Adipose-Derived Stem Cells for Therapeutic Applications

9

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Introduction

Adipose tissue contains several types of stem and progenitor cells, including the adipose tissue-derived stromal cells (ADSCs), the endothelial progenitor cells, and the hematopoietic and immune system cells. ADSCs share most of phenotypical and functional characteristics of the mesenchymal stromal cell (MSC): the bone marrow-derived mesenchymal stromal cell (BM-MSC) or MSC present in the cord blood, placenta, and umbilical cord. The basic function of ADSC is the preservation of the adipose tissue integrity by the production of adipocytes in the intensity proportional to their degradation. Recently it has been proven that the adipose tissue may contain more MSC-like cells than the bone marrow (which serves as the "gold standard" of cells available for autologous cellular therapies. ADSCs are not only able to differentiate into adipo-, chondro-, or osteogenic lineages but also participate in the formation of the endothelium; smooth, skeletal, or cardiac muscle; hepatocytes; or neural cells. It remains unclear in which extent adipose tissue serves as the natural depository of stem cells, supplying "on-demand" cells for tissue regeneration. ADSCs are the abundant source of autologous stem cells for regenerative medicine techniques, being present in humans throughout all their lifetime.

Adipose Tissue as a Source of Stem Cells

Adipose tissue derives from the mesodermal layer of the embryo [104, 122]. There are several types of adipose tissue, differing in localization and functions: white, mechanical, brown, mammary, and bone marrow. White adipose tissue provides mechanical insulation and energy supply and functions as an endocrine organ, producing the adipokine factors, such as leptin, adiponectin, resistin, osteopontin, lipocalin, and angiogenic-related factors. Mechanical adipose tissue is responsible for more specialized structural support, like palmary fat pads or retro-orbital supporting tissue. Brown adipose tissue plays a unique thermogenic function – being able to generate heat through expression of unique protein – it is localized around the aorta, heart, or kidney in newborn infants, and its volume decreases along with human maturation. Mammary adipose tissue is function specialized, providing the mechanical support and energy for the mammary glands during lactation. The role of the adipose tissue in bone marrow cavities is to replace in adults the space occupied in children by the bone marrow and to provide humoral support (cytokines) and contact regulatory signals for hematopoietic stem and progenitor cells.

Initially, the studies of cells isolated from the adipose tissue were concentrated on adipocytes and their precursors. As early as in 1966, Rodbell and Jones [137–139] were able to isolate the "stromal vascular fraction" (SVF) which was a heterogenous cell population with the predominance of adipocyte progenitors plus the admixture of the fibroblasts, pericytes, and endothelial and blood cells. Consecutive studies [41, 56] revealed that SVF cells have fibroblast-like morphology and are mitotically active source of adipocyte precursors capable to form adipose tissue in vitro. Some authors suggested [45] that under specific conditions, SVF is able to differentiate into non-adipogenic lineages. Almost a decade later, Zhuk et al. [188] demonstrated that the adipose tissue is a source of mesenchymal stromal-type cells (MSCs), capable to differentiate into adipo-, chondro-, myo-, and osteogenic lineages. Subsequently, the same authors demonstrated that the adipose tissue-derived ADSCs express the same marker composition (CD29+ CD44+, CD71+, CD90+, CD105+, SH3+, CD31−, CD34−, CD45−) as the bone marrow-derived mesenchymal stromal cell (BM-MSC) population [187]. The other less numerous population of adipose-derived cells is CD31+, CD34+, CD105+, and

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CD45− and consists of endothelial stem cells (ESCs), characterized by the low expansion rate in vitro [9].

ADSCs are integral component of the adipose tissue, being responsible for continuous replacement of aging adipocytes, resulting in remodeling, and continuous presence of the adipose tissue throughout all lifetime of human being. Several other stem cell populations may derive from the blood vessels (hematopoietic stem cells, immune system cells, or endothelial stem cells) or reside in "stem cell niches" in the adipose tissue following migration from other tissue locations.

Collection and Processing of ADSC

Elective suction-assisted lipectomy (liposuction) has been introduced as a technique of the elimination of the excessive amount of adipose during esthetic medicine treatment (body modification, weight reduction). Liposuction, being one of the basic tools of cosmetic surgery, offers the unique opportunity for collection of large quantities of stem cells from the waste material, without any ethical, medical, or religious contraindications. The same technique may be applied specifically for collection of autologous ADSC for regenerative medicine purposes. Liposuction is not only the less invasive technique as bone marrow aspiration; it allows to collect much higher numbers of cells of MSC characteristics when compared to bone marrow aspiration [24].

