodermal origin. There is increasing evidence for the ability of ADSC to differentiate into cells of nonmesodermal origin, such as neurons, endocrine pancreatic cells, hepatocytes, endothelial cells, and cardiomyocytes (Table 3). Accordingly, we suggest to describe this process by using the term "cross-differentiation" (cross-germ plasticity).

The transcriptional and molecular events triggering the lineage-specific mesodermal differentiation into adipocytes [47–49], myocytes [50–52], osteocytes [53, 54], or chondrocytes [53] are well-known and several reviews focus on that point. Although they are beyond the focus of this review, Figure 2 summarizes the main transcription factors involved in lineage-specific mesodermal differentiation. However, before a lineage-specific differentiation can occur, the MSC has to be "allocated" or "committed" to a certain lineage (e.g., the adipocyte lineage). In contrast to the transcriptional events causing lineage-specific differentiation, this process is only poorly understood [55]. Widely yet unidentified molecular rheostats, most probably transcription factors, are discussed to cause the commitment of the MSC to a specific lineage (Fig. 2).

Table 3. Experimentally used factors triggering the differentiation of adipose tissue-derived stromal cells

Type of differentiation	Differentiation factors
Adipogenic	Insulin, IBMX, dexamethasone, rosiglitazone, indomethacin
Chondrogenic	BMP-6, BMP-7, FGF-2, TGF-β_1, TGF-β_2, TGF-β_3, dexamethasone, IGF-1
Osteogenic	1,25$(OH)_2D_3$, β-glycerophosphate, ascorbic acid, BMP-2, dexamethasone, valproic acid
Myogenic differentiation	Specific microenvironment?
Cardiomyogenic differentiation	IL-3, IL-6, SCF
Vascular/endothelial	Specific microenvironment?
Neurogenic	Valproic acid, insulin, hydroxyanisole, hydrocortisone, EGF, FGF
Pancreatic/endocrine	Activin-A, exendin-4, pentagastrin, HGF, nicotinamide, high glucose concentration
Hepatic	HGF, OSM, DMSO
Hematopoietic	Specific microenvironment?

Abbreviations: 1,25$(OH)_2D_3$, 1,25-dihydroxy-cholecalciferol; BMP, bone morphogenetic protein; DMSO, dimethyl sulfoxide; EGF, epidermal growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IBMX, 3-isobutyl-1-methylxanthine; IGF, insulin-like growth factor; IL, interleukin; OSM, oncostatin M; SCF, stem cell factor; TGF, transforming growth factor.

