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Adipose tissue stem cells: the great WAT hope

William P Cawthorn^a, Erica L Scheller^a, and Ormond A MacDougald^{a,*}

^aDepartment of Molecular & Integrative Physiology University of Michigan Ann Arbor, MI 48109 United States of America

Abstract

The past decade has witnessed an explosion in research into adipose tissue stem cells (ASCs), facilitated by their ease of isolation from white adipose tissue (WAT) and fueled by their therapeutic potential. Recent developments have extended ASC multipotency to include endodermal and ectodermal cell types, along with generation of induced pluripotent stem cells. This expanding multipotency has been paralleled by burgeoning translational applications, ranging from tissue engineering to anti-cancer therapy, that are currently subject to clinical trials. However, this promise is tempered by potential pitfalls, such as tumorigenicity, and is further undermined by lingering uncertainties regarding the very identity of ASCs. Confronting these issues will be essential if we are to bypass the pitfalls and develop the promises of ASCs.

1 Introduction

The ability to isolate stem cells from adult tissues was first inferred from studies in the late 1960s, when osteogenic stromal cells were identified in bone marrow (BM) [1]. Subsequent studies demonstrated that these adherent cells possess at least some self-renewal capacity and are capable of forming mesenchymal cells, such as adipocytes, osteoblasts, chondrocytes and myocytes [2]. Based on these properties, these cells are known as mesenchymal stem cells (MSCs; see *Glossary*). Such BM MSCs have since become a benchmark for adult stem cell research. Indeed, based largely on BM MSCs, the International Society for Cellular Therapy (ISCT) has established minimal criteria for defining MSCs [3]: firstly, MSCs must adhere to plastic under standard culture conditions; secondly, MSCs must express the surface molecules CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR; and thirdly, MSCs must be capable of osteoblastogenesis, adipogenesis and chondrogenesis *in vitro*. BM MSCs are a promising tool for both basic research and regenerative medicine; however, their isolation from BM is an invasive, painful procedure that often results in a relatively low yield. More recent studies have identified MSCs with similar properties in almost all mammalian tissues [4], suggesting that MSCs with similar clinical potential might be more readily isolated elsewhere. Arguably the most promising of these MSCs are adipose tissue-derived stem cells (ASCs).

Human ASCs were discovered at the turn of the millennium, identified in the stromal vascular fraction (SVF) of white adipose tissue (WAT) as cells capable of *in vitro* differentiation into adipocytes, osteoblasts, chondrocytes and myocytes [5–7] (see *Glossary*

*Corresponding author Ormond A MacDougald University of Michigan Brehm Center, Room 6313 1000 Wall St Ann Arbor MI 48105 Tel: 734-647-4880 Fax: 734-232-8175 macdouga@umich.edu.

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for further definitions). This multipotency was soon confirmed in murine ASCs and extended to include differentiation and tissue repair *in vivo*, often after seeding ASCs onto polymeric 3D scaffolds or exogenous gene expression. Several earlier studies also reported neuronal differentiation of ASCs, largely based on morphology or expression of typical neural markers (reviewed in [8]). In addition to this multipotency, ASCs are relatively easy to isolate from readily available WAT. The combination of these beneficial properties has fueled an explosion in ASC research over the past decade, with particular focus on tissue engineering and regenerative medicine. Important aspects of ASC biology, including their characterization, multipotency, physiological relevance and clinical applications, have recently been reviewed extensively elsewhere [8–11]. Thus, in the present review we discuss the potential promises and pitfalls of ASCs, focusing on recent developments and controversies in this increasingly important field of research.

2 New promises of ASCs

2.1 Expanding cell fates

Early reports of ASC differentiation toward mesodermal and neuronal cell fates have more recently been extended to include other cells of non-mesodermal origin. Brzoska *et al* were among the first to report *in vitro* differentiation of ASCs into epithelial-like cells [12]. More recently, ASCs were shown to undergo *in vitro* differentiation into cells resembling corneal keratocytes [13], and into retinal pigment epithelial-like cells that are able to form pigmented granula [14] (Figure 1). The latter may lead to novel therapeutic applications, because ASCs were subsequently shown to engraft into retinas of rats with diabetic retinopathy [15]. Moreover, ASCs were recently found to form dental bud-like structures under prolonged 3D culture *in vitro* [16], revealing another ectodermal ASC fate. However, both the 2005 Brzoska study [12] and the latter studies [15, 16] used 'ASCs' that were CD34–; given that ASCs are likely CD34+ [8], it remains unclear whether genuine ASCs can form epithelial cells. Nevertheless, human ASCs were recently shown to differentiate toward renal tubular epithelium, both *in vitro* and after injection into mice with acute kidney injury [17]; rat ASCs can also engraft into glomeruli and promote recovery from acute kidney injury [18]. Therefore, ASCs may be able to form epithelial cells in injured tissues *in vivo* (Figure 1).

