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Cells in focus

Adipose-derived stem cells: Fatty potentials for therapy

Wee Kiat Ong[a], Shigeki Sugii[a,b,*]

[a] Fat Metabolism and Stem Cell Group, Laboratory of Metabolic Medicine, Singapore Bioimaging Consortium, A*STAR, 138667, Singapore
[b] Cardiovascular and Metabolic Disorders Program, Duke-NUS Graduate Medical School, Singapore

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ABSTRACT

Adipose-derived stem cells (ASCs) are the mesenchymal stem cell (MSC) population found in the stromal-vascular fraction (SVF) of fat tissue. White adipose tissue (WAT), with well-established roles in lipid storage and adipokine secretion, is advantageous over bone marrow as the source of MSCs due to relative abundance and ease of isolation of the tissue. ASCs reside perivascularly within WAT and physiologically undergo adipogenesis to support WAT expansion in response to increased energy intake. Apart from adipogenesis, ASCs can be induced *in vitro* to differentiate into osteoblasts, chondroblasts, myocytes, neurons and other cell types. ASCs can also be reprogrammed to induced pluripotent stem (iPS) cells more efficiently than other cell types. ASCs are immunoprivileged cells and secrete immunomodulatory, angiogenic, anti-apoptotic and haematopoietic factors that facilitate tissue repair. The multi-lineage differentiation capacity, unique immunobiological properties and secretome of ASCs offer tremendous therapeutic potentials in regenerative medicine.

Cell facts

- As much as 1% of adipose cells from WAT are ASCs with MSC properties.
- Like MSCs from other sources, ASCs are immunoprivileged and known to migrate to sites of injury upon systemic administration.
- The paracrine secretion of cytokines by ASCs promotes tissue regeneration and is of therapeutic importance in regenerative medicine.

1. Introduction

Adipose-derived stem cell (ASC) is the standard nomenclature proposed by the International Fat Applied Technology Society for the plastic-adherent, proliferative, multipotent cell population isolated from adipose tissue (Gimble et al., 2007). Other names and abbreviations used prior to the consensus include adipose-derived adult stem (ADAS) cells, adipose-derived stromal cells (ADSCs), adipose mesenchymal stem cells (AdMSCs), lipoblast and processed lipoaspirate cells (PLAs) (Gimble et al., 2007, Cawthorn et al., 2012a). A typical protocol of ASC isolation involves collagenase digestion of isolated WAT, followed by centrifugation to separate floating adipocytes and ASC-containing SVF in the pellet fraction (Rodbell, 1964; Halvorsen et al., 2000; Zuk et al., 2001).

ASCs are fibroblastic in morphology and possess the properties of MSCs traditionally isolated from the bone marrow (Halvorsen et al., 2000; Zuk et al., 2001). According to the International Society for Cellular Therapy, MSCs are defined as being (i) plastic-adherent in the standard cell culture condition, (ii) multipotent, *i.e.*, able to differentiate into osteoblasts, adipocytes and chondrocytes *in vitro* and (iii) positive for CD73, CD90 and CD105, and negative for CD11b or CD14, CD19 or CD79α, CD34, CD45 and HLA-DR in their cell surface immunophenotype (Dominici et al., 2006).

Currently, there are no definitive markers for ASCs. Despite the similarity between ASCs and bone marrow-derived MSCs (BMSCs), there have been reports that showed differential expression of cell surface markers between ASCs and BMSCs. For example in humans, BMSCs lack expression of CD49d that is present in ASCs, while ASCs lack expression of CD49f, CD104, CD106 that are present in BMSCs (Lindroos et al., 2011; Pachón-Peña et al., 2011; Cawthorn et al., 2012a). However, these are not always consistent across reports and may be in part affected by donor heterogeneity, methods and quality of ASC isolation, antibody sources, sensitivity of detection methods and cell culture conditions that include differences in media composition, oxygen supply, cell confluency and passage number (Lindroos et al., 2011; Baer and Geiger, 2012).