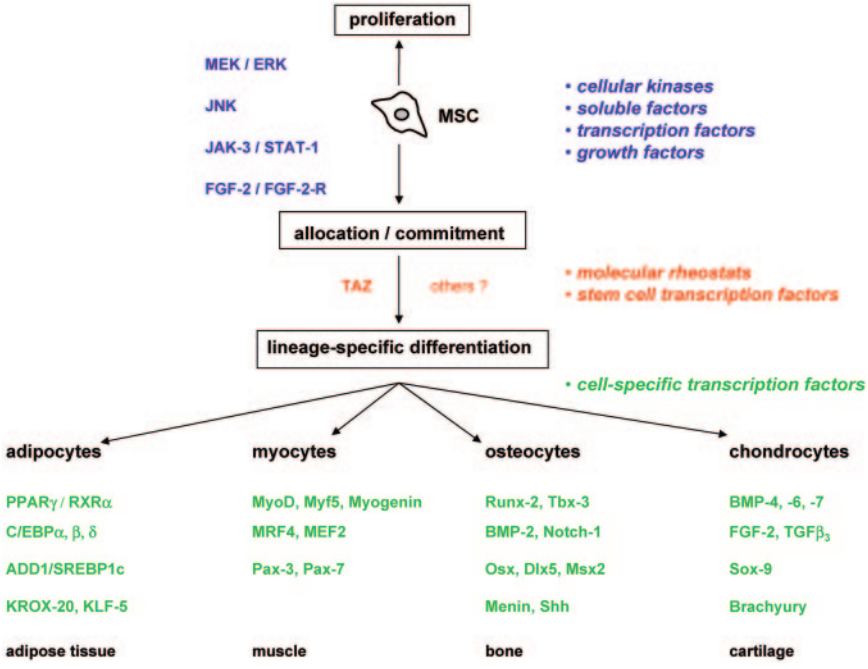

Figure 2. Molecular regulation of proliferation, allocation, and differentiation of adipose tissue-derived mesenchymal stem cells. The processes of proliferation, allocation, and lineage-specific terminal differentiation are regulated by a complex interplay involving stem cell transcription factors (molecular rheostats), cell-specific transcription factors, and a wide variety of cellular kinases, growth factors, and receptors. Whereas the lineage-specific differentiation triggered by tissue-specific transcription factors is well understood, the allocation/commitment of the mesenchymal stem cell to a specific lineage is poorly understood. Thus, unknown stem cell transcription factors, such as TAZ, allocating the stem cell to a specific lineage still await discovery (molecular rheostats). Abbreviations: ADD1/SREBP1c, adipocyte determination- and differentiation-dependent factor-1/sterol regulatory element-binding protein-1; BMP, bone morphogenetic protein; C/EBP, CCAAT enhancer-binding protein; Dlx5, distal-less homeobox-5; ERK, extracellular signal-regulated kinase; FGF-2, fibroblast growth factor 2; FGF-2-R, fibroblast growth factor 2 receptor; JNK, c-jun N-terminal kinase; KLF, Krüppel-like transcription factor; KROX-20, Krox-20 homolog *Drosophila* (previously); MEF2, MADS box transcription enhancer factor-2; MEK, microtubule-associated protein/extracellular signal-regulated kinase kinase; MRF4, muscle regulatory factor-4; Myf5, myogenic factor-5; MyoD, myogenic differentiation antigen; PPARγ, peroxisome proliferator-activated receptor γ; RXRα, retinoid X receptor α; Shh, sonic hedgehog; STAT-1, signal transducer and activator of transcription-1; TAZ, transcriptional coactivator with PDZ-binding motif; TGF, transforming growth factor.

In the case of adipocyte differentiation, although several transcriptional key events regulating the differentiation of preadipocytes into mature adipocytes have been identified in the last decade, master genes committing the multipotent mesenchymal stem cell to adipoblasts are still awaiting discovery. Recently, transcriptional coactivator with PDZ-binding motif (TAZ) was identified as an early "molecular rheostat" (Fig. 2) modulating mesenchymal stem cell differentiation [56, 57]. Whereas runx-2, the key osteogenic transcription factor, triggers MSC to an osteogenic differentiation program, adipogenic differentiation is mainly promoted by PPARγ. It is mainly of interest how these two transcription factors are regulated to determine these alternative cell fates. Hong et al. [57] demonstrated that TAZ coactivates runx-2-dependent gene transcription and inhibits PPARγ-dependent gene transcription. As a net result, osteogenic differentiation is favored. By modulating TAZ expression in cell lines, mouse embryonic fibroblasts, primary MSC in culture, and in zebrafish in vivo, Hong et al. [57] were successful in triggering osteogenic versus adipogenic differentiation. These results indicate that TAZ functions as a real molecular rheostat that allocates MSC to either osteogenic or adipogenic differentiation. In this context, β-catenin signaling and Wnt3a

are important mediators in reducing the osteogenic differentiation in ATMSC [58].

Adipogenic Differentiation

ADSC can be isolated from human subcutaneous adipose tissue and readily differentiated into cells of the adipocyte lineage. Most importantly, these ADSC-derived adipocytes develop important features known from mature adipocytes, such as lipolytic capacity upon catecholamine stimulation, anti-lipolytic activity mediated by α_2-adrenoceptors, and the secretion of typical adipokines, such as adiponectin and leptin [19]. Furthermore, ADSC retain their adipocyte differentiation capacity through multiple passages [19].