The adipose tissue may be obtained by tumescent lipoaspiration [81], ultrasound-assisted lipoaspiration [125], laser-assisted or water-assisted liposuction [2], or surgical resection – all these methods are considered as useful for stem cell collection (in our experience the highest percentage of viable cells is obtained by surgical resection, and the highest, although acceptable, cell mortality results from laser-assisted or ultrasound-assisted procedures). The best results are obtained when the storage time from adipose tissue collection till processing does not exceed 24 h [10].

All the existing protocols for adipose tissue-derived cell separation [8, 54, 123, 188] are based on the enzymatic digestion (collagenase, trypsin) and density gradient separation of ADSCs. Surgical isolation and mechanical dissection of fat, applied in pioneer works [74, 98], was replaced by various liposuction techniques, but all the rest of processing techniques remained basically unchanged. Following lipoaspiration, the mixture of the adipose tissue and balanced salt solution is washed with PBS (purification and removal of anesthetics and epinephrine used during tumescent liposuction) and digested with collagenase. Depending on the technique protocol, cells are isolated by centrifugation, erythrocytes removed by density gradient separation or by addition of erythrocyte lysis buffer, and resulting population of ADSC is expanded in plastic-adherent cultures in media without addition of any growth factors. Cytokine deprivation in in vitro culture allows for further purification of cell population by elimination of residual hematopoietic stem cells originating from blood vessels.

The increasing demand for ADSCs for cell-based therapies resulted in construction of automated systems for adipose-derived cell separation, which can be used at the bedside, without the access to of stem cell laboratory [21, 112]. The advantage of automated devices is (more or less) closed processing system and the possibility of applying cell-based therapies by the groups having no experience in stem cell processing. The disadvantage of automated ADSC processing "on the bedside" is temptation to neglect the verification step of obtained cellular material (tests of cell numbers, viability, phenotype characteristics, etc.) in situations, when cells are isolated by the machine and directly transplanted into patients by surgeons. The other disadvantage of automated system is the cost of the cell isolation procedure and lower flexibility of the procedure when dealing with the material of nontypical quantity or quality.

Phenotypical and Functional Characteristics of ADSC

The procedure of isolation of the adipose tissue-derived cells does not allow to purify the homogenous cell population, resulting in the separation of mixture of mesenchymal stromal cells (MSCs), adipocyte progenitors, fibroblasts, pericytes, and endothelial and blood cells. Such heterogenous population is described by the term "stromal vascular fraction" (SVF) [41, 56, 137–139] or "processed lipoaspirate" (PLA) [118, 188]. The population of adipose tissue-derived stromal cells (ADSCs) is purified by culture in plastic-adherent manner in media non-supplemented with growth factors. Cells which need the supplementation of culture media with growth factors (hematopoietic stem cells) will commit apoptosis, and the more differentiated cells will achieve mature stage and, being nonproliferating, will be eliminated during consecutive passages. The final cell population is composed predominantly of MSC type of cells and is described by various authors as adipose-derived adult cells (ADACs), adipose-derived stem cells (ADSCs, nomenclature advocated by International Fat Applied Technology Society), or adipose mesenchymal stem cells (AdMSCs). Since the "stemness" of adipose-derived cells is not formally proven, the acronym of "adipose-derived stromal cells" (ADSCs) seems most appropriate, reflecting both the adipose tissue origin and mesenchymal stromal characteristics of the cells.

There are several papers discussing the availability of ADSC in comparison with the bone marrow MSC (BM-MSC). The frequency of non-hematopoietic stem cells in human bone marrow, measured by CFU-F assay, varies between 1 in

25,000 and 1 in 100,000 [6, 7, 21, 118]. In contrast, ADSCs are present in frequency of 1 in 50 in population of adipose tissue-isolated nucleated cells [157]. The direct comparison of CFU-F numbers formed by ADSC or BM-MSC plated in the same frequencies of initial cells, revealed the sevenfold higher frequency of ADSC-derived CFU-F in comparison to BM-MSC-derived CFU-F [77]. Based on the frequency of MSC in the bone marrow, and frequency of adipose-derived cells, and on the approximate volume of the adipose tissue or bone marrow collected, it may be concluded that the adipose tissue is a more efficient source for cell collection for therapeutic purposes than the bone marrow [157].

