# Human adipose-derived stem cells: current challenges and clinical perspectives *

## Células-tronco derivadas de tecido adiposo humano: desafios atuais e perspectivas clínicas

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**Abstract:** Adult or somatic stem cells hold great promise for tissue regeneration. Currently, one major scientific interest is focused on the basic biology and clinical application of mesenchymal stem cells. Adipose tissue-derived stem cells share similar characteristics with bone marrow mesenchymal stem cells, but have some advantages including harvesting through a less invasive surgical procedure. Moreover, adipose tissue-derived stem cells have the potential to differentiate into cells of mesodermal origin, such as adipocytes, cartilage, bone, and skeletal muscle, as well as cells of non-mesodermal lineage, such as hepatocytes, pancreatic endocrine cells, neurons, cardiomyocytes, and vascular endothelial cells. There are, however, inconsistencies in the scientific literature regarding methods for harvesting adipose tissue and for isolating, characterizing and handling adipose tissue-derived stem cells. Future clinical applications of adipose tissue-derived stem cells rely on more defined and widespread methods for obtaining cells of clinical grade quality. In this review, current methods in adipose tissue-derived stem cell research are discussed with emphasis on strategies designed for future applications in regenerative medicine and possible challenges along the way.
Key words: Adipocytes; Adipose tissue; Adult stem cells; Tissue therapy

**Resumo:** As células-tronco adultas ou somáticas detêm grande promessa para a reparação e regeneração de tecidos. Atualmente, o interesse dos cientistas é contínuo na investigação da biologia de células-tronco mesenquimais, tanto em aspectos básicos, quanto no potencial de aplicações terapêuticas. As células-tronco adultas derivadas do estroma do tecido adiposo, em comparação com as células-tronco derivadas do estroma da medula óssea, apresentam como vantagem o método fácil de obtenção da fonte tecidual. As células-tronco adultas derivadas do estroma do tecido adiposo apresentam potencial para se diferenciarem em células de tecidos mesodérmicos, como os adipócitos, as cartilagens, os ossos e o músculo esquelético e não mesodérmicos, como os hepatócitos, as células pancreáticas endócrinas, os neurônios, os hepatócitos e as células endoteliais vasculares. Entretanto, os dados disponíveis na literatura científica sobre as características das células-tronco adultas derivadas do estroma do tecido adiposo e os procedimentos para sua obtenção e manipulação no laboratório são inconsistentes. É necessário o desenvolvimento de metodologias e procedimentos eficazes de isolamento dessas células para obtenção de células em quantidade e qualidade suficientes para aplicação terapêutica. Nesta revisão, são discutidos os métodos correntes de coleta de tecido adiposo, isolamento e caracterização de células-tronco adultas derivadas do estroma do tecido adiposo, com ênfase na futura aplicação em medicina regenerativa e nos possíveis desafios nesse recente campo da ciência.
Palavras-chave: Adipócitos; Células-tronco adultas; Tecido adiposo; Terapia tissular

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## INTRODUCTION

By definition, stem cells are characterized by being primitive (undifferentiated or non-specialized) and by having the ability to generate not only new stem cells, but also a diverse range of specialized cell types under certain physiological and experimental conditions. [1-2] In this process of self-renewal and differentiation, the stem cell can go through two basic division processes: a) the deterministic model, which corresponds to the division of a stem cell that generates a new stem cell and a cell that will differentiate (progenitor cell) and b) the random or stochastic model, in which some stem cells generate only new stem cells while others generate differentiated cells.

Regarding their differentiation potential, stem cells are classified into four basic categories: a) totipotent, capable of differentiating into all the tissues that form the human body, including the placenta and embryonic membranes (zygote-derived cells); b) pluripotent, present in the inner cell mass of a blastocyst and capable of differentiating into any of the three germ layers (ectoderm, mesoderm, and endoderm); c) multipotent, which differentiate into various cell types of a single germ layer; d) oligopotent, which differentiate into few cells of a single germ layer, and e) unipotent, which differentiate into only one type of cell of a single germ layer. [2-3]

