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Adult Human Adipose Tissue Contains Several Types of Multipotent Cells

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Abstract Multipotent mesenchymal stromal cells (MSCs) are a type of adult stem cells that can be easily isolated from various tissues and expanded in vitro. Many reports on their pluripotency and possible clinical applications have raised hopes and interest in MSCs. In an attempt to unify the terminology and the criteria to label a cell as MSC, in 2006 the International Society for Cellular Therapy (ISCT) proposed a standard set of rules to define the identity of these cells. However, MSCs are still extracted from different tissues, by diverse isolation protocols, are cultured and expanded in different media and conditions. All these variables may have profound effects on the selection of cell types and the composition of heterogeneous subpopulations, on the selective expansion of specific cell populations with totally different potentials and ergo, on the long-term fate of the cells upon in vitro culture. Therefore, specific molecular and cellular markers that identify MSCs subsets as well as standardization of expansion protocols for these cells are urgently needed. Here, we briefly discuss new useful markers and recent data supporting the rapidly emerging concept that many different types of progenitor cells are found in close association with blood vessels. This knowledge may promote the necessary technical improvements required to reduce variability and promote higher efficacy and safety when isolating and expanding these cells for therapeutic use. In the light of the discussed data, particularly the identification of new markers, and advances in the understanding of fundamental MSC biology, we also suggest a revision of the 2006 ISCT criteria.

Keywords Multipotent mesenchymal stromal cells · Hematopoietic progenitor cells · White fat progenitor cells · Endothelial progenitor cells · Pericyte · Markers

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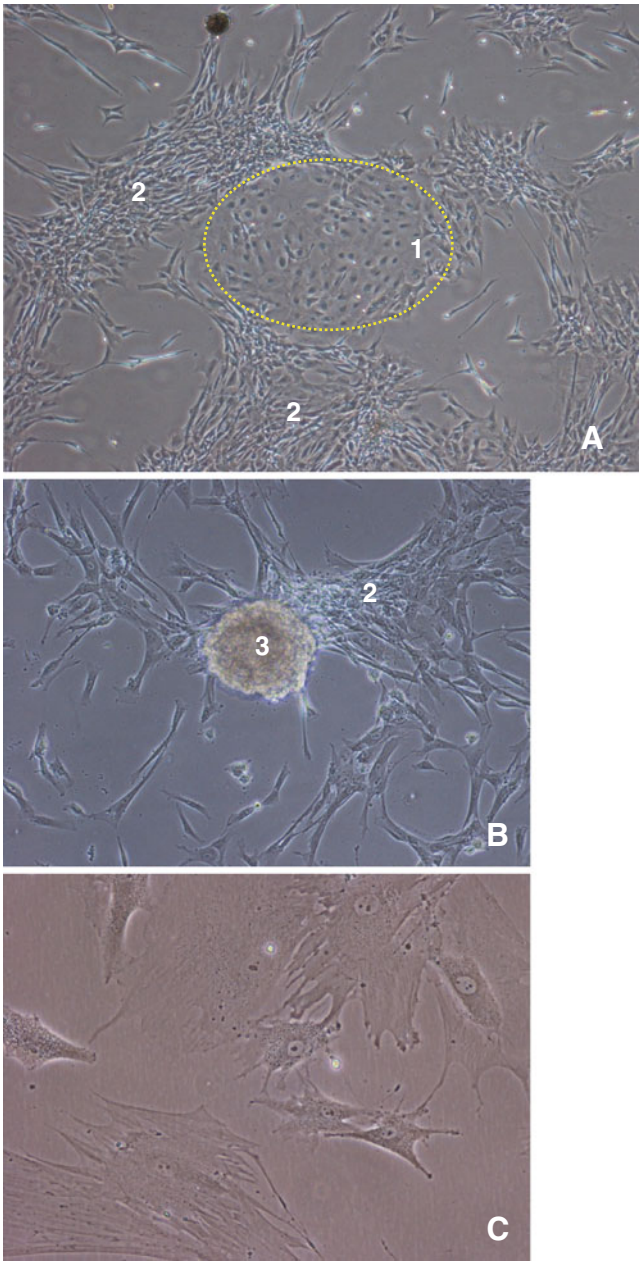
Introduction

In human and in other mammalians, the stromal vascular fraction (SVF), a heterogeneous mixture of cells isolated by enzymatic dissociation of adipose tissue, has been shown to contain multipotent cells [1, 2].