To date, a human white adipocyte cell line is not commercially available. Thus, most researchers are currently using several rodent cell lines (e.g., mouse 3T3-L1 preadipocytes) or SVF prepared from total adipose tissue followed by hormonal differentiation programs. However, rodent adipocytes and human adipocytes demonstrate species-specific differences, and mature adipocytes differentiated from SVF cannot be expanded. Based on these limitations, ADSC can effectively serve as a source for human white fat cells, and donor-specific cell banks might be easy to establish. Soft tissue defects after trauma, burn injury, or surgery still remain a challenge in plastic and reconstructive surgery, and innovative therapies are needed. Adipose tissue engineering using ADSC subjected to adipogenic differentiation seems to be a highly promising approach [59]. Choi et al. [59] designed injectable poly-lactic-*co*-glycolic acid spheres, attached MSC after adipogenic differentiation on these spheres, and were successful in generating newly formed adipose tissue in nude mice.

Autologous ADSC therapy might also be used for the treatment of fistulas in patients suffering from Crohn disease. In a pilot study [60] on five patients with Crohn disease, the external opening of six of eight fistulas could be closed by inoculation of the fistulas with autologous lipoaspirate-derived ADSC. The results of this uncontrolled phase I clinical trial do not allow the demonstration of effectiveness but might give motivation to undertake in vivo studies with autologous ADSC in patients suffering from wound healing defects and fistulas.

To date, artificial or biological implants suitable for the correction of soft tissue defects after trauma, tumor resection, or deep burns is lacking. In contrast to mature adipocytes, preadipocytes seem to have several characteristics that make them more suitable for this purpose than mature adipocytes. Morphologically, preadipocytes resemble fibroblasts, and they do not have large cytoplasmic lipid droplets. Since preadipocytes are smaller than mature adipocytes, they might allow a quicker revascularization after transplantation. Furthermore, transplanted preadipocytes maintain their ability to differentiate into mature adipose tissue in vivo, whereas the transplantation of mature adipocytes often gives poor results, such as oil cysts or transplant shrinkage. Preadipocytes have a significantly lower oxygen consumption than mature adipocytes [61], and this advantage in respiration and the better revascularization of undifferentiated adipose tissue cells might allow the future development of innovative transplants.

Chondrogenic/Osteogenic Differentiation

Since bone and cartilage tissue engineering requires large amounts of osteogenic/chondrogenic precursor cells, new sources of progenitor cells are needed. Compared with BMMSC, ADSC have the same ability for osteogenic differentiation, and this ability is maintained with increasing donor age [29]. There are only rare data directly comparing the effectiveness of ADSC and BMMSC in osteogenic and chondrogenic differentiation. In one study, ADSC were reported to have a slightly inferior potential for osteogenesis and chondrogenesis [62]. In a functional study [63], ADSC also had a inferior ability in the treatment of partial growth arrest in a murine experimental model compared with MSC derived from bone marrow or periosteum.

When ADSC were cultured in atelocollagen honeycomb-shaped scaffolds (three-dimensional culturing), osteogenic differentiation could be successfully triggered, as determined by alkaline phosphatase expression, osteocalcin secretion, and calcium phosphate deposition [64]. Depending on the media formulations used, ADSC can differentiate into a chondrocyte-like phenotype expressing cartilage-specific genes, such as aggrecan and type II collagen. Early activation of ERK and subsequent activation of JNK (two mitogen-activated protein kinase family members) represent molecular events triggering osteogenic differentiation and blocking adipogenic differentiation of MSC [65]. Subcutaneous adipose tissue-derived stromal cells synthesize cartilage matrix molecules, such as collagen type II, type VI, and chondroitin 4-sulfate [66], and maintain this expression when transplanted into nude mice as alginate cell constructs after preconditioning using chondrogenic media formulations [66].