Cell Surface Markers

Phenotypically, ADSCs express surface markers characteristic for MSC category, and, apart from minor differences, their phenotype is similar to BM-MSCs. Both ADSCs and BM-MSCs express markers common for cells having multilineage potential: STRO-1, CD105, and CD166 [26, 47, 48, 103, 128, 155]. The other markers suggesting the therapeutic potential of ADSC are CD29 (beta-1 integrin), important for inducing angiogenesis [4], intercellular adhesion molecule-1 ICAM-1 (CD54) immunoglobulin supergene family [141], and CD44 (hyaluronate receptor involved in development of extracellular matrix) [187]. ADSCs are HLA-DR negative, mostly MHC Class I positive [5], being of low immune reactivity when transplanted in HLA mismatch situation.

ADSCs fulfill the criteria for being multipotential stromal cells, proposed by the International Society for Cellular Therapy (in vitro plastic adherence; expression of CD105, CD73, and CD90 and lack of expression of CD45, CD34, and CD14 or CD11b, CD79a, or CD19 and HLA-DR surface molecules; and capacity of differentiation to osteoblasts, adipocytes, and chondroblasts [30, 57]).

The extended characterization of ADSC surface markers [5, 23, 24, 47, 110, 108, 187] revealed the presence of CD9, CD10, CD13, CD29, CD34, CD44, CD49d, CD49e, CD54, CD55, CD59, CD73, CD90, CD105, CD117, CD146, CD166, and STRO-1 markers and the absence of lineage-specific, hematopoietic, and endothelial markers CD3, CD4, CD11c, CD14, CD15, CD16, CD19, CD31, CD33, CD38, CD45, CD56, CD62p, CD104, and CD144. The expression of VLA-4 (CD49d) and its receptor VCAM-1 (CD106) is reciprocally reversed when comparing ADSC to BM-MSC: ADSCs express CD49d+/CD106- pattern [157], whereas BM-MSCs are CD49d-/CD106+ [23]. The concentration of CD34 marker was higher in freshly isolated cells (SVF) and remained present at reduced levels throughout the culture period of ADSC [110] or have been already unobserved by the others in at least 95 % of cultured cells [77]. Low percentage of CD34-positive cells may reflect the presence of subpopulation of endothelial progenitor cells (EPCs) – the possibility supported by the finding that adipose-derived CD34+ and CD133+ cells are able to form endothelial colonies in vitro or induce angiogenesis in vivo [11, 109, 130, 134, 160]. The concentration of EPC positively correlates with body index, suggesting the entrapment of these cells in the adipose tissue resulting in reduced angiogenic potential in obesity [168].

It has been also documented [15] that ADSCs express Toll-like receptors (TLR-1, TLR-2, TLR-3, TLR-4, TLR-5, TLR-6, and TLR-9) identified both by flow cytometry and real-time PCR. TLRs affect ADSC proliferation and differentiation and play a nonimmune role in signaling on ADSC, but their exact role as structures present on ADSC remains mostly unclear.

Proteome and Transcriptome Analysis

Mass spectrometry analyses revealed the similarities of ASC proteomes and proteomes of fibroblasts and MSCs [25, 161, 172]. Transcriptomes of ADSC and BM-MSC were studied by gene microarrays [75, 99, 175] or Affymetrix gene chips [40]. Both methods have revealed that ADSC and BM-MSC share a common transcriptome [40, 175], expressing stem cell-associated gene markers (Oct4, Sox2, and Rex1) [62].