The heterogeneous nature of these cells is immediately apparent upon examination of the individual cell morphologies (see Fig. 1a). Indeed, three distinct cell types can be found in cultured cells extracted from the SVF [3, 4]: very small round cells that rapidly self-renew (RS cells), spindle-shaped cells (SS cells), and slowly replicating, large and flattened cells (FC cells, see Fig. 1b). Interestingly, within the SVF there are also cells which can form sphere clusters and even grow in free-floating conditions (see Fig. 1c, [4, 5]).



◀ **Fig. 1** Exemplary cell culture appearance of the main cell populations found in the SVF. Typical phase-contrast photomicrographfhs of living adipose tissue-derived cultured cells of the SVF at passage 0. **A** The SVF contains heterogeneous populations of cells: in this pictures, together with the typical spindle-shaped cells (SS cells, *1*), adipose tissue-derived multipotent mesenchymal stromal cells (AT-MSCs), there is a different population of cuboidal shape cells (*2*, *circled with yellow dotted line*). These cells are usually of endothelial origin and are quickly lost in the subsequent passages. **B** Typical appearance of a spheroidal colony (*3*) surrounded by spindle-shaped AT-MSCs (*2*). These spheroidal colonies are probably generated by very small round cells that rapidly self-renew (RS cells). Interestingly, the spheroids can also detach from the monolayer and grow in free-floating conditions. **C** Photomicrograph of slowly replicating, large and flattened cells (FC cells). It has been shown that small RS cells or cells of the spheroids manifest the highest multipotentiality [4, 5, 82]. On the other hand, FC cells are much more mature with subsequent loss of multipotent characteristics [82]. Immunocytochemistry has confirmed that FC cells lack CD73 expression, whereas RS cells are positive for all three MSCs typical markers CD73, CD90, and CD105 [82]. Interestingly, the newly described mesenchymoangioblast also forms spheroid colonies in semisolid medium [52]

In 2006, the International Society for Cellular Therapy (ISCT) proposed to call these cells "multipotent mesenchymal stromal cells" (MSCs), whereas the term "mesenchymal stem cell" should only be reserved for cells that meet specified criteria for stem cells [6]. So, the acronym MSC is commonly applied to plastic-adherent cell preparations isolated from bone marrow (BM) or other tissue that are positive for several antigens, such as CD73, CD90, and CD105, that lack expression of hematopoetic antigens, and that are able to differentiate at least into osteoblasts, adipocytes, and chondroblasts under specific in vitro differentiating conditions. Furthermore, albeit controversial, MSCs have been reported to differentiate even into cells of non-mesodermal origin such as hepatocytes and neurons [2, 7, 8].

A surprising new paradigm elaborated in these last years is the possibility that stem and progenitor cells (including MSCs!) may reside within the vessel wall. Even more striking, it appears likely that several distinct stem/progenitor populations may reside in this location both during embryo development as well as in the adult life. Experimental results from several laboratories suggest that MSCs also belong to the population of progenitors cells found within the vessel wall [9–11]. In fact, it has been shown that MCSs are most probably derived from perivascular cells (pericytes/mural cells), which are traditionally defined as extensively branched cells located in non-muscular microvessels, capillaries, and postcapillary venules [12, 13].

The focus of this short review is to give an overview of the progenitors/stem cells associated with the vasculature of human subcutaneous adipose tissue. Furthermore, we will discuss some useful and well-known markers, which could help to characterize these different cell types. For clarity, the discussion is organized according to the classifications shown in Fig. 2.

Types of Progenitors Cells Found Associated with the Vasculature

Adipose Tissue-Derived Multipotent Mesenchymal Stromal Cells Three different subpopulations of MSCs have been recently identified in the vasculature of adipose tissue (SVF) by Zimmerlin et al. [14] and were called "subendothelial progenitors" (SP), "subendothelial progenitors transitional population" (SP-TP), and "supra-adventitia-adipose stromal cells" (SA-ASC), respectively (reviewed