The basic difference about the nature of stem cells lies in the existence of **embryonic stem cells** (totipotent or pluripotent) [1] and **adult or somatic stem cells**, precursor cells of the already developed organism. [1-3] Recently, to improve the plasticity [I] [4] of adult stem cells, researchers were able to increase the differentiation potential of these cells, by a) somatic cell nuclear transfer [5] and b) genetic reprogramming of somatic stem cells to the embryonic state, by the introduction of pluripotency-determining genes (*OCT-4, SOX-2, KLF-4, cMYC*). These cells are called induced pluripotent stem cells or IPs cells. [6]

Pluripotent stem cells are the only ones capable of differentiating *in vitro*, inherently and spontaneously, into cells of the three germ lineages. Pluripotent stem cells are characterized by a high proliferation capacity, typical morphology and expression of specific markers (e.g. SSEA-3, SSEA-4, OCT-4, SOX-2, NANOG, KLF4), and the ability to form teratomas. [2,3,7,8]

Adult stem cells (ASC) have been isolated and characterized in various body tissues, such as bone marrow, umbilical cord, encephalon, epithelium, dental pulp and, more recently, adipose tissue. However, ASC have a limited capacity of differentiating into the various tissues of the human body. [9] Some scientists call them post-natal cells because they are found in the umbilical cord and other tissues of newborn babies. ASC are responsible for maintaining tissue homeostasis by renewing cells lost due to maturation, aging or damage. [9] For this reason, they hold great promise for tissue regeneration and repair.

A type of ASC that has been receiving more attention in preclinical and clinical studies about cell therapy is the mesenchymal stem cell [II] (MSC), which can be isolated from many biological sources, such as the umbilical cord, bone marrow, adipose tissue and fetal liver. [9,10] According to the scientific literature, [9-11] MSC are considered great candidates for cellular therapy due to the following criteria: a) they are easily harvested; b) they can be harvested from the patient himself; c) possibility of harvesting an adequate number of cells for transplantation, due to the high cellular proliferation *in vitro*, d) multipotent capacity of cell differentiation, e) easy laboratory handling, f) they have little immunogenicity, and g) they have the ability to integrate into the host tissue and interact with the surrounding tissue. However, the critical issue about these criteria is that scientists still ignore all the factors involved in cell differentiation and self-renewal (controlled by specific genes) and the chronic *in vivo* effects of MSC infusion. But the capacity that MSCs have to differentiate into mesodermal tissue, [10,11] such as musculoskeletal, bony, cartilaginous, and adipose tissue, strengthens their potential of use in regenerative medicine.

Circumjacent factors (internal and external factors) are extremely important for their maintenance, that is, their self-renewal and differentiation. This complex and dynamic microenvironment, which sends and receives signals via cellular and non-cellular mediators, is called niche. [12] Scientists still ignore the mechanisms that establish niches.

Another factor that limits the use of ASC is the low amount of telomerase, because telomeres are shortened in these cells; this limits their cell proliferation capacity. [7] Scientists have recently suggested age as a limiting factor for the use of ASC due to the accumulation of intrinsic events, such as DNA mutations, as well as extrinsic factors (niche alterations). It is thought that with aging the mechanisms responsible for the suppression of cancer development, such as senescence or apoptosis, may induce the decline of

[I] Plasticity: recently-discovered capacity of stem cells to expand their potential beyond tissues from which they are derived
[II] MSC: Mesenchymal or stromal stem cells

the replicating function of stem cells. [13]

The stromal vascular fraction (SVF) of adipose tissue has been the focus of recent research on **adipose-derived stem cells – ADSC**. [9,11,14,15] Some studies indicate that this tissue compartment is a rich source of pluripotent cells [14-16], although some scientists question the pluripotentiality of ADSC. [17] ADSC, however, share characteristics of ASC and their application in pharmacological and clinical studies, particularly in the search for treatment of degenerative diseases, has been considered. [14,16]

The adipose tissue is highly complex and is constituted by mature adipocytes, preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, monocytes, and macrophages, [18] and lymphocytes. [19] There is confusion in the scientific literature about the terms used to describe multipotent stem cells of adipose tissue, such as ADSC, human processed lipoaspirate (PLA) cells, preadipocytes or SVF. The acronym SVF corresponds to ADSC and describes the cells obtained immediately after the digestion of collagenase. [20,21] In this review, we will adopt the term ADSC for stem cells present in the adipose tissue.