The discovery of ASCs has resulted in increasing appreciation of WAT as a valuable source of adult stem cells with MSC potency for use in regenerative medicine due to several advantages. ASCs are abundantly present in WAT where they constitute as much as 1% of

* Corresponding author at: 11 Biopolis Way #02-02, Singapore Bioimaging Consortium, 138667, Singapore. Tel.: +65 6478 8763; fax: +65 6478 9957.
E-mail address: shigeki_sugii@sbic.a-star.edu.sg (S. Sugii).

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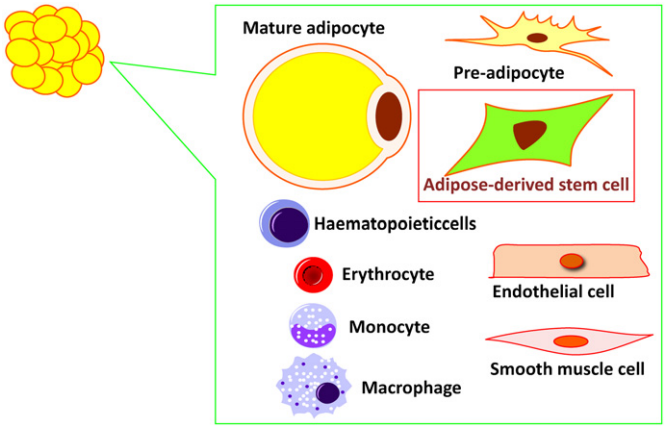

Fig. 1. Cell types in adipose tissue. A subpopulation of adipose cells is ASCs that possesses the properties of MSCs and can commit to become pre-adipocytes and subsequently differentiate into mature adipocytes. Other cell types include haematopoietic cells (*e.g.*, erythrocytes, monocytes and macrophages), endothelial cells and smooth muscle cells.

human adipose cells when compared to only 0.001–0.002% BMSCs in bone marrow (Fraser et al., 2006). ASCs can also be harvested by a minimally invasive procedure involving liposuction from subcutaneous depots.

2. Cell origin and adipogenesis

Several lines of evidence suggest a perivascular location and origin of ASCs within WAT (Lin et al., 2010; Zimmerlin et al., 2010; Cawthorn et al., 2012a). The perivascular niche (local environment) is important in determining ASC fate, function, maintenance and phenotype (Baer and Geiger, 2012). WAT stores excess energy in the form of lipids in its major cell type, adipocytes, that also secrete metabolic hormones collectively known as adipokines. The two main depots of WAT in humans are (1) subcutaneous depots in the buttocks, thighs and abdomen and (2) visceral/intra-abdominal depots around the omentum, intestines and perirenal areas (Gimble et al., 2007). The subcutaneous fat depot physiologically stores excess lipids and thus prevents their deposition into other organs, whereas excess visceral fat accumulation leads to pathological metabolic profiles due to its lipolytic nature (Després and Lemieux, 2006). Apart from adipocytes and multipotent ASCs, other cell types present in WAT include the committed adipocyte precursor cell (pre-adipocyte), haematopoietic cell types (*e.g.*, erythrocyte, monocyte and macrophage), endothelial cell and smooth muscle cell (Fig. 1).

ASCs undergo adipogenesis, *i.e.*, differentiation into mature adipocytes, to maintain adipocyte cell numbers by replacing dead cells under a normal metabolic state and to increase adipocyte number enabling hyperplastic WAT expansion when there is increased energy intake (Cawthorn et al., 2012a). The adipogenic capability of ASCs depends on their WAT depot origin and donor characteristics such as age, sex and metabolic status (Katz and Mericli, 2011; Cawthorn et al., 2012b). For example, ASCs from subcutaneous fat differentiate better into mature adipocytes than those from visceral fat by the standard *in vitro* adipogenesis protocol (Macotela et al., 2012). Adipogenesis occurs in two stages, namely (1) commitment of ASCs to unipotent pre-adipocytes and (2) terminal differentiation of pre-adipocytes into mature adipocytes (Fig. 2).