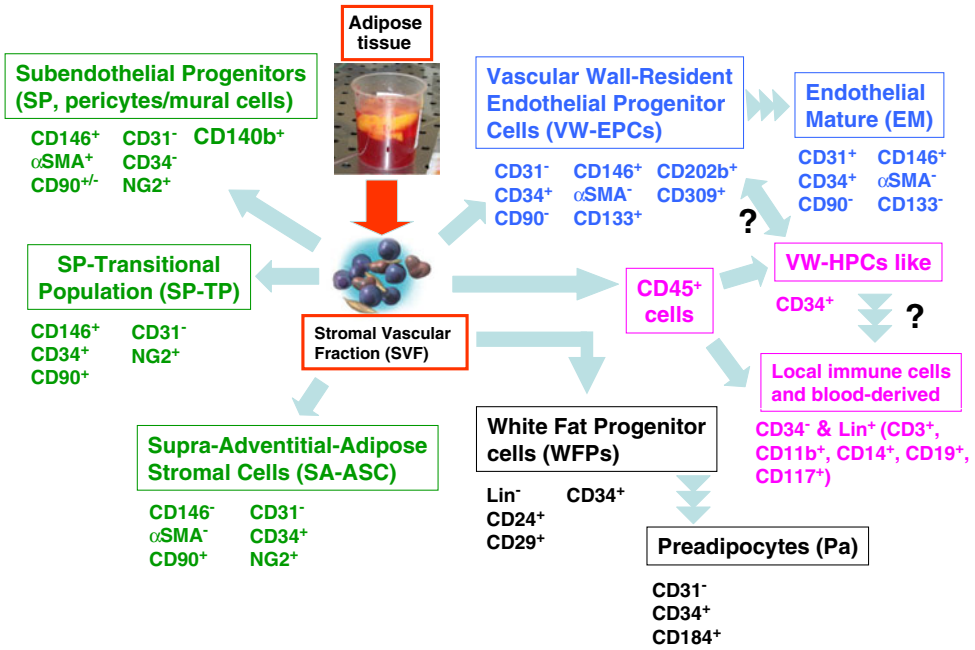


Fig. 2 Scheme showing the different types of progenitor/stem cells found in the freshly isolated stromal vascular fraction (SVF). The so-called SVF is extracted from adipose tissue by enzymatic digestion and contains a highly heterogeneous mixture of cells such as endothelial cells, smooth muscle cells, mast cells, macrophages, and other blood-derived cells (B and T cells, granulocytes, monocytes), fibroblasts, and preadipocytes. Mature adipocytes, since they contain lipid droplets, have the propensity to "float" and are lost during the extraction procedure if the centrifugation steps are done at no more than 400×*g* for 5 min. The four major groups of multipotent cells found in the SVF and described so far are shown: *green*, subendothellial progenitors (SP, sometimes summarily referred as "pericytes" or "mural cells") and other related cells (subendothelial progenitor-transitional population, SP-TP, and supra-adventitial-adipose stromal cell, SA-ASCs); *blue*, vascular wall resident endothelial progenitor cells (VW-EPCs); *violet*, vascular wall hematopoietic progenitor cells (VW-HPCs); *black*, white fat progenitor cells (WFPCs). Some of the common markers used to distinguish these different populations are shown. Special aspects of few of them are discussed in the text. CD name/other name(s) of few markers: CD117: c-Kit; CD140b: PDGFRβ; CD184: CXCR4; CD202b: Tie-2; CD309: VEGF-R2 (KDR)

in [9–11, 15]; Fig. 2). The SP, which represent the cells that are usually named MSCs or pericytes/mural cells, are found normally in the intima region of microvessels [11, 14].

In large vessels, similar cells are found also in the media and adventitia associated with the vasa vasorum [16, 17]. SP are heterogeneous with respect to their self-renewal and differentiation potential. Thus, the MSC pool comprises not only putative "multipotent mesenchymal stromal cells" but also subpopulations at various stages and on different paths of differentiation.

It appears that the SP are the more potent whereas SA-ASC are the more prevalent cells. The third population, ST-TP, may represent a transitional population between SP and SA-ASC [14]. The exact location of MSCs in vivo is still matter of debate. Indeed, some authors [12, 18, 19] consider the inner intimal layer ("the pericyte theory"), whereas others [20–22] consider the adventitia as location of these multipotent cells. Conceivably, both observations are correct. In fact, small-sized vessels, such as capillaries (range in diameter from 4 to 10 μm), arterioles (10 to 100 μm), and venules (10 to 100 μm), normally consist of endothelial cells surrounded by a basement membrane and a variable coverage of mural cells [16] (capillary less, arterioles and venules more; see Fig. 2a–c). On the other side, the walls of larger vessels (range in diameter from 100 μm to 10 mm, see Fig. 2d) consist of three specialized layers: intima, media, and adventitia [16]. Possibly, in these larger and more mature vessels, the MSCs are simply more concentrated in the adventitia. Thus, the location of these cells could depend on the size and maturation stage of the vessels.