In Vitro Proliferation and Differentiation of ADSC

ADSCs grow in vitro without supplementation with any growth factors. Fibroblastoid-like cells adhere to plastics and are passaged following trypsinization through a culture period up to 20 passages, or >4 months without visible loss of telomere length [37, 62]. The stable, low senescence level of ADSCs in culture was confirmed by the observation of the absence (<5 %) of β-galactosidase-positive cells in cultures from passage 1 to passage 15 [188]. Data on the telomerase activity are not consistent [40, 62, 75] and may depend on the observation protocols. Cell doubling time varies from 2 to 4 days [62, 110] being longest at the beginning of the culture. In both in vitro and in in vivo animal models, ADSCs are able to differentiate into several "mesenchymal" and "non-mesenchymal" lineages. Since only the minority of experiments were based on the analysis of single cell-derived clonal population of cells [187], the evidence of multilineage differentiation may be assigned rather to the "ADSC cell population" than to the single cell. It has been, nevertheless, proven that ADSC is able to differentiate into other mesenchymal cell lineages – the phenomenon interpreted by some authors as transdifferentiation or plasticity [130, 133, 145, 146].

Differentiation potential of cells residing in the adipose tissue resembles this of the MSC or MSC-like cells residing in the bone marrow (BM-MSC), umbilical cord Wharton jelly (umbilical cord stromal cells, UCSC), cord blood (unrestricted somatic stem cells, USSC), or placenta (reviewed in [131]). It is not surprising that cells resident in the adipose tissue are capable of adipogenic differentiation [28, 51, 144, 151, 187, 188] and, similarly to the other "MSC-type" cells, may differentiate into osteogenic [31, 50, 52, 53, 58, 70, 82, 83, 88, 124, 153, 164, 187, 188] or chondrogenic [32, 35, 128, 174, 175, 187, 188] lineages. The other directions of their differentiation in vitro are myogenic (skeletal muscle [90, 112, 187, 188], smooth muscle [1, 42, 65, 91], and cardiac muscle [44, 129, 156, 158, 179, 187, 188]), neurogenic [4, 71, 86, 142, 145–147, 187], pancreatic [165], and hepatic [152, 162, 163] lineages. It seems to be unclear, if observed angiogenic potential of adipose tissue-derived cells [3, 115, 130, 167] should be attributed to ADSC, EPC, or both cell types, since both are present in adipose tissue and both are capable of endothelial differentiation [130]. It has to be stressed, however, that the majority of experiments describing the differentiation potential of ADSC did not result in the observation of the formation of functional mature cells or tissues but allowed to deduce the differentiation capability from the identification of some structural markers or genetic profiles specific for the cell lineages – so the suggested "differentiation potential" does not mean that ADSCs are able to produce fully functional cells of specific lineage.

Interaction with Hematopoietic and Immune System

The earliest recognized function of mesenchymal stromal cells was formation of "niches" in the bone marrow, where MSC functioned as bone marrow microenvironment, supporting homing and proliferation of hematopoietic stem cells. It has been reported that co-infusion of BM-MSC and hematopoietic stem cells enhanced hematopoietic recovery in chemotherapy-treated patients [84]. ADSCs, being the MSC-type cells, are able to support hematopoiesis in lethally irradiated mice [18]. In intraperitoneal infusion of large quantities (10^7), ADSCs resulted in survival of 40 % of lethally irradiated mice [19], whose hematopoietic cells were of endogenous origin. In all reported experiments, ADSC did not differentiate per se into hematopoietic cells but, similarly to the physiological role of bone marrow MSCs, supported hematopoiesis, playing the role of hematopoietic microenvironment cells.

Mesenchymal stromal cells play an immunomodulatory role when infused into patients with graft-versus-host disease (GVHD) following bone marrow transplantation. It has been observed [87] that in vitro-expanded bone marrow MSCs are able to reduce GVHD symptoms and are efficient in treatment of steroid-resistant GVHD in bone marrow transplanted cancer patients. Comparison of BM-MSC and ADSC revealed similarity in the immunomodulatory properties of both cell types – ADSC did not provoke in vitro alloreactivity of incompatible lymphocytes, suppressed mixed lymphocyte reaction, and suppressed lymphocyte proliferative reaction to mitogens [132]. These findings opened the perspectives for ADSC clinical applications for treatment of patients with severe therapy-resistant GVHD [38, 180].