Differentiation into endodermal cell types has also been reported. For example, ASCs can undergo hepatic differentiation *in vitro*, as assessed by gene expression, morphology and functional assays [19–21]. ASC-derived hepatocytes can also engraft into livers and reconstitute some hepatocyte functions in immunodeficient mice, often after induction of liver injury [19, 21–23]. Thus, human ASCs have been proposed as a potential therapy for liver injury [23]. Similarly, ASCs can form islet-like cells, either in response to specific culture conditions [24, 25] or through exogenous expression of Pdx1, a pancreatic transcription factor [26, 27]. These ASC-derived islet-like cells express pancreatic genes, such as insulin and glucagon; display β -cell functions, such as glucose-dependent insulin release; and can restore pancreatic function after transplantation into diabetic mice or rats [24–27] (Figure 1). However, ASCs can restore normoglycemia in diabetic rats even without prior *in vitro* differentiation into islet-like cells [15].

2.2 ASCs as pluripotent cells

This seemingly ever-expanding list of possible cell fates begs the question: might ASCs be pluripotent? Unlike embryonic stem cells (ESCs; see *Glossary*), it is clear that ASCs are not genuinely pluripotent in the context of normal development. However, recent studies demonstrate that ASC pluripotency can be artificially induced in experimental contexts. In 2009, Sun and colleagues generated induced pluripotent stem (iPS) cells (see *Glossary*) by

viral transduction of human ASCs under feeder-free conditions, finding this to be faster and more efficient than induction of pluripotency in human fibroblasts [28]. Subsequent studies have confirmed and extended these observations with both human and mouse ASCs [29–31] (Figure 1). The relatively high efficiency of ASC-derived iPS cell formation may partly result from ASCs' high expression of pluripotency factors, such as basic-FGF, TGF β , fibronectin and vitronectin [29]. More recently, mapping of the DNA methylome (see *Glossary*) revealed that DNA methylation patterns are similar among iPS cells derived from ASCs or other somatic cell types [32], suggesting similar degrees of epigenetic reprogramming among these cell types. Considering their relative ease of isolation, ASCs may therefore become the preferred cell type for generating iPS cells. A potential drawback to these studies is that they ectopically expressed *OCT4*, *SOX2* and *KLF4*, with or without *c-MYC*, through viral transduction of ASCs. Though effective, viral transduction may elicit detrimental effects as a result of insertional mutagenesis. However, recent studies have bypassed this issue by generating iPS cells through non-viral methods, such as use of chemical agents that promote pluripotency [33]. The impact of such chemical agents on induction of ASC pluripotency has yet to be reported; however, a recent study has bypassed the need for viral induction by using a single minicircle DNA vector, encoding *OCT4*, *SOX2*, *LIN28* and *NANOG*, to generate iPS cells from human ASCs [34]. Although the efficiency of this approach is lower than that of viral transduction, the relative safety benefits of minicircle-based transduction, or indeed of other non-viral approaches, may facilitate potential clinical applications of ASC-derived iPS cells.

2.3 Beyond cells and tissues: ASCs as a tool for cloning

An obvious implication of iPS cells is the potential to bypass ethical and political issues associated with ESCs. Although ASCs are not yet a substitute for ESCs, the gap between these cell types may have further narrowed in light of a very recent study in which cloned beagles were produced through ASC nuclear transfer [35] (Figure 1). The utility of this approach had been previously predicted [36] and awaits development through future studies. That ASC nuclei can generate a fully cloned organism further suggests that ASC pluripotency may extend beyond the context of iPS cells. As such, further investigation of ASC nuclear transfer is clearly warranted.