Both terms, MSCs and pericytes/mural cells, are unfortunately used to indicate the same type of cells. Pericytes is the term for vascular mural cells embedded within the vascular basement membrane of blood microvessels, where they make specific focal contacts with the endothelium [16]. Although related in function, and assumed to belong to the same lineage as vascular smooth muscle cells (VSMCs) that surround larger blood vessels, pericytes can be distinguished from VSMCs by their unique location relative to the endothelium, their morphology, and, to some extent, by their marker expression [16]. However, the distinction based on morphology and position between

pericytes and VSMCs is not absolute, and there is evidence that pericytes are present also in large vessels. Indeed, pericyte-like cells have been identified in the inner intimal layer, the outer layer of the media, and in vasa vasorum in the adventitia of large, medium, and small arteries and veins. Cells with pericyte-like characteristics have also been identified in the kidney (mesangial cells), liver (Ito cells), and bone marrow (reticular cells) [16].

Pericytes are commonly regarded as the structural components of blood vessels that regulate vascular contractility and support the stability of blood vessels. Their functions are, however, much more diverse. They can sense angiogenic stimuli, guide sprouting tubes, elicit endothelial survival functions, and even exhibit macrophage-like activities [16].

Recently, numerous lines of evidence suggested a close relationship between MSCs and pericytes. In fact, MSCs have a perivascular location and express pericytic markers [12]. So, it may be best to describe MSCs as cells with pericytic properties, or in other words, they are "dressed up" like pericytes. Probably, at least mature pericytes and smooth muscle cells are descendants of MSCs.

Hopefully, the Mesenchymal and Tissue Stem Cell Committee of the ISCT will introduce soon rules to clearly name this type of cells. Few, and meanwhile very popular molecular markers, have been chosen to immunophenotype MSCs. Although none of these markers is specific for this kind of cells, as shown in Fig. 2, their combination allows to distinguish MSCs from the other progenitors present in the vasculature.

White Fat Progenitor Cells Despite the clinical importance of obesity and its linked metabolic complications [23], the characterization of white fat progenitor cells (WFPs) was accomplished only in 2008 [24–27].

WFPs are hypothetically descendants of the SA-ASCs, reside in the adipose vasculature, and were defined as $Lin^{-}CD24^{+}CD29^{+}CD34^{+}$ (see Fig. 2) in the mouse [25, 26]. Apparently, the same markers can be used to characterize human WFPs [21].

In mammals, the adipose organ is composed not only of white adipocytes, but also of brown adipocytes, which are specialized in the basal and inducible energy dissipation and thermogenesis. While sharing major regulators of differentiation, brown and white adipocytes have significant transcriptional, secretory, and morphological differences [28]. Remarkably, brown adipose tissue developed relatively late in the course of evolution, in parallel with the capacity of homeothermy and thermoregulation [23]. Thus, it is not surprising that brown and white adipocytes have a distinct origin. As mentioned before, white adipocytes can develop from early progenitor cells ($Lin^{-}CD24^{+}CD29^{+}CD34^{+}$) most probably of mesenchymal origin. However, it has been shown that white adipocytes can develop also from neuroectoderm [29, 30] or from hematopoietic progenitors [31].

To date, two types of progenitors of brown adipocytes have been identified. The first type is characterized by the expression of a regulatory factor found in muscle-cell precursors (myogenic factor 5, Myf5) and displays a myogenic transcriptional signature [32]. So, it is likely that brown adipocytes arise from myogenic precursors in response to unknown signals.

The second type of progenitor cell for brown adipocytes is myf5-negative, which expresses the CD34 surface protein [23, 33], and possibly, shares a common ancestor with the white adipocytes. In addition to white and brown fat serving different purposes, white adipose in different locations in the body can serve different functions. In fact, primary preadipocytes from various depots in the body show distinct global transcriptional profile, even after culturing [34]. Many of the genes that are differentially regulated are implicated in lipid metabolism suggesting that adipocytes in different depots may have different biological roles. A different developmental origin (mesenchymal [25, 26], hematopoietic [31], or ectodermal [29, 30]) of the white adipocytes progenitors may explain these results.