Some scientists believe that the adipose tissue is a promising source of ASC for therapeutic applications because it is available in large amounts (100 ml up to 1 liter) through liposuction and with minimal morbidity. [22,23] However, very few review studies in the scientific literature explore the cellular and molecular characterization of ADSC. [20,21] In tissue bioengineering, the use of ADSC has been considered in the treatment of chronic degenerative diseases; for instance, in X-linked muscular dystrophy in the mouse. [24] They are also promising in reconstructive surgical treatment, of traumatic or non-traumatic origin, as in hereditary or acquired lipoatrophy. [25,26]

The objective of this review is to gather information from the scientific literature about tissue preparation for cellular and molecular characterization, the capacity of differentiation of ADSC and their future applicability in regenerative medicine, as well as the possible challenges in this recent scientific field.

## THE ADIPOSE TISSUE

In human adults, brown adipose tissue (BAT) is practically absent. However, the localized deposit of white adipose tissue (WAT) is present in various areas of the organism, involving or infiltrating organs and internal structures. [27,28] Anatomically, BAT is distributed in the organism as subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). In addition to adipocytes, BAT contains a matrix of connective tissue (collagenous and reticular fibers), nervous tissue, stromal-vascular cells, lymphatic nodules, immune cells (leukocytes, macrophages), fibroblasts, and stem cells. [27,28]

Over the last few years, it has been observed that the adipose tissue is not only an energy storer and provider, but also a dynamic, hormone-producing organ, which is involved in a variety of physiological and physiopathological processes, because it is able to secrete many proteins called adipokines (cytokines). [27]

The protein structure and the physiological function of the adipokines identified so far are highly varied. Adipokines may be grouped based on their main function: immunologic (e.g. adipsin), cardiovascular, metabolic or endocrine (e.g. adiponectin). [18]

### 1. Adipogenesis

The analysis of adipogenesis (adipose tissue differentiation process) aims at unfolding the molecular and cellular basis of adipose tissue development. [27] The components involved in cell-cell interaction or in the cellular matrix are important to the regulation of the differentiation process of adipocytes. [27] Both hormonal and nutritional signs may affect this differentiation in a positive and negative manner.

The differentiation of the preadipocyte into adipocyte is a highly controlled process. Adipogenic transcription factors, including the peroxisome proliferator-activated receptor (PPAR) gamma and the sterol regulatory element -binding protein (SREBP-1c), and the *CCAAT/enhancer binding protein* - C/EBPs play a key role in the complex transcriptional cascade that occurs during adipogenesis. [28,29]

The PPAR γ are responsible for the differentiation of adipocytes and lipid storage. The PPAR γ are the key regulators of adipocyte differentiation, stimulating an increase in the expression of various genes. The activation of these receptors leads to the reduction of adipokine secretion (tumor necrosis factor-alpha and leptin) and to the increase of adiponectin. [28.29]

The PPAR γ may be activated by synthetic compounds denominated thiazolidinediones (TZD), which are clinically used as antidiabetic agents. [28,29]

The SREBP protein (orphan nuclear receptor) is a transcription factor originally cloned from the adipose tissue of a mouse. The SREBP transcription factor is important to lipid homeostasis because it increases the expression of the gene responsible for lipogenic enzymes; however, its adipogenic function and the relation with PPAR Á remain unclear. [28,29]

C/EBPs are members of the b-zip family (DNA binding domain), which contains a leucine zipper-like domain necessary for dimerization. The isoforms of C/EBP (·, , e ‰) are highly expressed in the adipocytes and induced during adipogenesis. C/EBP · is important in the differentiation of preadipocytes into adipocytes and acts in the conversion of fibrob-

lasts into adipocytes. C/EBP, also induces adipogenesis, probably by stimulating PPAR γ expression, whose gene contains C/EBP sites in its promoter region. It has been shown that PPAR γ is a potent stimulant of the adipocyte cell differentiation cascade and acts synergically with C/EBP · to promote it or to induce the differentiation of fibroblasts into adipocytes. [28,29]