The mechanism underlying commitment of multipotent ASCs to the adipocyte lineage and identity of the pre-adipocyte are still unclear. Bone morphogenic proteins (BMPs), Wnt, transforming growth factor β (TGFβ), insulin-like growth factor 1 (IGF1), interleukin 17 (IL-17), fibroblast growth factor 1 (FGF1), FGF2 and activin are among the signaling molecules that have been reported to promote pre-adipocyte commitment (Lowe et al., 2011, Tang and Lane, 2012). It was recently demonstrated that pre-treatment with BMP2 and BMP4 enhanced the adipogenic capability of mouse Lin^- ($CD31^-$ $CD45^-$ $Ter119^-$) $Sca1^+$ $CD34^+$ cells sorted from visceral SVF to the level comparable to those from the subcutaneous counterparts (Macotela et al., 2012).

No consensus has been reached regarding molecular markers of the pre-adipocyte. One of the first few markers suggested for pre-adipocytes is CD34. Several studies demonstrated that $CD31^-$ $CD34^+$ SVF cells from humans and mice differentiate better than $CD31^-$ $CD34^-$ cells *in vitro*, suggesting the correlation of CD34 expression with pre-adipocyte commitment (Cawthorn et al., 2012a). Friedman's group demonstrated that Lin^- $CD24^+$ $CD29^+$ $CD34^+$ $Sca\text{-}1^+$ $CD105^-$ $CD117^-$ SVF subpopulation are capable of *in vivo* WAT reconstitution in lipodystrophic mice, indicating that these additional surface markers define pre-adipocytes (Rodeheffer et al., 2008). Another group showed that, like ASCs, pre-adipocytes reside in the adipose vasculature in mice and express pericyte markers and proteins such as CD140β (PDGFRβ), chondroitin sulfate proteoglycan (NG2) and alpha-smooth muscle actin (α-SMA) (Tang et al., 2008). Zinc-finger protein-423 (Zfp423) has been proposed to be a functional determinant of pre-adipocyte commitment as Zfp423 was found highly expressed among most adipogenic mouse cell lines like 3T3-L1 by comprehensive transcription factor profiling (Gupta et al., 2010). Ectopic Zfp423 expression potentiates non-adipogenic cell lines to undergo adipogenesis, while its deficiency markedly diminishes adipogenic capability *in vitro* and *in vivo*.

The subsequent events that mediate terminal differentiation into adipocytes are relatively well studied with availability of 3T3-L1 as the pre-adipocyte cell model. Essentially, the process involves a cascade of transcriptional events mediated by peroxisome proliferator-activated receptor γ (PPARγ), members of CCAAT/enhancer binding proteins (C/EBPs) and other transcription factors (Lowe et al., 2011; Tontonoz and Spiegelman, 2008). PPARγ is the master regulator in this regard as all pathways that promote adipogenesis converge to activate PPARγ, which in turn upregulates adipogenic genes such as adipocyte fatty acid binding protein (aP2), leptin, adiponectin, CD36 and glucose transporter type 4 (GLUT4) (Tontonoz and Spiegelman, 2008).

3. Functions and therapeutic potentials

ASCs can also undergo osteogenesis, chondrogenesis and differentiation into other cells with a mesodermal origin, such as myocytes (cardiomyocytes, smooth muscle and skeletal muscle cells) and tendocytes, upon *in vitro* induction. In addition, ASCs are also known to differentiate into pancreatic endocrine cells, neurons, epithelia and endothelia *in vitro* (Lindroos et al., 2011; Baer and Geiger, 2012). The capacity for multi-lineage differentiation allows conversion of ASCs to specialized cells of interest (*e.g.* insulin-secreting cells and hepatocytes), which would be useful for tissue and cell replacement therapy (Lindroos et al., 2011).

Alternatively, undifferentiated ASCs can be administered either *via* the systemic circulation or directly to the site of intended tissue regeneration for subsequent *in vivo* differentiation into appropriate cell types. Systemically administered ASCs by default tend to be retrieved in organs such as liver, lungs, heart, and brain (Katz and Mericli, 2011). ASCs express some of the chemokine receptors and ligands and may be directed to sites of injury and inflammation in a mechanism similar to the transendothelial migration and diapedesis of leukocytes (Katz and Mericli, 2011).