In conclusion, additional studies are needed to further characterize the progenitors of white and brown adipocytes in humans and to understand the relationship of WFPs with the other progenitor families. Furthermore, the understanding of the developmental origin and of the differential accumulation of progenitor cells of the different fat depots in the body could help in selecting the most appropriate fat tissue for specific cell therapeutic applications.

Vascular Wall Resident Endothelial Progenitor Cells EPCs were first discovered by Asahara and colleagues in 1997 [35], who isolated them from peripheral blood using magnetic beads selection on the basis of cell surface antigen expression. EPCs are known to consist of different subsets which display different surface markers even though they share the common stem cell properties of clonal expansion, high proliferative rate, pluripotency, and resistance to stress. First evidence for the presence of vascular wall resident endothelial progenitors was brought by Alessandri et al. [36] in 2001. A couple of years later, the studies of Ingram et al. [37, 38] revealed the presence of a complete hierarchy of EPCs in the wall of human adult blood vessels and umbilical cord blood. Final crucial evidence for the presence of EPCs in the adult was provided by Zengin et al. [39] who reported their existence in a distinct zone of the vascular wall termed "vasculogenic zone (VZ)" [10, 39, 40] (see Fig. 3d). Moreover, it has been reported that this region

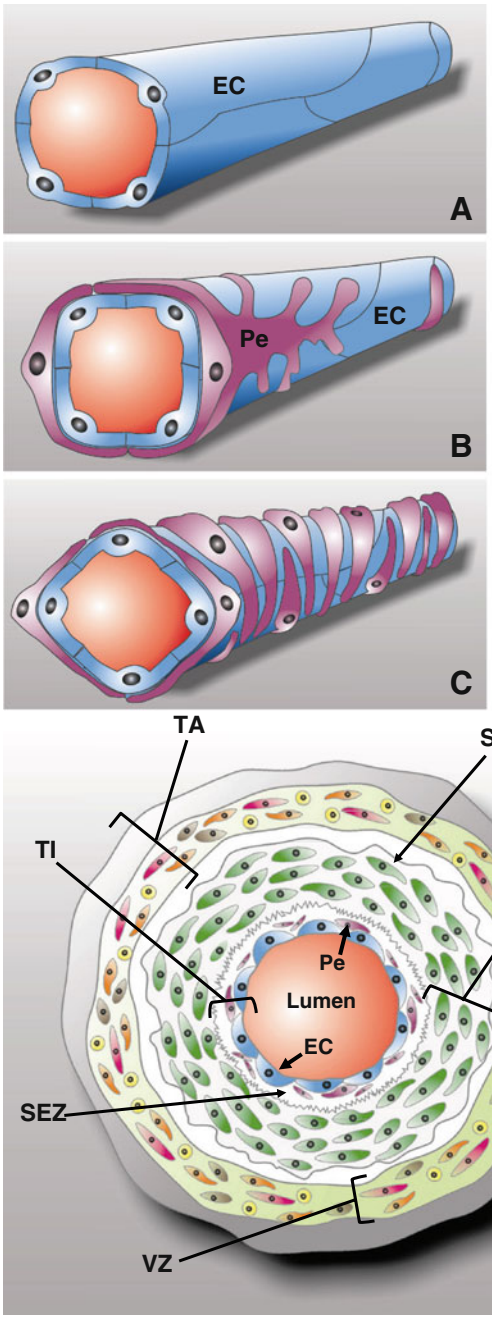


Fig. 3 Very schematic and idealized drawings showing the wall composition of immature and mature vessels. **A**. Nascent vessels consist of a tube of endothelial cells (EC, *blue*). These mature into the specialized structures of capillaries, arteries, and veins. **B** Capillaries (diameter from 10 to 100 μm) are composed of only a single layer of endothelial cells (EC) and a basement membrane (not shown). Few pericytes (Pe, *violet*)/mural cells are scattered around this kind of vessels. **C** Arterioles (diameter from 10 to 100 μm) have an increased coverage of pericytes compared with capillaries. Venules (diameter from 10 to 100 μm) are similar to arterioles (not shown). **D** The walls of larger vessels (diameter from 100 μm to 10 mm) consist of three specialized layers: a tunica intima (TI) containing endothelial cells (EC) and pericytes (Pe), a tunica media (TA) of smooth muscle cells (SMCs), and a tunica adventitia of fibroblast and other cells, together with elastic laminae. In large vessels, the adventitia layer has its own blood supply, known as vasa vasorum (not shown). Two locations have been considered as possible location for multipotent progenitor cells: the intima and the adventitia. The exact location of MSCs in vivo is at the moment matter of debate. Some authors consider the inner intimal layer ("the pericyte theory"), whereas others consider the adventitia as residence of these cells. The so-called "vasculogenic zone (VZ)" is a distinct region between the smooth muscle and adventitial layers. According to Zengin et al., this zone harbors endothelial progenitors cells (EPCs), hematopoietic progenitor cells (HPCs), and MSCs