ADSC and Oncogenesis

There exists evidence on oncogenic potential of bone marrow-derived MSC. MSC may be involved in cancer induction or expansion in several ways – as normal cells supporting cancer growth by migrating towards tumors, modifying tumor environment (vasculogenesis), and immunosuppression or as cells undergoing spontaneous malignant transformation (reviewed in [114]). ADSCs, being a subpopulation belonging to the MSC family, do not differ significantly from BM-MSC in the probability of promotion or induction of carcinogenesis, although the experimental evidence, concerning ADSC role in oncogenesis, is much more scarce than their bone marrow-derived counterparts. Extensive study on the interrelation between ADSC and breast cancer cells [117] revealed that ADSCs are able to home to tumor site even when injected intravenously and incorporate into tumor vessels, where they differentiate into endothelial cells. Direct contact of ADSCs with tumor cells results in enhancement of secretion from ADSCs of stromal cell-derived factor 1 (SCF-1), which acts in a paracrine fashion on the cancer cells enhancing their motility, invasion, and metastases. It has been also documented that ADSCs, similarly to their interactions with breast cancer, were recruited towards cancer cells through SDF1/CXCR4 axis and supported cancer growth by increasing tumor vascularity when cocultured with prostate cancer cells in athymic mice [97].

Standard ex vivo expansion procedure, when ADSCs are cultured for 6–8 weeks, is "safe" and does not lead to the phase of cell transformation events. It has been documented, however, [143], that after in vitro expansion lasting 4–5 months, human or mouse ADSC spontaneously bypassed the senescence and crisis phase, showing altered phenotype and chromosome instability and losing contact inhibition capacity. At this stage, cells were able to induce cancer when injected into immunodeficient mice. The general conclusion from the observations on long-term expansion of ADSC is that the cells, expanded "traditionally" for the period of 6–8 weeks, may be considered as a valuable tool for tissue regeneration and engineering, but the prolonged in vitro culture may cause the risk of spontaneous

transformation and induction of cancerogenesis in transplant host [143]. Contrary to the observations of the immortalization of ADSC, after prolonged in vitro culture, the aberrant, tumorigenic cell line was isolated as early as from third-passage cells [121] – the result suggesting the need of rigorous testing of in vitro-expanded ADSCs prior to their clinical applications.

Clinical Applications of ADSC

Subcutaneous Tissue Formation

The adipose tissue is present physiologically in multiple locations in human body, being responsible for multiple functions (mechanical, endocrine, thermoinsulatory, and energy supplying). Typical surgical procedures (liposuction, lipotransfer) are performed for cosmetic rather than medical purposes – the exemption is the application of lipotransfer technique for treatment of breast cancer patients after mastectomy, where injection of the adipose tissue not only partially reconstructs the amputated breast but locally supports better healing and prevents formation of connective tissue scar between the skin and muscles. Enrichment of lipotransferred autologous adipose tissue with ADSC isolated from the same patient [105, 184] reduces the atrophy of implanted tissue and supports the formation of new adipocytes in the region of implantation. Immunosuppressive potential of implanted ADSC may also minimize the inflammatory reaction in the implantation area. There is some consideration [101] if the implantation of ADSCs may increase the risk of cancer recurrence; however, such speculations seem to be not substantiated by the observations. Similar technique of ADSC enrichment of implanted adipose tissue was used for corrective treatment after artificial breast implants removal caused by various complications (like capsular contracture), and the results were described as satisfactory [185]. As a support and a carrier for transplanted ADSCs, "injectable scaffolds" consisting of cell-binding polyglycolic acid (PGA) [14], poly (lactic-co-glycolic acid) or PLGA [127], hyaluronic acid [49], fibrin [149], matrigel [76], or alginate gel [182] are applied. The in vivo study has shown that ADSCs attached to micronized acellular dermal matrix (Alloderm) and cultured for 14 days in adipogenic differentiation media were able to differentiate into mature adipocytes when implanted subcutaneously into dorsal cranial region of nude mice [183]. For the applications, when the elastic, mechanically resistant, non-immunogenic, and slow degradable scaffold is needed, 3-D scaffolds of silk fibroin were developed [106]. Interesting, although not yet validated, is the exploitation of the ability of ADSC to produce a variety of growth factors, regulatory factors, and collagen for skin antiaging therapy [126].