2.4 Paracrine effects on host tissues

While ASC differentiation potential seemingly knows no boundaries, the ability of ASCs to promote tissue regeneration and repair may depend more on their ability to directly impact host tissues through paracrine mechanisms. Indeed, ASCs secrete myriad proteins and other bioactive factors that can modulate host tissue biology [37] (Figure 1). For example, murine ASCs secrete brain-derived neurotrophic factor (BDNF) to induce nerve repair and axon growth [38]. This may explain how these cells can enhance regeneration of host neural tissues [39] despite their reported inability to form functionally mature neurons *in vivo* [40]. Studies investigating paracrine effects of ASCs have largely focused on neovascularization and immunosuppression.

Neovascularization—A general mechanism through which ASCs promote host tissue repair is by stimulating neovascularization. For example, ASC secretion of vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) is implicated in their ability to repair scarred myocardium after myocardial infarction, despite minimal ASC-derived cardiomyocyte formation [41, 42] (Figure 1). Human ASCs also promote revascularization of ischemic mouse hindlimbs, at least in part through HGF secretion [43, 44]. Additionally, these pro-angiogenic effects likely contribute to the ability of ASCs to facilitate recovery from cerebral vascular injuries [45], which, along with their neuroprotective effects, underscores the potential of ASCs for treatment of stroke. Similar

paracrine effects on vascularization may account for ASC utility in recently reported novel applications, such as treatment of erectile dysfunction [46, 47] (Figure 1).

Immunosuppression—ASCs also promote tissue repair and regeneration through immunosuppressive effects, exemplified by ASC-mediated suppression of proliferation and activation of lymphocytes and peripheral blood mononuclear cells [48–50]. Mechanistically, ASCs may suppress immune responses through both cell-contact-dependent and –independent mechanisms. For example, several studies find that ASCs fail to suppress lymphocyte or peripheral blood mononuclear cell proliferation when these cells are separated from ASCs by transwells [48, 51], suggesting that cell-cell contact is required. In contrast, other reports show that transwell-based separation does not prevent ASC-induced immunosuppression, and that ASC-conditioned medium attenuates lymphocyte proliferation [52, 53]. This suggests that ASCs secrete immunosuppressive factors. Proposed candidates include prostaglandin E2 [52, 54], leukemia inhibitor factor [55] and kynurenine, a product of tryptophan metabolism [53] (Figure 1). Indeed, during co-culture with lymphocytes, ASCs increase their secretion of each of these molecules, and blocking their production or activity significantly blunts suppression of lymphocyte proliferation by ASCs [52–55]. Based on these immunosuppressive properties and lack of adverse ASC immunogenicity *in vivo* [56], there is immense interest in the potential of ASCs for treatment of inflammatory diseases (Figure 1). These include graft-versus-host disease [10, 49], Crohn's disease [57], sepsis [58], transplant tolerance [59, 60], encephalomyelitis [61] and rheumatoid arthritis [62, 63]. Indeed, numerous clinical trials are currently further investigating the therapeutic efficacy of ASCs [9–11]. Nevertheless, the conflicting findings regarding the requirement of cell-cell contact highlight the need for further investigation into mechanisms underlying ASC-mediated immunosuppression.

2.5 ASCs and anti-cancer therapy

The studies discussed above clearly demonstrate both the utility of ectopic gene expression to modulate ASC fate, and the ability of ASCs to directly impact host tissue biology. These characteristics are now seeing novel applications of ASCs as tools for gene therapy. Some of the earliest studies of ASCs alluded to this possibility. For example, exogenous expression of BDNF improved the ability of human ASCs to promote motor recovery after cerebral ischemia [64], and adenoviral BMP2 expression enhanced ASC-mediated bone regeneration [65]. More recent studies have investigated ASCs as gene delivery vehicles. For example, VEGF-transfected ASCs have been used to improve survival and quality of human autologous fat transplants [66], and ASCs virally transduced for expression of human alpha-1 antitrypsin (hAAT) can engraft into host livers and restore serum AAT levels in AAT-deficient mice [67] (Figure 1). Because these applications of ASCs have recently been reviewed elsewhere [10], we will avoid extensive discussion herein. However, one related area of current interest clearly warrants further consideration: the use of ASCs in anti-cancer therapy.