harbors also hematopoietic progenitors cells [10, 39, 40], which can generate cells for the local immune system, such as macrophages and MSCs. So, in addition to the so far identified sources for EPCs, namely peripheral blood and bone marrow, also EPCs widely distributed in the vascular walls may contribute to the adult local and systemic pool.

Vascular Wall Resident Hematopoietic Progenitor Cells To our knowledge, the first report on the existence of a population of CD34+/CD45+ cells in white adipose tissue was published in 2003. Indeed, Cousin et al. [41] reported that mouse adipose tissue contains a significant percentage (up to 3.5% of the SVF population, a number of cells which are much higher than in peripheral blood) of CD34 and CD45 double positive cells which were able to differentiate into hematopoietic colonies in methylcellulose. Furthermore, they reported also that cells of the SVF extracted from murine inguinal adipose tissue were able to efficiently rescue lethally irradiated mice resulting in the reconstitution of the major hematopoietic lineages. Apparently, this first report was dismissed by the scientific community. However, in 2006, another group found a relevant number (5.2±1.3%) of CD34+CD45+ cells, in the SVF prepared from human abdominal subcutaneous adipose tissue. Furthermore, they showed that 0.75±0.1% of the double positive cells expressed also CD117 [42]. This interesting marker, the stem cell factor receptor c-kit, which is typically expressed in normal human bone marrow by hematopoietic stem cells and progenitor cells of all lineages, was detected on MSCs also by other researchers (see for example [5, 43–45]). Vascular biologists also reported the existence of progenitor cells in the vasculature which express CD45 and are not just blood-derived differentiated cells [39, 40]. In 2008, Miñana et al. [46] found primitive mesodermal progenitors within the SVF of human adipose tissue which exhibited in vitro hematopoietic and hemangioblastic activities. Moreover, Han et al. [47] provided experimental evidence that adipose tissue may be an alternative extramedullary tissue for hematopoietic stem and progenitor cells (HSPCs), and therefore, the SVP in adipose tissue may be a novel resource for obtaining functional

and transplantable HSPCs. Finally, Majka et al. [31] challenged the paradigm of a resident progenitor of stromal origin for all white adipocytes and questioned the developmental origin of tissue-resident adipocyte progenitors with mesenchymal characteristics. Indeed, the authors showed that hematopoietic progenitors were responsible for the de novo development of a subset of white adipocytes in adults and the generation of a tissue-resident stromal adipocyte progenitor population. Thus, Majka et al. [31] propose a model where hematopoietic stem cells in the bone marrow give rise to myeloid intermediates which migrate to the adipose tissue, loosing in this process their hematopoietic markers, and adopting the appearance of resident stromal adipocyte precursors. Interestingly, these myeloid lineage cells are reminiscent of an intriguing circulating population called "monocytes-derived multipotential cells," which have the potential to differentiate into a variety of cells, have fibroblast-like morphology in culture, and were described for the first time in 2003 [48–50].

A second important observation from the studies of Majka et al. [31] is the preferential accumulation of bone marrow progenitor-derived adipocytes in visceral versus subcutaneous fat depots, with a gender-specific effect, with female accumulating more cells than male mice.

Taken together, data presented by different groups in the last years demonstrate that the adipose tissue harbors hematopoietic stem and progenitor cells. Further studies are needed to fully characterize these cells (see Fig. 2), to understand their exact location, and to uncover their relationships with the other progenitor cells present in the adipose tissue. All this knowledge could set the basis for future exploitation of the SVF extracted from fat tissue as an alternative resource of HSPCs.