Bone Formation

The bone formation phenomenon was observed prior to the experiments with ADSC differentiation, in patients with progressive osseous heteroplasia, which is characterized by spontaneous formation of calcified nodules in the adipose tissue [72, 154]. In vitro, both human and animal ADSCs may be stimulated to differentiate into osteogenic lineage [28, 51, 144, 151, 187, 188], producing cells of osteogenic phenotype characterized by the presence of bone markers: alkaline phosphatase, osteopontin, osteonectin, type I collagen, bone sialoprotein, osteocalcin, BMP-2, BMP-4, and BMP receptors I and II. In vivo ADSCs differentiate into the bone when implanted ectopically into rodents [55]: rat-isolated ADSCs, seeded in polyglycolic acid, form the bone when implanted subcutaneously [89]. Similarly, human ADSCs in HA-TCP scaffolds differentiate to osteocytes in immunodeficient mice [31, 33]. ADSCs, when seeded in apatite-coated PLGA scaffolds and surgically implanted, were able to repair surgically created critical-size calvarial defects in mice [20]. In contrary to these observations, poly-L-lactic scaffolds colonized with non-differentiated ADSCs were unable to repair experimental rat palatal bone defects, while similar implants containing osteogenically differentiated cells fully reconstructed the bone defects in vivo [17]. Basing on these in vivo experiments, ADSCs were collected from a 7-year-old girl with large, bilateral calvarial defect, combined with iliac crest bone fragments and fibrin glue on resorbable mesh, and autologously implanted, treatment resulting in marked ossification and regeneration of defect to near-complete continuity after 3 months following surgery [92].

Cardiac Repair and Angiogenesis

Morbidity and mortality, resulting from cardiovascular diseases (CVDs), account for approximately 30 % causes of deaths, constituting major medical, social, and economical problem. At the beginning, the rationale of stem cell therapy of cardiac infarct was to implant cells, which will be able to transdifferentiate into cardiomyocytes and regenerate the necrotic region of the cardiac muscle. The obvious candidates, according to the cell plasticity concept, were hematopoietic stem cells from the bone marrow or umbilical cord blood. The effects observed in animal experiments were the increase of muscle mass in regenerating heart muscle, improvement of cardiac hemodynamics, and, surprisingly, very low frequency of the presence of myocardial cells of donor origin. Detection in the adipose tissue of the MSC-type cells capable of myogenic differentiation resulted in in vivo experiments based on intracardiac transplantation of ADSC in models of coronary disease or myocardial infarction [170]. It has to be determined, if the beneficial effects of

treatment with ADSC results from differentiation of ADSC into myocardium or in paracrine mechanisms supporting endocrine repair [11, 13, 109, 119, 134, 156, 158, 169]. In 2004 the cardiomyogenic potential of ADSC has been documented [130, 175]; since then multiple studies have confirmed the phenomenon of direct formation of cardiac muscle by ADSCs [111, 173, 186]. It has been shown [100, 179] that the brown adipose is the best source of cells capable of cardiomyocyte differentiation. Treatment with ADSCs significantly improves functional parameters of regenerating heart, such as neovascularization [12, 13, 109, 130, 186], collateral perfusion [66, 67], and hemodynamic parameters (ventricular end-diastolic dimension, ejection fraction, cardiac output) [22, 107, 148, 169, 173]. ADSC transplantation into the heart does not increase arrhythmogenic tendency of the cardiac muscle [39, 73]. Some improvements may result from secretion humoral factors (angiogenic cytokines) by ADSC [134] or direct formation of endothelium and, in consequence, angiogenesis [12, 13].

The same mechanisms allow using ADSC for treatment of animal model of severe hind limb ischemia [119, 134]. Considering the importance of treatment of cardiac ischemia and infarct and the beneficial effects of ADSC on cardiac muscle regeneration, there is a real possibility of expanding the role of autologous ADSC in cardiac muscle regeneration and treatment of diseases with ischemic background.