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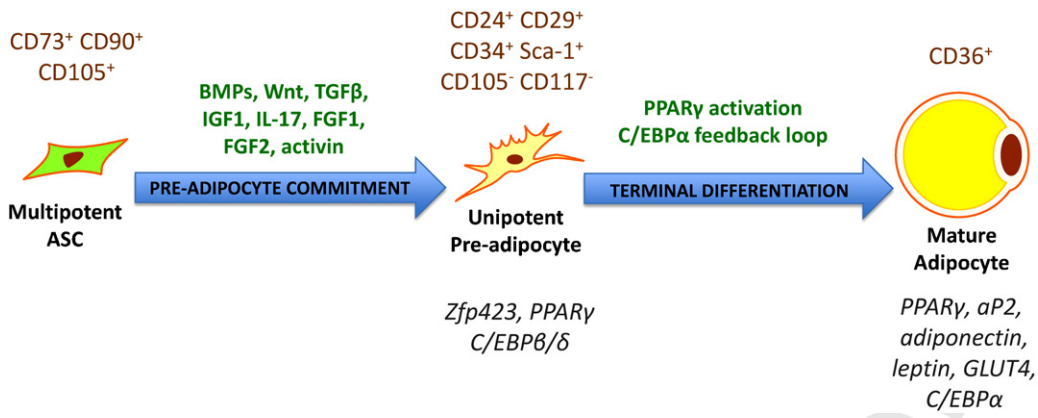

Fig. 2. Adipogenesis of ASC. Expression of representative cell surface markers (top) and genes (bottom) in the ASC, pre-adipocyte and mature adipocyte is shown.

Apart from the obvious application of ASCs as precursors of differentiated cells for cell replacement, the unique immunobiology and secretome of ASCs are increasingly appreciated for their therapeutic potential (Fig. 3). Like MSCs from other sources, ASCs are immunoprivileged due to lack of expression of class II major histocompatibilty complex (MHC-II) and co-stimulatory molecules on the cell surface (Lindroos et al., 2011). This potentially allows allogeneic transplantation of ASCs into immunocompetent recipients with minimal immune reactions in the host, as exemplified by an *in vivo* study using a rat spinal fusion model (McIntosh et al., 2009). In addition, ASCs are also immunomodulatory and can promote tissue repair through immunosuppressive effects exerted *via* direct cell-to-cell interaction or secreted factors such as prostaglandin E2 (PGE2), leukemia inhibitory factor (LIF) and kynurenine (Salgado et al., 2010; Cawthorn et al., 2012b). The immunosuppressive property can be exploited to prevent and treat acute graft-*versus*-host disease (GVHD) in allogeneic stem cell transplantation, autoimmune diseases and inflammatory diseases such as Crohn's disease, sepsis and rheumatoid arthritis (Lindroos et al., 2011; Cawthorn et al., 2012b).

Besides immunosuppressive molecules, ASCs also secrete an array of soluble factors that promote tissue regeneration at the injury site *via* a paracrine mechanism. The secretome includes angiogenic factors [*e.g.*, vascular endothelial growth factor (VEGF)], anti-apoptotic factors [*e.g.*, insulin-like growth factor-1 (IGF-1)], hematopoietic factors [*e.g.*, colony stimulating factors and interleukins] and hepatocyte growth factor (HGF) that is hematopoietic, angiogenic and promotes mammary epithelial duct formation (Kilroy et al., 2007; Salgado et al., 2010; Baer and Geiger, 2012). It is gaining appreciation that the therapeutic effects of ASC therapy *in vivo* are largely attributed to the paracrine and immunomodulatory effects of ASCs rather than the cell replacement *per se* (Katz and Mericli, 2011).

ASCs are also ideal for reprogramming into induced pluripotent stem (iPS) cells. The reprogramming efficiencies of ASCs are substantially higher than those reported for fibroblasts from humans (up to 100-fold) and mice (~5-fold) (Sugii et al., 2010). Remarkably, the process can be performed without the requirement for feeder cells (Sugii et al., 2010, 2011). This is possible because ASCs intrinsically secrete a high level of self-renewal supporting factors such as FGF2, LIF, fibronectin and vitronectin, and thus can serve as feeders for both autologous and heterologous pluripotent stem cells (Sugii et al., 2010).