A Common Precursor for Mesenchymal Stem/Stromal and Endothelial Cells While some MSCs are known to originate from neural crest precursors during embryogenesis [51], the direct mesodermal precursor of these cells was not well defined until recently. Now, Vodyanik et al. [52], using embryonic stem cells directed towards mesodermal differentiation, were able to characterize a precursor for mesenchymal and endothelial cells, which the authors named "mesenchymoangioblast." They also found a new specific cell surface marker, APLNR (apelin receptor, also known as angiotensin receptor like-1) which should facilitate the work with these cells. So, in respect to the scheme showed in Fig. 2, the mesenchymoangioblast should be placed at a higher hierarchical level. Thus, these cells may finally offer the opportunity to study the cellular and molecular pathways for the development of mesodermal lineage cells.

Markers for the Characterization of Vascular Wall Resident Progenitor Cells

In this section, we would like to briefly review recent published data on cellular markers used to characterize MSCs. The interested readers are referred to Pacilli and Pasquinelli [15] and Lin et al. [22] for a description of additional markers which are not discussed here.

Neuron-glia Antigen 2 A disialoganglioside found mainly in the nervous system, this marker is also expressed on neural crest cells, which are thought to give rise to a subset of MSCs and consequently to adipocytes [29, 51]. Perivascular cells, including mature and immature SMCs, and pericytes express neuron-glia antigen 2 (NG2). On the other hand, it is not expressed by endothelial cells of the microvasculature [52, 83]. Interestingly, this marker was found to be confined to perivascular cells along arterioles and capillaries, and continuous expression was not observed along venules beyond the immediate postcapillary vessels. To our knowledge, this is the only marker which can differentiate venous smooth muscle and pericytes from other capillary- and arteriole-associated perivascular cells [53, 54]. Typically, NG2-positive pericytes co-express the PDGF-β receptor, a finding which is consistent with the identification of the PDGF-β receptor and its ligands as crucial elements for pericytes survival and development.

In conclusion, NG2 belongs to the very few reliable markers for detecting pericytes. In fact, CD146, which is also typically used as "pericytic marker," is in reality expressed in the whole human endothelium, irrespective of its anatomical site or vessel diameter. Finally, NG2 could be very useful to better understand the developmental origin of perivascular cells, since apparently some arise from the neural crest and some others not [51].

Mesenchymal Stem Cell Antigen 1 This marker has been proved to be suitable to purify and characterize MSC subsets from primary bone marrow. Recently, it has been demonstrated that mesenchymal stem cell antigen 1 (MSCA-1) is identical to tissue non-specific alkaline phosphatase (TNAP) [55]. This protein belongs to a large family of dimeric enzymes which catalyzes the release of inorganic phosphate upon phosphomonoesters hydrolysis. TNAP is bound to the exoplasmic face of the plasma membrane by a glycosyl-phosphatidylinositol anchor. It is present in the bone, liver, kidney, and endometrium and is expressed at high levels on the surface of embryonic stem (ES) cells. During differentiation of ES cells, the expression level of TNAP decreases. Interestingly, the expression of alkaline phosphatase activity was used as a marker to characterize MSCs in two landmark papers published recently [12, 56].

CD271 (L-NGFR, Low-affinity Nerve Growth Factor Receptor, p75NTR, Neutrophin Receptor) Monoclonal antibodies to CD271 stain primitive MSCs with high specificity from adult BM, defining a subset of cells with higher clonogenic efficiency, proliferative and differentiative potential in comparison to the whole MSCs population. CD271 is a type I transmembrane protein of 75 kD and is expressed also by many other cells type including neurons, Schwann cells, oligodendrocytes, astrocytes, follicular dendritic cells, and melanocytes. Interestingly, CD271, like NG2, is also expressed on neural crest cells. This marker is becoming very popular and is used for immunomagnetic positive selection of highly enriched primitive MSCs preparations from adipose tissue or bone marrow [57].

CD34 This cell surface protein has been used for almost 30 years as a marker for the characterization and purification of hematopoietic stem and progenitors cells [58–60]. Recently, it has been increasingly used to identify other stem/progenitor cells, like muscle satellite cells and epidermal precursors [61]. Despite its importance as a stem-cell marker, the functions of CD34 (and of its family members) are still elusive. Several papers have shown that CD34 is highly expressed also in freshly isolated SVF cells [18, 21]. However, this marker is quickly lost in cultured cells [19], and interestingly, the same is observed with satellite cells [61].