Cartilage Repair

In general, the diseases originating from cartilage defect, resulting from injury, autoimmunity, or degenerative disease (osteoarthritis), have strong negative impact both at the patient's level and at the social and economical levels. There have been published several attempts of inducing of cartilage repair using autologous stem and progenitor cells. In young patients with isolated cartilage lesions, the use of culture-expanded autologous chondrocytes seems most promising. In elderly patients, suffering from the massive denudation of articular cartilage, the availability of autologous expanded chondrocytes is, however, reduced and insufficient for therapy, so there is demand for another autologous cell source. The candidate cells must be available in adult donor, and their collection must be safe and relatively uncomplicated; these cells must have the potential for differentiation into chondrogenic lineage both in vitro and in vivo. Such cells must be also available in patients with osteochondral defects, so the original disease must not influence the numbers and qualities of cells collected for treatment. The candidate cells, fulfilling the criteria, are ADSCs collected from patient's adipose tissue [120]. Comparison of the chondrogenic potential of BM-MSCs and ADSCs isolated from various locations confirmed that all these cells are able to differentiate into chondrocytes in vitro, but their differentiation potential depends on the source [113, 171, 177]. Some authors claim the superiority of ADSC over BM-MSC [24], but prevailing data suggest that BM-MSCs have superior chondrogenic potential when compared with ADSC [16, 59, 61, 85, 99, 126, 135, 150, 175]. The exception is intrapatellar fat pad, which is a much better cell source than subcutaneous adipose tissue [34, 113]. The future of ADSC as a candidate for cellular repair of cartilage is unclear; some findings suggest that improvement in in vitro/in vivo stimulation of chondrogenic differentiation of ADSC may increase their importance as candidates for clinical applications [36, 78].

Central Nervous System Repair and Regeneration

Limited natural capacity of self-renewal of neural system, combined with high frequency of accidents and diseases resulting in neural system dysfunction, emphasizes the importance of development of the new methods for stem cell application in neurological disorders. ADSC is capable of differentiating into neuropoietic lineage as well as regulating the neural repair and restoration of local circulation in central nervous system. Several authors documented that ADSCs are able to differentiate in vitro into neural cells [32, 60, 64, 71, 99, 181, 187], interact with neural cells on paracrine level [68], or produce Schwann-type cells [178]. There exists no evidence that adipose-derived cells, differentiating into neural cells, derive from the neural crest lineage [176].

There is also, unfortunately, no evidence that so-called neural cells observed in vitro are indeed mature functional neural cells – most authors recognize cells of "neural morphology" after identification selected markers present on early neural cells, like microtubule-associated protein, neuronal nuclear antigen, β-tubulin III [60], neurofilament 1 (NF1), nestin, neuron-specific enolase (NSE) [181], or neurosphere formation [71]. The other data derive from in vivo animal experiments, where ADSCs are implanted into regions of injury of neural system. The intensively researched problem is the possibility of amelioration of brain stroke effects by local application of ADSCs. Possible therapeutic effects may result from direct replacement of ischemia-eliminated brain cells, regulation of neural cell regeneration in paracrine manner, or reconstitution of local microcirculation by angiogenesis mediated or formed by ADSCs. When human ADSCs were injected into lateral ventricle of healthy rats, they were able to migrate to multiple areas including the contralateral cortex and could be locally identified up to 30 days following implantation. Similar implantation of ADSCs into the brain 1 day after MCA occlusion (the experimental model of stroke) resulted in cell migration into the ischemic area and localization at the border between the

intact and injured brain tissue [69]. Injection of ADSCs did not change the infarct size but significantly improved the recovery in motor and somatosensory behavior aspects, suggesting that at least there exists the mechanism of local trophic support from ADSCs [69]. In other experiment, ADSCs not only improved neurological functions of infarcted rats but also markedly attenuated brain infarct size [93]. Immunomodulatory effect of ADSCs was exploited in the compassionate study on three patients with multiple sclerosis (the disease caused by the autoimmunity mechanisms). Multiple intravenous or intrathecal infusions of autologous ADSCs, combined with allogeneic CD34+ and MSCs, resulted in marked improvement in disease status of all patients, although the observation is very preliminary and statistically not significant [136]. ADSCs were tested for their ability to accelerate the spinal cord fusion (treatment for lumbar compression fractures) in rat model. Local application of scaffolds colonized by autologous or allogeneic ADSCs into the injury site reduced inflammatory cell infiltration and accelerated posterior spinal fusion process [102]. ADSC may act through the different mechanisms, like local regulation by paracrine manner [80, 94], participation in local angiogenesis, or immunomodulatory effects; the phenomenon of direct ADSCs differentiation to neural cells cannot also be excluded. Nevertheless of the mechanisms of ADSC actions, the preliminary in vivo results suggest the usefulness of both autologous and allogeneic ADSCs in treatment of central nervous system diseases and injuries.