**VED STEM CELLS**

The adipose tissue is an abundant source of cells for autologous transplants and is easily obtained through liposuction, a cheaper and less invasive procedure than bone marrow puncture. Nonetheless, there is still no consensus among researchers about the nomenclature and plasticity of ADSC. [17,20,21]

Comparative analysis of MSC, bone marrow stromal cells (BMSC), ADSC, and umbilical cord stem cells (UCSC) showed that ADSC are not different in relation to morphology and immunologic phenotype in comparison with BMSC and UCSC. [10,15]. However, the frequency of ADSC in the adipose tissue is higher than that of BMSC [III] in the medullary stroma. [30-33] The cell proliferation rate of ADSC is higher than that of BMSC. [31]

Approximately 2 - 6 x$10^8$ cells in 300 mL of adipose tissue can be obtained from lipoaspirate. However, this number of ADSC may vary depending on the method for harvesting stem cells. In fact, data from the literature about the isolation of ADSC indicate that according to the surgical procedure [IV] and the laboratorial method employed, a different number of ADSC may be obtained from the same amount of adipose tissue, [30-35] as well as ADSC with varied characteristics and viability. [34] The viability of ADSC is, however, apparently not altered after cryopreservation of the aspirated material. [35]

Despite the existence of very few clinical studies, the number of ADSC appears to remain the same in relation to the anatomical region. [32] Nonetheless, the human adipose tissue shows metabolic differences, according to the anatomical localization. [27] In addition, age is another factor that may interfere in cell composition, since it has been observed that younger individuals have a higher number of ADSC as compared to older subjects. [13] In mice, researchers noticed differences in cell composition and differentiation capacity of ADSC, based on anatomical regions. [15]

Therefore, it appears that human adipose tissue is probably constituted by different subtypes of stem cells, based on the anatomical region. Nevertheless, more comparative studies about the nature of the cells and the potential of differentiation of ADSC isolated from adipose tissue of distinct anatomical regions are needed. Attention should also be focused on the type of surgical and/or laboratorial procedure employed.

The number of stem cells that may be obtained from adipose tissue is clinically relevant since they have a higher cellular proliferation rate than BMSC. [3]

**1. Identification, molecular characteristics and differentiation of ADSC**

Rodbell *et al.* (1960) [36] introduced the initial method for isolating cells from adipose tissue. At first, this method was performed only with adipose tissue samples of laboratory animals (mice, rabbits). Later, this method was modified for use with human adipose tissue samples (Figure 1).

The adipose tissue is grinded and washed extensively in phosphate-buffered saline (PBS) containing penicillin/streptomycin (P/S). Next, with the addition of collagenase, the digestion phase begins. The tissue is incubated at 37$^0$C from 30 to 90 minutes. After this time period, it is necessary to neutralize the activity of collagenase by adding fetal bovine serum (FBS). [20,21,33]

Before transferring the sample to the centrifuge tube, it is advisable to mix it to disintegrate adipose tissue aggregates. After the material is centrifuged, it is possible to separate adipocytes from the SVF. SVF is formed by a heterogeneous cell population, including circulating blood cells, fibroblasts, periocytes and endothelial cells, as well as ADSC. [21,33]

The final step for isolating ADSC is the selection of the adherent population inside the SVF. After the separation of SVF from the adipocytes is complete, the sample (SVF) is incubated in ice for 10 minutes in lysis buffer. Then, the sample is washed in PBS containing P/S and, once again, centrifuged. Next, cellular expansion is initiated in appropriate culture medium (e.g. *DMEM-LG- Dulbecos's modified Eagle's medium).* Cells are cultured in cell culture plaques and culture-expanded for up to 15 passages [V]. The ADSC obtained this way may be used in various protocols of cell characterization (Figure 1). [33,36,37] The various surface proteins considered to be ADSC markers are shown in table 1. [9,10,17,30,31,33,34]

Zhu Y *et al*. (2008) [32] observed that ADSC can be maintained in culture with many growth stages, without passage, for more than a month. During this period, right after the second week, the authors observed that there was an increase in protein synthe-

[III] The incidence of BMSC is estimated to be about 1 in every 100,000-500,000 nucleated cells from bone marrow aspirate in adults