ASCs home toward tumor cells *in vitro* and engraft into tumor sites after injection *in vivo* [68, 69], suggesting that ASCs might prove useful for directing anti-cancer transgenes to tumorigenic regions. In 2007, Kucerova *et al* showed that ASCs can indeed facilitate anti-cancer therapy through expression of prodrug converting enzymes [68]. Thus, ASCs were retrovirally transduced for expression of a cytosine deaminase::uracil phosphoribosyltransferase fusion gene (CD::UP), which activates the prodrug 5-fluorocytosine. Thereby modified, ASCs delivered the transgene to tumors *in vivo* and exerted strong anti-tumor effects in response to 5-fluorocytosine [68]. Similar bystander killing approaches have since been used, including use of thymidine kinase-expressing ASCs to facilitate ganciclovir-mediated tumor death [70]. Related studies have generated

anti-tumorigenic ASCs that express oncolytic viruses [71], proapoptotic ligands [72–74] or anti-angiogenic effectors [73]. Importantly, these effects appear to target tumors specifically, with no adverse impact on either ASCs or normal host tissues. Thus, genetically modified ASCs are promising as a future anti-cancer therapy (Figure 1).

3 Perils and pitfalls of ASCs

Although ASC multilineage potential and ability to modulate host tissue biology clearly hold immense therapeutic promise, these characteristics may be a double-edged sword. For example, ASCs are reported to primarily undergo osteogenic and chondrogenic differentiation *in vivo* [75], which might result in formation of undesirable cell types in target tissues (Figure 1). Indeed, one study found that use of ASCs to facilitate autologous lipoinjection was associated with cyst formation or tissue calcification in 4 of 70 patients [76]. This is reminiscent of intramyocardial calcification following transplantation of non-selected BM cells into infarcted myocardium [77]. Treatment of graft-versus-host disease with BM MSCs has also been implicated with increased risk of relapse [78], suggesting that ASC-mediated immunosuppression might pose similar problems. However, to our knowledge, no additional studies have reported aberrant differentiation or increased relapse associated with ASC treatment. In contrast, several studies suggest another possible peril of ASCs: their ability to facilitate tumorigenesis.

3.1 ASCs and tumorigenesis: friend or foe?

Numerous recent studies find that ASCs enhance tumorigenesis when co-transplanted with tumor cell lines into nude mice (Figure 1). In 2008, Yu *et al* found that ASCs promote tumor growth by enhancing tumor cell proliferation and suppressing apoptosis [79]. Similarly, following transplant of 4T1 mammospheres into mammary fat pads, co-injection of mouse ASCs promoted tumor growth, invasiveness and vascularization, likely through ASC-derived stromal cell-derived factor-1 (SDF-1) signaling through CXCR4 on the tumor cells [69]. Such SDF-1/CXCR4 signaling has also been implicated in the ability of human ASCs to promote tumorigenesis of melanoma cell lines, with increased VEGF secretion also making possible contributions [80]. Two recent studies also found that human ASCs co-injected with prostate cancer cells can engraft into tumors and promote tumorigenesis [81, 82]. Moreover, human ASCs can enhance invasiveness of breast cancer tumor cells *in vitro*, an effect mediated at least in part through ASC-derived CCL5 [83, 84]. Similarly, ASCs were recently found to augment growth of active tumor cells from metastatic breast cancer clinical isolates, although they did not affect growth of resting tumor cells from these samples [85].

Related studies indicate that tumor cells also modulate ASCs. Two very recent papers suggest that exosomes (see *Glossary*) secreted from ovarian or breast cancer cells can induce ASCs to increase expression of SDF-1, CCL5, VEGF and TGF β [86, 87]. The ability of breast cancer cells to stimulate SDF-1 secretion from ASCs is also reported in an independent study [69]. That tumor cells can modulate ASC function is consistent with the observation that MSCs associated with human ovarian tumors differ from MSCs of healthy individuals [88]. For example, carcinoma-associated MSCs have increased BMP expression, which is implicated in their ability to promote tumorigenesis *in vitro* and *in vivo* [88]. Whether this is also true for transplanted ASCs, remains to be established.