Other Therapeutical Applications of ADSC

Experimental and clinical applications of ADSC resemble those exploited earlier with the use of BM-MSC. ADSCs seem to be the cell population, which may be widely used for gene therapy. In autologous transplantation model, gene-transfected ADSC guarantees relatively high safety, and their reported ability to maintain stable telomere length [37, 62] and long proliferation time in in vitro systems guarantees long-term delivery of gene product. Parallel experiments with infection of both MSCs and ADSCs with E1A-deleted type 5 adenovirus constructs containing the BMP-2 (bone morphogenic protein-2) gene or the bacterial beta-galactosidase (lacZ) gene resulted in 55 % transduction efficiency for ADSC in comparison with 35 % efficiency for BM-MSC [31], which resulted in threefold higher expression of BMP2 protein by ADSCs than by BM-MSCs. Experiments on stability of lentiviral vector-transduced cells revealed the presence of transduced cells in culture over 100 days at transduction efficiency of 98 % [116].

There are rather scarce data on the differentiation of ADSC into several cells and tissues, like skeletal and smooth muscle, hepatocyte-like cells, or pancreas endocrine cells. When transplanted into mdx mice (murine model of Duchenne muscular dystrophy), ADSC helped to regenerate the muscle and induced expression of dystrophin [140], although their role in muscle repair is still rather unclear. In vitro, ADSC differentiates into cells of myogenic phenotype, resembling the characteristics of skeletal muscle, the process observed when ADSCs are directly contacting primary muscle cells [27, 90]. Observations of in vitro capacity of ADSC to differentiate into smooth muscle cells [1, 42, 65, 91] were clinically exploited in attempted urinary incontinence treatment and bladder reconstitution [63], with results not substantially different to those obtained when used BM-MSC. There exist a scarce data on hepatopoietic differentiation potential of ADSC. In vitro, ADSC cultures in the presence of HGF, OSM, and DMSO form cells of hepatocyte-like phenotype, expressing albumin and α-fetoprotein, capable to take up low-density lipoprotein and to produce urea [152]. Following these observations, ADSCs, intravenously injected into mice, were detected in injured liver, and their integration into the liver was augmented by partial hepatectomy [79]. Preliminary data confirm the ability of ADSC to differentiate into cells of pancreatic endocrine phenotype partially maintaining pancreatic endocrine cell functions. Following the stimulation with activin-A, extendin-4, HGF, and pentagastrin, cells expressed pancreatic endocrine transcription factor Isl-1; developmental transcription factors Pax-6, Ipf-1, Ngn-3; and expressed pancreatic hormones insulin, glucagon, and somatostatin [165]. The data are too preliminary and need to be extended and confirmed, but even now they give some hope for the use of ADSC for cell-based therapy for type 1 diabetes mellitus. There exist also several reports of preliminary results after ADSC treatment of such varying diseases as Crohn's disease (occlusion of rectovaginal fistula) [43, 46], wound healing [95], erectile dysfunction [96], tissue engineering (bypass graft construction [29], production of skin substitutes [166]), or feeder layer for induced pluripotent stem cells (iPSCs) [159]. All these reported ADSC therapeutic applications have one common characteristic – they need much more research for data collection and validation before their potential usefulness may be evaluated.

Conclusions

The phenotype, functional characteristics, and differentiation potential of ADSC are enough similar to their BM-MSC counterparts to conclude that the differences between ADSC and MSC are not important in the aspects of their applications for cellular therapy. The advantages of ADSC over MSC lay in the possibility of collection of much larger numbers of cells without endangering patient's health. The other advantage is higher purity of isolated ADSC population – bone marrow aspirates consist of much higher numbers of hematopoietic cells than MSCs, and the most efficient method of primitive

BM-MSC isolation (bone grinding) is impossible to use when considering collection from living donor. The most promising clinical applications of ADSC, according to presently available data, are treatment of cardiac ischemia and myocardial infarction, central nervous system repair following accidents or stroke, treatment of immunology-related diseases (graft-versus-host disease, multiple sclerosis), and techniques of bone and joints replacement and repair using scaffolds seeded with ADSCs and their more differentiated progeny. In the esthetic medicine/plastic surgery, ADSCs are the "cells of choice" for corrections of irregularities in subcutaneous tissue distribution.

In general, availability of large numbers of autologous cells in any patient's age, safe protocols of cell collection, in vitro expansion and differentiation, multilineage differentiation potential, and in vivo immunomodulatory capacity make ADSC the almost ideal cell type for cellular therapy, gene therapy, and regenerative medicine.

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