[IV] Surgical resection or tumescent liposuction or ultrasound assisted liposuction

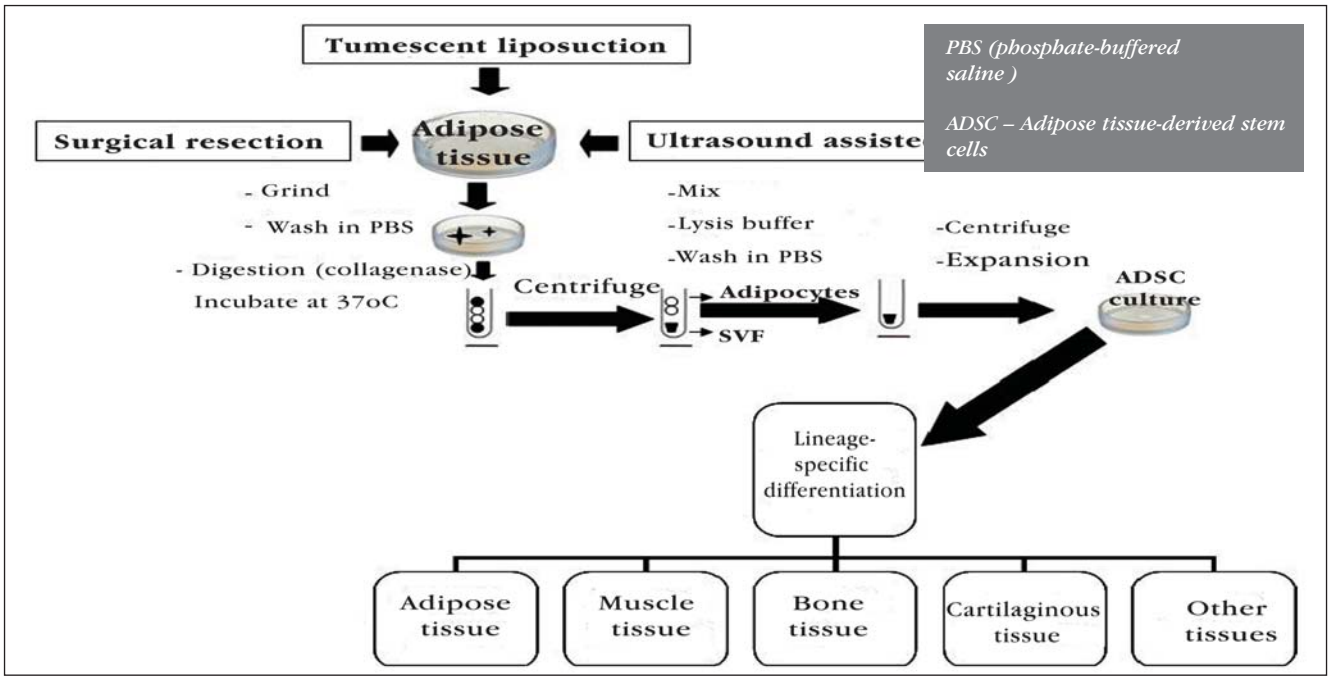


**Figure 1:** Flowchart of the laboratorial procedure to isolate adipose tissue-derived stem cells

sis and, consequently, an increase in the expression of surface proteins.

As a minimal prerequisite, based on flow cytometry data analysis, MSC must express typical antigens such as CD73, CD105, and CD90. ADSC also express high levels of these antigens; CD13, CD29, CD44, CD105, and CD166 are the most frequent (Table 1). [9,10,30,31,33,34] Nonetheless, ADSC do not express hematopoietic antigens such as CD34, CD45, and HLA-DR, a profile also found in MSC.

Zhu *et al.* (2008) [32], after 25 passages, performed subculture of ADSC every 14 days, instead of every five days, intent on obtaining a greater amount of ADSC, keeping their phenotype and capacity of proliferation and differentiation.