Interestingly, MSC derived from synovial adipose tissue of joints exhibit a higher potential for chondrogenic differentiation (as determined by a higher STRO-1 and CD106 expression, a higher proliferation rate and colony-forming efficiency, and a higher amount of cartilage matrix production) than do MSC derived from subcutaneous adipose tissue [67]. The molecular master regulators that allocate the ADSC to the chondrogenic lineage are widely unknown with a role for Brachyury, bone morphogenetic protein (BMP)-4, transforming growth factor β_3 (TGFβ_3), and Smad-1, -4, and -5.

BMP-6 strongly upregulates the expression of aggrecan-1 and α1 chain of collagen II [68] and thus seems to provide an important growth factor for chondrogenic tissue engineering. BMP-7 belongs to the TGF-β superfamily of polypeptides and is also known to induce chondrocyte differentiation [69, 70]. Treating ADSC with recombinant BMP-7 stimulates chondrogenic differentiation and upregulates aggrecan gene expression [71], the predominant large chondroitin sulfate proteoglycan, a marker protein for chondrogenic differentiation. Similarly, FGF-2 enhances chondrogenesis and the proliferation of ADSC [34] by inducing the expression of N-cadherin, FGF-receptor-2, and the transcription factor Sox-9.

Human [72, 73] and mouse [74] adipose tissue-derived stromal cells can acquire typical osteoblast-like differentiation hallmarks, such as mineralized extracellular matrix production (calcium phosphate deposits), expression of the osteoblast-associated proteins osteocalcin and alkaline phosphatase [72], and response to mechanical loading [73]. Following osteogenic differentiation, ADSC can acquire bone cell-like functional properties, such as responsiveness to fluid shear stress [75], and increase their expression of both alkaline phosphatase and mechanosensitive genes, such as osteopontin, collagen type Iα1, and COX-2 after mechanical loading. These results indicate that ADSC have the potential to differentiate into real mechanosensitive bone-like cells and might therefore provide a promising tool for bone tissue engineering. When subcutaneous and visceral ADSC were directly compared regarding their osteogenic potential, visceral ADSC were found to possess a greater osteogenic potential than those isolated from subcutaneous adipose tissue [26]. However, the (transcription) factors that initially commit the ADSC to the osteocytic lineage are widely unknown. Menin, Shh, and Notch-1 were reported to be involved during the acquisition of an osteogenic phenotype [54].

BMP-2 is known to stimulate osteogenic differentiation [76–78]. Treating ADSC with recombinant BMP-2 stimulates osteogenic differentiation [71, 77] and upregulates runx-2 and osteopontin gene expression [71]. Runx-2 represents the earliest transcription factor during osteogenic differentiation, whereas osteopontin is one of the most abundant noncollagenous proteins found in bone extracellular matrix. BMP-2 receptor activation results in pleiotropic intracellular effects, such as the activation of R-Smad, multiple kinase activation, and modulation of osteogenic transcription factor activity (e.g., Runx-2, Osx, Dlx5, and Msx2) [78]. Runx-2 represents the central regulator of bone formation and mediates temporal activation/repression of cell growth and the expression of phenotypic genes through osteoblast differentiation [54]. Genetically modified, lipoaspirate-derived, human ADSC overexpressing BMP-2 were successfully used for healing critically sized femoral defects in a nude mouse model [79]. Tbx3 is a transcription factor known to be involved in the ulnar mammary syndrome when mutated. Tbx3 plays an important role in osteogenic differentiation and proliferation of human ADSC [80]. However, the mechanism standing behind its effects is completely unknown. FGF-2 inhibits osteogenic differentiation of ADSC [81], in contrast to its stimulatory effects on chondrogenic differentiation [34], mentioned above. Valproic acid can inhibit histone deacetylase (an enzyme that regulates differentiation processes in mammals) and increase osteogenic differentiation in human ATMSC in a dose-dependent manner [82]. Valproic acid-treated ADSC undergoing osteogenic differentiation increased their expression of osterix, osteopontin, BMP-2, and runx-2 [82]. Therefore, inhibitors of histone deacetylase might be of future interest in bone engineering.