While these results are undoubtedly concerning, one drawback to many of the above studies is that they are based on *in vitro* experiments or on implantation of cultured tumor cell lines into host organisms; far less evidence exists for ASC-stimulated tumorigenesis beyond this context. For example, even 17 months after injection into aged, tumor-prone SCID mice, human ASCs survive and differentiate into fibroblasts or adipocytes, but remain at the site of

injection and neither fuse with host tissues nor form teratomas [89]. Similarly, ASCs delivered to a skin wound did not affect growth of distal tumor implants [90], and treatment of acute liver injury with rat ASCs was associated with no abnormal tissue growth in kidneys or other organs, even three months after ASC administration [18]. Equally, human ASCs caused no adverse side effects up to 13 weeks after injection into SCID mice, and did not form tumors up to 26 weeks post-injection into Balb/c-nude mice [91]. Importantly, this latter study further tested the safety of ASC transplantation in human patients with spinal cord injuries and found that, up to three months post-transplantation, no adverse effects were associated with ASC treatment [91]. These observations suggest that ASCs do not impact tumor growth if injected beyond the tumor environment, indicating that they may not cause adverse side effects in therapeutic applications. Ongoing clinical trials should shed further light on the safety and efficacy of ASCs [9].

3.2 What you don't know can hurt you

While this expanding knowledge reveals potential pitfalls of ASCs, it is our lingering ignorance of these cells that may pose the greatest roadblocks. A fundamental issue is the lack of standard criteria for definition, isolation and culture of ASCs: whereas many studies define ASCs as a distinct subpopulation of adipose tissue SVF, other studies describe whole, unsorted SVF as 'ASCs'; in some studies, it is unclear whether the ASCs studied are whole SVF or a subpopulation. This is important, because WAT SVF is a heterogeneous, functionally diverse cell population (see *Glossary* and [92]). Moreover, ASCs have been analyzed as freshly isolated SVF, or after different durations in culture, a distinction that can greatly impact ASC characteristics [93, 94]. ASC biology may also vary depending on their WAT depot of origin or other donor characteristics, such as age (*Box 1*). Further inconsistencies exist for ASC surface marker expression, which differs to MSC surface markers suggested by the ISCT criteria [3, 8].

Two recent studies have analyzed ASC characteristics on a global level. In 2010, Rosen and colleagues globally mapped chromatin state, transcription factor localization (*cistrome*; see *Glossary*) and gene expression profiles (*transcriptome*) of ASCs and mouse 3T3-L1 preadipocytes, before, during and after adipogenesis [95]. In a separate study, Lister *et al* mapped the DNA methylome of ESCs, ASCs, ASC-derived adipocytes, ASC-derived iPS cells, and iPS cells derived from other cell types [32]. In the Rosen study, the global analyses were focused on adipogenesis and comparisons between ASCs and 3T3-L1 cells, rather than on broader characterization of ASCs in comparison to WAT SVF. Equally, the work by Lister *et al* provides insight into the extent of epigenomic reprogramming that occurs during induction of pluripotency, rather than focusing on ASC characterization *per se*. Future studies should globally characterize the transcriptome, proteome, cistrome and methylome of different subpopulations of WAT SVF, comparing ASCs (e.g. as defined in [8]) with non-ASC populations (e.g. CD34⁺ cells). These analyses could be extended to compare cells from SVF of different WAT depots or from distinct donor groups (e.g. different ages). Such an approach would comprehensively address much of the uncertainty regarding ASC identity, an issue that must be resolved before the clinical potential of ASCs can be fully realized.

5 Future directions

As ASCs progress from the bench to the clinic, focus is shifting on how to develop large-scale manufacturing processes with relevant quality controls for production of ASCs in accordance with Good Manufacturing Practices [10, 11]. Such steps are necessary to ensure that ASCs consistently exhibit the safety, reproducibility and quality required for widespread clinical use. For example, ASCs are frequently cultured using fetal bovine serum (FBS), which may render them unsuitable for downstream therapy in humans owing to the

potential risks associated with exposure to xenogenic products [10, 11]. Indeed, clinical applications may depend on the ability to culture ASCs in autologous human serum or under serum-free conditions, which may alter ASC properties in comparison to culture with FBS. Therefore, future studies must investigate the functionality of ASCs cultured under conditions suitable for their clinical use in humans.