Some authors noticed an improvement in the growth rate and viability of ADSC with the use of antioxidants and low concentration of calcium. [37] Several proteins may stimulate the proliferation of ADSC, such as:

a) Fibroblast growth factor-2 (FGF-2), by FGF-2 receptor; [38]
b) Sphingophosphorylcholine, by c-jun N terminal kinase activation (JNK); [39]
c) Platelet-derived factor, by JNK activation, [40] and
d) Oncostatin M, [41] by the activation of microtubule-associated protein kinase (MEK), the extracellular signal-regulated kinase (ERK) and by JAK3/STAT1 **[VI].**

ADSC are able to maintain their self-renewal (or self-replication) *in vitro* [42] because they secrete growth factors. The oncogenic potential of ADSC may be reduced by the inhibition of MEk1 protein, but this does not affect the differentiation potential of ADSC. The longevity of human ADSC may be prolonged by the overexpression of the catalytic subunit of the telomerase gene. [43] Moreover, ADSC secrete other potent growth factors, such as the vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and growth factor-1, which is similar to insulin growth factor-1 (IGF-1). [44] The tumoral necrosis factor may significantly increase the secretion of VEGF, HGF, and IGF-1 of ADSC by the activation of p38, which is dependent on the protein kinase mechanism. [44]

There are some molecular differences between ADSC and BMSC, although the profile of the expression of surface proteins [VII] and genes [VIII] appears to be similar [10,33] (Table 1). It is estimated that less than 1% of the genes are expressed differently by ADSC and BMSC. [31] There has not been a successful specific phenotypic characterization of ADSC in the literature, and comparative studies between the expression of the gene and surface proteins of ADSC and BMSC are rare. [10,30,31]

More knowledge about the mechanisms that regulate the biology of ADSC is necessary to perfect and standardize laboratory techniques for the isola-

[V] Passage: in the cell culture, it is the process in which cells are dissociated, washed and cultured in new culture plaques, after a growth and cell proliferation cycle. The number of passages that a cultured cell lineage goes through indicates its age and likely stability

**TABLE 1:** Profile of the expression of protein markers in adipose tissue-derived stem cells

| Positive Expression | Negative or defficient expression |
|---|---|
| **CD9** | **CD11b** |
| **CD10** | **CD14** |
| **CD13** | **CD19** |
| CD29 | **CD31** |
| CD44 | **CD34** |
| **CD49** | **CD45** |
| **CD54** | **CD79 ALFA** |
| **CD55** | **CD80** |
| **CD59** | **CD117** |
| CD73 | **CD133** |
| CD90 | **CD144** |
| CD105 | **HLA-DR** |
| **CD106** | **c-kit** |
| **CD146** | **MyD88** |
| CD166 | **STRO-1** |
| **HLA1** | **LIN** |
| **FIBRONECTIN** | **HLAII** |
| **ENDOMUCIN** | |
| **ASTHMA** | |
| **VIMENTIN** | |
| **COLLAGEN-1** | |

* Based on the scientific literature, Barry & Murphy, 2004,9 Kern et al., 200610; De Ugarte et al., 200330 Lee et al., 2004;31 Oedayrajsingh et al., 2006; 33 Gronthos et al., 2001,34 all the cellular surface markers were verified in vitro after cell expansion. They were not evaluated in primary cells. All the attempts to differentiate the molecular phenotype of these cells and those similar to fibroblasts were not successful. As a minimal prerequisite, ADSC typically express mesenchymal markers (in bold) and express, in a deficient way, markers of endothelial and hematopoietic lineage (Source: Schäffler & Büchler, 2007 [20]).

tion, characterization and manipulation of ADSC. Such knowledge is prerequisite, not only to the culture and differentiation of a specific cell lineage, but also to the development of a more efficient therapy.

It is important to highlight that ADSC share immunosuppressive properties with BMSC, [45] as well as the deficient HLA-DR expression. Therefore, it is likely that in the future ADSC may be made available for allogeneic transplants due to the lower risk of rejection. [45]

The process through which ADSC differentiate into other cells is denominated lineage-specific differentiation [9] and begins with the activation of certain transcription factors (Figure 2). Recently, researchers [17,25,46-49] have shown in vitro the ability of ADSC to differentiate into mesenchymal cells (adipocytes, myocytes, osteocytes, and condrocytes) and non-mesenchymal cells (hepatocytes, neurons, pancreatic cells, endothelial cells, and cardiomyocytes – Table 2). [20] Rodriguez *et al.* (2005) showed *in vivo* that the implantation of ADSC in mice restored the expression of dystrophin in the muscle cells of the mouse.