However, in addition to the specific differentiation factors, both the artificial extracellular matrix substitutes and the three-dimensional environment used for cell culture are critical for a successful chondrogenic and osteogenic differentiation. Chitosan particle-agglomerated scaffolds, fibrin scaffolds, and β-tricalcium phosphate scaffolds were reported to be suitable for ADSC-derived cartilage and osteochondral tissue engineering [62, 83, 84].

Myogenic/Cardiomyogenic Differentiation

Cultured adipose tissue SVF cells have the potential for differentiation into a cardiomyocyte-like phenotype with specific cardiac marker gene expression and pacemaker activity [85] when cultured in a semisolid methylcellulose medium containing interleukin (IL)-3, IL-6, and stem cell factor. Moreover, the differentiated cells were capable of responding to adrenergic and cholinergic stimuli. The transplantation of monolayered ADSC onto the scarred myocardium in murine myocardial injury models results in cardiomyocyte differentiation, angiogenesis, expression of cardiomyocyte-specific markers, and improvement of cardiac function [86, 87]. Using ADSC isolated from mouse brown adipose tissue, infarction area could be reduced and left ventricular function could be improved after transplantation of these cells in a mouse model of myocardial infarction [88]. However, these data were obtained exclusively from animal models of murine origin and cannot be transferred into the human system.

Rodriguez et al. [89] were the first to report on the potential of ADSC to regenerate muscle and to express dystrophin when transplanted into mdx mice (a murine model of Duchenne muscular dystrophy). By using specific inductive media, ADSC can be differentiated into a myogenic phenotype resembling the characteristics of skeletal muscle [90, 91], such as the formation of myotubes. Moreover, ADSC seem to posses an intrinsic myogenic potential for skeletal muscle reconstitution. Direct contact with primary muscle cells is necessary for this differentiation process [90, 91]. When incorporated into muscle fibers after experimental-induced ischemia, ADSC can restore dystrophin gene expression in *mdx* mice [90].

Vascular/Endothelial Differentiation

Not only BMMSC but also ATMSC have the potential for endothelial differentiation [21]. In mice, adipose tissue-derived stromal vascular cells have a considerable proangiogenic potential regarding vessel incorporation, postischemic neovascularization, and vessel-like structure formation [92, 93]. Concerning the secretion profile, adipose tissue-derived stromal cells secrete significant amounts of angiogenesis-related mediators, such as VEGF, HGF, placental growth factor, FGF-2, TGF-β, and angiopoietin-1 [32, 43, 94, 95]. The secretion of angiogenesis-related cytokines probably makes these cells suitable both for regenerative cell therapy and for treating ischemic disorders [32, 43]. Ongoing studies using murine ischemia models [32, 43, 92, 93, 96] have already demonstrated an equal ability of ADSC compared with BMMSC in restoring the blood flow in these animals.

Neurogenic Differentiation

Incubation of ADSC under neuroinductive conditions can create a cell population expressing the neuronal differentiation marker type III β-tubulin [97]. Using a complex neurogenic differentiation protocol, both murine and human ADSC develop a neuronal phenotype and a positive staining for glial fibrillary acidic protein (GFAP), nestin, NeuN, and intermediate filament M [98]. However, confirmatory studies and investigations clarifying the molecular mechanisms are lacking. Moreover, many artifacts could explain the observed phenotype. Intraventricular injection of human ADSC transfected with a retrovirus overexpressing the human telomerase gene in ischemic rat brain showed enhancement of functional recovery in these animals [39]. Kang et al. [99] isolated human ADSC by liposuction, induced neural differentiation with azacytidine, and transplanted these microtubule-associated protein 2- and GFAP-expressing cells into rat brains. Since this procedure improved motor recovery and functional deficits in rats with artificial induced ischemic brain injury [99], genetically engineered ADSC might function as vehicles for future therapeutic gene transfer to the brain. Although these results are encouraging, more detailed and confirmatory studies are necessary before speculating on the future clinical implications.