Although ASCs clearly hold immense therapeutic promise, the developmental relevance of these cells remains poorly established. The existence of multipotent cells in WAT is supported by progressive osseous heteroplasia, a disease in which ectopic bone forms within adipose tissue [105]. However, whether multipotent ASCs play a role in normal development of WAT or other tissues remains unknown. Indeed, while self-renewal and multipotency are hallmarks of stem cells, there is little evidence that ASCs display these properties *in vivo*; hence, whether ASCs are genuine stem cells remains questionable. Nevertheless, knowledge gleaned from ASC research provides important insights into the process of preadipocyte commitment, as we have recently argued elsewhere [8]. Thus, further consideration of ASCs from a physiological perspective could reveal additional insights into the biology of WAT.

7 Concluding remarks

Over the past decade, the groundswell of ASC research has pushed these cells to the forefront of regenerative medicine. The original identification of ASCs as mesenchymal stem cells is being challenged by their ever-expanding differentiation potential, with ASC pluripotency moving closer to reality. Although this may reveal new therapeutic applications, ASC clinical potential likely owes more to their ability to modulate host tissue biology than to their differentiation capacity. An emerging development of this concept is the use of genetic manipulation to enhance the impact of ASCs on host tissues, with targeted anti-cancer therapies showing particular promise. But while paracrine effects may underlie ASC-mediated tissue regeneration and repair, they also carry potential risks, such as stimulation of tumor formation. However, ASC-mediated tumorigenesis and immunosuppression are subject to conflicting reports, which highlights our incomplete understanding of the mechanisms through which ASCs modulate host tissue biology. Indeed, greater understanding of ASC-host interactions could be exploited to improve the therapeutic efficacy of ASCs, and may also reveal new therapeutic applications of these cells. Several clinical trials are currently investigating the efficacy and safety of ASCs in treating various diseases, which should reveal the extent to which the promises or pitfalls of ASCs extend from the bench to the clinic. Finally, a major hurdle to both basic and clinical ASC research remains the lack of standard criteria regarding isolation, culture and characterization of ASCs. Future efforts must therefore address these issues if we are to fully capitalize on the potential of this promising cell type.

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Glossary

Adipose tissue stem cells (ASCs)

Multipotent cells that can be isolated from adipose tissue SVF. *In vitro*, ASCs are capable of differentiation into cells of mesodermal, endodermal or ectodermal origin; however, whether WAT contains

	cells that are genuinely multipotent <i>in vivo</i> remains to be firmly established.
Cistrome	A profile of <i>cis</i> -acting targets (i.e. DNA-binding sites) of <i>trans</i> -acting factors (i.e. transcription factors) across the whole genome of an organism or cell type.
Embryonic stem cells (ESCs)	pluripotent stem cells, which can produce all cell types emerging from the three embryonic germ layers. ESCs are isolated from the inner cell mass of blastocysts of an early-stage embryo.
Exosomes	Small (30–100 nm) vesicles secreted by numerous mammalian cell types into the extracellular environment. Exosomes may contain both protein and RNA molecules and exert effects including cell-cell communication, cell proliferation and immune system modulation.
Induced pluripotent stem (iPS) cells	pluripotent stem cells that have been derived from non-pluripotent cells, such as adult somatic cells, through induction by exogenous gene expression and/or treatment with chemical agents.
Mesenchymal stem cells (MSCs)	Multipotent stem cells that are capable of forming adipocytes (fat cells), osteoblasts (bone-forming cells), chondrocytes (cartilage cells) and myocytes (muscle cells) and which exist in almost all post-natal tissues and organs.
Methylome	A profile of DNA methylation across the whole genome of an organism or cell type.
Prodrug	A pharmacological agent delivered in an inactive form that is metabolized <i>in vivo</i> into the active drug.
Stromal-vascular fraction (SVF)	The heterogeneous, non-adipocyte cell population of adipose tissue, which includes committed preadipocytes, vascular and immune cells and ASCs.
White adipose tissue (WAT)	One of two broad classes of adipose tissue found in mammals, the other type being brown adipose tissue. WAT contains white adipocytes, which specialize in storage of energy as triacylglycerol and in secretion of numerous endocrine factors that impact many aspects of metabolism. Thus, WAT plays a central role in the regulation of energy homeostasis.