Before the transcription events, the process through which stem cells can be coaxed or destined to a specific lineage is still not fully understood. [8] However, some tissue transcription factors are known. In stem cells, the process of proliferation, coaxing, and terminal differentiation (lineage-specific) are regulated by a complex network of molecular interaction that involves gene transcription modulators, transcription factors, protein kinases, growth factors and cell receptors (Figure 2).

Runx-2, for instance, is a key transcription factor for osteogenesis involved in the differentiation of ADSC into osteocytes, [50] whereas PPAR γ is a factor involved in the adipogenic differentiation of ADSC. Hong *et al.* (2006) [50] showed that TAZ (co-activating transcription factor) is capable of activating the Runx-2 transcription factor, as well as of inhibiting the transcription of the PPAR γ-codifying gene.

## 2. Therapeutic Perspectives

Embryonic and somatic stem cells are useful to investigate questions in basic science and clinical research. The use of somatic stem cells, particularly due to practicality and the uninvolvement of ethical and immunogenic issues, is one of the most promising areas of research about tissue regeneration and cancer development. In this field, there is a growing interest in the use of ADSC in studies about the development of neoplasms, degenerative diseases, and therapeutic applications in reconstructive surgery (Table 2). [20]

In addition, ADSC may be used in: i) basic studies that seek to understand the molecular, genetic and epigenetic mechanisms involved in the control of the intrinsic processes of stem cells, ii) the study of the physiopathology of human genetic diseases, and iii) development and tests of new drugs. Even though therapeutic strategies with ADSC have not been widely tested in humans, technical-scientific advances in the areas of cellular and molecular biology, facilitated by studies

[VI] JAK3 - Janus Kinase 3- enzyme found in immune cells, responsible for the signaling process that results in the differentiation of leukocytes. STAT1 - Transcription factor – signal transductor and activator of the transcription that mediates cell responses to interferons. STAT1 interacts with the tumor suppressive protein p53 and regulates the expression of the genes involved in growth control and apoptosis

[VII] Determined by FACS (fluorescence-activated cell sorting)