4. Associated pathologies

Gene mutations in PPARγ, AGPAT2, seipin/BCSL2, and lamin A result in inherited lipodystrophy, a condition characterized by substantial reduction in fat development (Virtue and Vidal-Puig, 2010; Garg, 2011). It is plausible that the disease arises from defects in adipogenesis, but it is not certain which, if any, properties of ASCs are

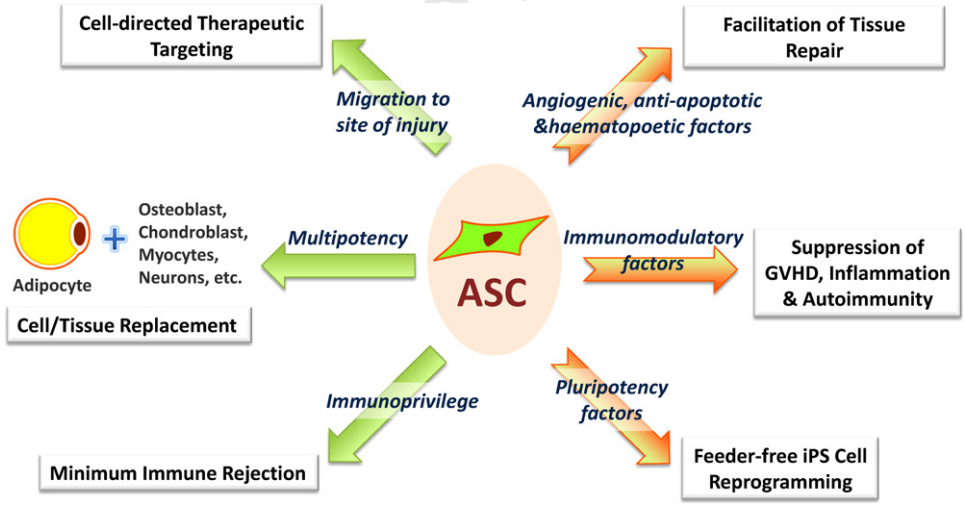

Fig. 3. Biological properties and therapeutic potentials of ASC. The right half summarizes secretome of the ASC, and the left half summarizes multi-lineage differentiation capacity and immunobiology of the ASC.

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affected in lipodystrophic patients. On the other extreme, adipogenesis capacity is over-saturated and hypertrophy of adipocytes occurs in obesity (Slawik and Vidal-Puig, 2007). The inability to properly store excess lipids in both lipodystrophy and obesity leads to lipid spillover into non-adipose tissues, causing lipotoxicity and subsequent metabolic complications such as insulin resistance, type 2 diabetes, steatotic liver disease and heart failure (Slawik and Vidal-Puig, 2007). Involvement of ASCs in the impaired adipose tissue expandability is only beginning to be explored. It was reported that the number of mature adipocytes is set during childhood and stays constant throughout adulthood regardless of fat mass changes in human (Spalding et al., 2008), but it is yet not clear how ASCs and progenitor cells contribute to this process.

Dysregulation of ASC adipogenesis can lead to ectopic adipose tissue formation as in the case of lipoma. This benign adipose neoplasm may be a result of positive balance in adipocyte turnover due to accelerated adipogenesis without enhanced apoptosis (Suga et al., 2009). Infantile hemangioma, the most common benign tumor of infancy, is characterized by an initial phase of endothelial cell and MSC proliferation, and gradual replacement by fat-like tissue during the next involuting phase (Boscolo and Bischoff, 2009). It would be of interest to determine if hemangioma-derived MSCs show characteristics and origin similar to ASCs. On the other hand, formation of ectopic bone in the subcutaneous adipose depots is observed in progressive osseous heteroplasia. Osteoblasts and chondrocytes are present in addition to adipocytes in this rare congenital disease associated with mutations in *GNAS1* gene that encodes the α subunit of the G protein Gs (Gimble et al., 2007). The phenotype of this genetic disorder thus points to the multipotent differentiation capacities of ASCs *in vivo*. Collectively, further studies on etiology of these human diseases should help understand the identity and nature of ASCs.

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