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Box 1 – WAT depot and other donor characteristics impact ASC biology

In humans, WAT is dispersed throughout the body, with the largest depots found intra-abdominally (visceral WAT, or vWAT) and subcutaneously (scWAT, around the buttocks, thighs and abdomen). Other depots are located in the face and extremities, the intra-orbital space, and in bone marrow. These different WAT depots have distinct biological properties, which extend to depot-specific differences in ASCs. Microarray analyses have revealed distinct global gene expression patterns between SVF of scWAT and vWAT of humans [96]. Unsurprisingly, functional distinctions also exist between SVF of different depots. For example, compared to scWAT SVF, vWAT SVF is more osteogenic, less proliferative and has lower capacity for white and brown adipogenesis [97–100]. Differences in ASC neovascularization potential may also explain why scWAT is more angiogenic than vWAT [101]. These depot-specific differences in SVF also reflect differences in mature adipocytes from each depot, and are maintained over 40 population doublings in immortalized SVF cells [96, 99]. These observations suggest that depot-specific differences are stably pre-programmed, perhaps through epigenetic mechanisms. Other factors, such as age and body mass index (BMI), may also impact ASC number, viability and function, although findings have been inconsistent [97, 102–104]. Nevertheless, these studies underscore that WAT depot and other donor characteristics can modulate ASC biology, with obvious ramifications on ASC therapeutic potential.

Figure 1. The promises and pitfalls of adipose tissue stem cells

Benefits of ASCs are shown in green boxes and potential pitfalls are highlighted in red boxes. ASC multipotency has recently been extended to include cells of endodermal and ectodermal origin, including renal tubular epithelial cells, retinal pigment epithelial cells, hepatocytes and islet-like cells. The ability to differentiate into these cell types is revealing new potential clinical applications of ASCs, as indicated. Differentiation into islet-like cells has been achieved through specific culturing conditions, or through transduction for expression of Pdx1. Transduction of ASCs for expression of reprogramming factors, such as Oct4, Sox2, Klf4, c-Myc, Lin28 and Nanog, allows generation of iPS cells from ASCs; this may lead to additional clinical applications. However, ASC multipotency carries the risk of aberrant differentiation, resulting in calcification of soft tissues, or cyst formation. Exogenous expression of other genes, such as VEGF, BDNF, BMP2 or hAAT, can also enhance the therapeutic potential of ASCs. In addition, ASCs are being engineered to express anti-cancer genes, such as prodrug converting enzymes, oncolytic viruses, apoptotic ligands and anti-angiogenic effectors. In addition to multipotency and ease of genetic manipulation, ASCs secrete numerous factors that may exert beneficial or deleterious effects. Thus, ASCs have been reported to stimulate tumorigenesis, at least in part through secretion of SDF-1, CCL5, TGF β and, possibly, BMPs. In contrast, secretion of BDNF is implicated in ASC-mediated nerve repair; HGF and VEGF exert pro-angiogenic effects; and LIF, Kyn and PGE₂ mediate immunosuppression by ASCs, although some studies suggest that cell-cell contact is required for ASCs to suppress lymphocyte or peripheral blood mononuclear cell activation. Neovascularization may also underlie the tumorigenic effects of ASCs. In any case, ASC-mediated immunosuppression, neovascularization and, to a lesser extent, nerve repair, are strongly implicated in many of the reported clinical applications of ASCs.

Pdx1, pancreatic and duodenal homeobox 1; Sox2, SRY-box-containing gene 2; Oct4, octamer-binding transcription factor 4; Klf4, Krüppel-like factor 4; c-Myc, myelocytomatosis oncogene; Lin28, Lin-28 homolog A; Nanog, Nanog homeobox; VEGF, vascular endothelial growth factor; BDNF, brain-derived neurotrophic factor; BMP, bone morphogenetic protein; hAAT, human alpha-1 antitrypsin; CD::UP, cytosine deaminase::uracil phosphoribosyltransferase; HSV-TK, herpes simplex virus thymidine kinase; TRAIL, TNF-related apoptosis-inducing ligand; MDA-7, melanoma differentiation-

associated gene 7; PEDF, pigment epithelium-derived factor; LIF, leukemia inhibitory factor; Kyn, kynurenine; PGE2, prostaglandin E2; HGF, hepatocyte growth factor; SDF-1, stromal cell-derived factor-1; CCL5, chemokine (C-C motif) ligand 5; TGF β , transforming growth factor- β .