[VIII] Determined by micro-arrangements

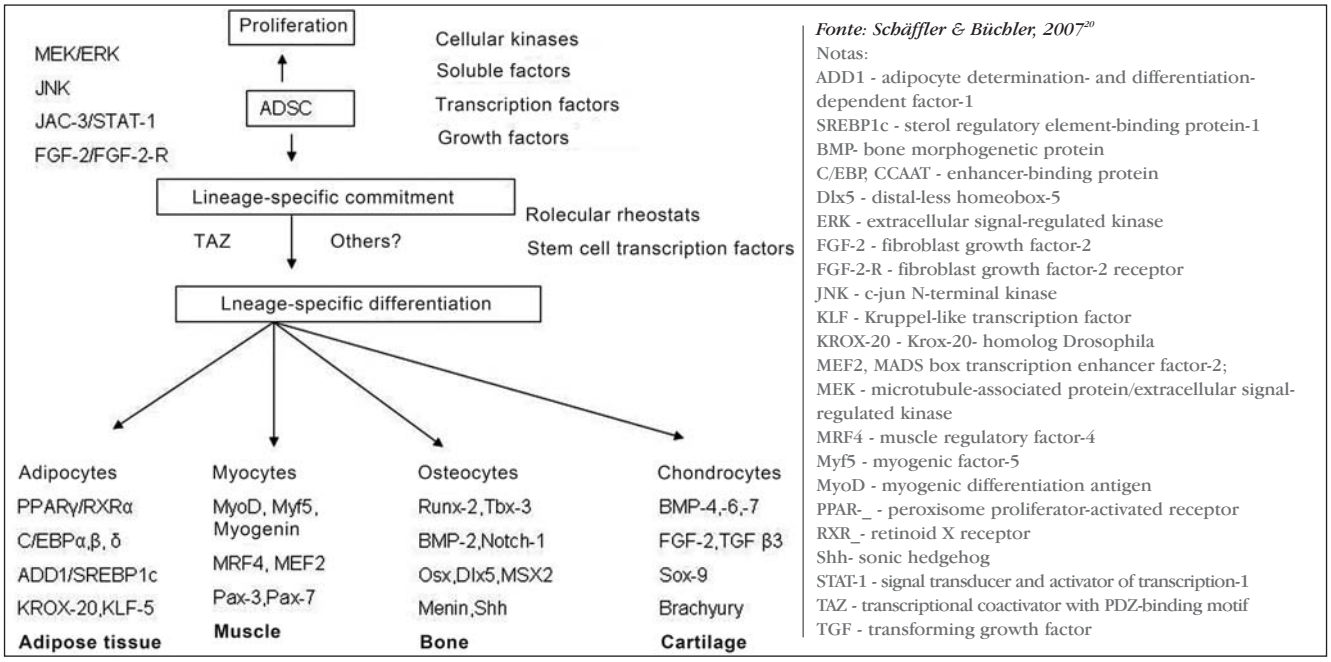


**Figure 2:** Molecular regulation of the proliferation, lineage-specific coaxing, and differentiation of adipose tissue-derived stem cells

about human somatic stem cells, may positively influence the advance of clinical research with ADSC.

## CONCLUSION

Despite great advances, many important issues about the use of ADSC in tissue bioengineering remain unclear. These questions must be explored before clinical tests to better direct clinical strategies. Among the greatest current challenges, we may cite: how to control the processes of proliferation and differentiation of ADSC *in vitro* and *in vivo*? Which factors control these processes? Which niche factors determine the control of its behavior? Which factors stimulate the migration to and integration of ADSC into the sites of tissue injury? What capacity do ADSC have to form tumors?

An important factor to improve ADSC research is the standardization of surgical and laboratory methods used to isolate and characterize these cells. Along these lines, comparative studies are extremely relevant and should be conducted to evaluate surgical procedures employed to obtain adipose tissue and to locate the best adipose tissue donor area. Laboratory techniques for tissue preparation to isolate and identify ADSC, cell culture techniques in scale and purity degrees for clinical application, and the methods that evaluate the quality of the cells to be implanted (e.g. evaluation of cell differentiation potential, presence of genetic or epigenetic alterations, etc) should also be standardized.

The comprehension of the molecular mechanisms that regulate the self-renewal and differentiation of ADSC, as well as their interaction with the cell niche, is equally relevant. This basic knowledge may help the development of new therapies and the evaluation of biosecurity issues in future clinical protocols.❑

**Table 2:** Experimental factors to induce the differentiation of adipose tissue-derived stem cells

| Differentiation factors | type of differentiation | clinical potential |
|---|---|---|
| Insulin, IBMX (3-isobutil-1-methylxanthine), dexamethasone, rosiglitazone, indomethasone | Adipogenic | Reconstructive surgeries, skin filling |
| bone morphogenetic protein, fibroblast growth factor, transforming growth factor, dexamethasone, insulin growth factor | Chondrogenic | Reconstructive surgeries, joint repair in degenerative diseases |
| 1,25 dihydroxycholecalciferol b β, glycerophosphate, ascorbic acid, bone morphogenetic protein 2, valproic acid | Osteogenic | Bone tissue regeneration in degenerative diseases, tumors and traumas |
| ? | Myogenic | Regeneration in degenerative diseases such as Duchenne muscular dystrophy |
| stem cell factor, interleukin-3, interleukin-6 | Cardiomyogenic | Regeneration of cardiac muscle after acute myocardial infarction |
| ? | Vascular/endothelial | Neovascularization/ ischemic diseases |
| valproic acid, insulin, hydrocortisone, epidermal growth factor, FGF | Neurogenic | Chronic-degenerative diseases and central and peripheral nervous system traumas |
| Activin-A, exedin-4, pentagastrin, hepatocyte growth factor, nicotinamide, glucose | Pancreatic/endocrine | Diabetes mellitus type 1 |
| hepatocyte growth factor , oncostatin, dimethyl sulphoxide | Hepatic | Hepatic regeneration |
| ? | Hematopoietic | Hematopoetic reconstitution |

Source: Schäffler & Büchler, 2007 [20]

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