Pancreatic/Endocrine Differentiation

Timper et al. [100] were successful in differentiating human ADSC into cells with a pancreatic endocrine phenotype using the differentiation factors activin-A, exendin-4, HGF, and pentagastrin. The proliferating MSC expressed the pancreatic endocrine transcription factor Isl-1 and the pancreatic developmental transcription factors Pax-6, Ipf-1, and Ngn-3. Most importantly, the differentiated cells expressed the endocrine pancreatic hormones insulin, glucagon, and somatostatin. These cells might be used to establish cell-based therapies for type 1 diabetes mellitus in the future. However, confirmatory and functional studies have to be performed, and conclusions from these preliminary data have to be drawn very cautiously.

Hepatic Differentiation

ADSC treated with HGF, oncostatin M (OSM), and dimethyl sulfoxide have the potential to develop a hepatocyte-like phenotype expressing albumin and α-fetoprotein [101]. Furthermore, these hepatocyte-like cells have the ability to take up

low-density lipoprotein and to produce urea [101]. The molecular events behind this in vitro differentiation are far from clear. HGF is a potent mitogen that acts via the HGF receptor c-Met, a transmembrane protein with an intracellular tyrosine kinase domain. HGF plays an important role in liver regeneration and embryonic development. OSM is a member of the IL-6 cytokine family regulating hepatocyte differentiation. Expanding on these in vitro data, intravenously injected ADSC show integration into the liver in mice, an effect that can be enhanced after partial hepatectomy that promotes liver regeneration [102]. Although the amount of available data is still low, these results should encourage basic research groups to extend these investigations.

Hematopoietic Differentiation

Of course, ADSC cannot acquire the potential to undergo a complete hematopoietic differentiation program as do BMMSC. However, ADSC might support hematopoiesis in some way. Lethally irradiated mice can be reconstituted by cells isolated from adipose tissue [103]. Using this experimental approach, ADSC from subcutaneous adipose tissue were reported to support the complete differentiation of hematopoietic progenitors into myeloid and B lymphoid cells [103]. However, these cells were unable to maintain the survival and self-renewal of hematopoietic stem cells. Thus, ADSC could be a future tool for the short-term reconstitution of hematopoiesis. Even the treatment of severe and therapy-resistant acute graft-versus-host disease with human ADSC seems to be possible [104] by using the immunosuppressive properties of ADSC [105].

CONCLUSIONS

The easy and repeatable access to subcutaneous adipose tissue provides a clear advantage for the isolation of MSC, and both isolation and culture techniques are easy to perform. Compared with BMMSC, ADSC have an equal potential to differentiate into cells and tissues of mesodermal origin, such as adipocytes, cartilage, bone, and skeletal muscle. Based on this progress, several clinical implications for cell therapy and tissue engineering are highly promising. Although sparse data exist on ADSC differentiation into tissues of nonmesodermal origin, an initial effort has been made to differentiate ADSC into hepatocytes, endocrine pancreatic cells, neurons, cardiomyocytes, hepatocytes, and endothelial/vascular cells. Whereas the lineage-specific differentiation into cells of mesodermal origin is well understood on a molecular basis, the molecular key events and transcription factors that initially allocate the ADSC to a specific lineage are almost completely unknown. Decoding these molecular mechanisms is of great interest for a more effective development of novel cell therapies.

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Review criteria: PubMed/Medline was searched for the terms and issues to be covered in this review. In addition, information from the Cochrane Library, National Center for Biotechnology Information nucleotide and protein database, Online Mendelian Inheritance in Man database, and patent specifications was used.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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Concise Review: Adipose Tissue-Derived Stromal Cells—Basic and Clinical Implications for Novel Cell-Based Therapies

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