Despite its importance as a stem-cell marker, the functions of CD34 (and of its family members) are still elusive. The implications of a loss in CD34 expression remain therefore unclear, but some data indicate that CD34 + MSCs are more proliferative, while CD34− MSCs possess greater plasticity [21]. Suga et al. [21] suggest that CD34 expression in adipose-derived MSCs correlates positively with endothelial characteristics and negatively with pericytic features. Interestingly, in the contest of myogenic progenitor cells (satellite cells), Ieronimakis et al. [61] reported that the absence of CD34 marks a state of reversible activation which is not necessarily a hierarchy step down towards differentiation. So, the expression of CD34 marks the cells which are dormant, i.e., in a kind of "reserve state." In other words, the downregulation of CD34 in satellite cells indicate the reversible switch from dormancy ("reserve state") to activation towards "primed" cell which can advance further in the myogenic pathway. Thus, since CD34 plays an important role in cell trafficking, its downregulation may signalize the propensity of the progenitor cell to migrate and to engraft within regions where its differentiation potential and capacity is needed. If this concept is true also for other progenitor cells expressing CD34, the three subpopulations of MSCs depicted in Fig. 1, SP, SP-TP, and SA-ASC, should be at a similar hierarchical level regarding the differentiation potential.

CD146 The S-endo 1-associated antigen CD146, also referred to as MelCAM or MUC18, is a cell surface molecule belonging to the immunoglobulin superfamily that is constitutively expressed in the whole human endothelium, irrespective of its anatomical site or vessel caliber [84]. In recent years, CD146 has been proven as a very useful marker for the characterization and purification of MSCs [12, 56]. Recent experiments [62] suggest that CD146 may be considered as a marker of multipotency for MSCs. In human, this antigen is detected also on several other cell types, including melanoma cells, smooth muscle cells, fetal myogenic cells [63], follicular dendritic cells, and on small subpopulations of lymphocytes [64]. CD146 has been suggested to function as a very important cell adhesion protein in vascular endothelial cell activity and angiogenesis [65, 66]. The data describing the role of CD146 in tumors is conflicting. In fact, it promotes invasion, metastasis, growth, and survival of certain cancers, such as melanoma and prostate, whereas it may act as a suppressor of breast cancer progression [65, 66]. Other roles suggested for CD146 include implantation of the blastocyst and placentation [65, 67]. Cerletti et al. [63] reported that high expression of CD146 in human fetal muscle is associated with myogenic and myoendothelial cells (in adult cells this marker is barely detectable). Interestingly, in vitro downregulation of CD146 in activated human fetal satellite cells increases myoblast fusion and formation of multinucleated cells. Cerletti et al. [63] speculated that in fetal cells, CD146 may mediate migration of myogenic precursors from other sites (i.e., somites or dorsal aorta) to the limb musculature. Since one of the major established roles of this marker is cell trafficking and regulating cell invasion, it could also be possible that the expression of this protein signalize the "primed" status of the cells, that is, its propensity to migrate and to differentiate.

CD56 This marker is known to be typically expressed on natural killer, neural, and muscle cells. In spite of this, Hans-Jörg Bühring and collaborators found that a small subset of $CD271^{bright}$ MSCs from the bone marrow react with an antibody (39D5) which recognizes a CD56 epitope not expressed on natural killer cells [68]. Cells positive for CD271, $CD56_{39D5}$, and a third marker, MSCA-1, gave rise to CFU-F with high efficiency [69, 70]. It would be interesting to test, whether the 39D5 antibody reacts also with MSCs extracted from tissues different than bone marrow.

Finally, it is remarkable that the classical CD56 epitope is used for the characterization and purification of human myogenic progenitor cells [71]. Furthermore, it was also used to characterize the newly described mesenchymoan-

gioblast [52]. This could suggest that this surface protein may have a common function in different types of progenitor cells.

Conclusions In recent years, several new markers and panels of monoclonal antibodies with good selectivity for MSCs have been described. Although all these markers can be used for enrichment of MSCs, the isolated cells are still heterogeneous and many of them will not give rise to CFU-F [68]. It is therefore important to identify new markers which will facilitate the characterization and the efficient purification of the different types of multipotent cells found in the vasculature of different tissues. This will also improve the safety of cell therapies, since it could be possible that some subpopulation may be not always suitable for some specific applications.

It has been reported that MSCs alter their surface proteins based on cell culture conditions (e.g., culture media, cell density, number of passages, contact with tissue culture plastic). Freshly isolated SVF cells and early passaged MSCs have relatively higher expression of CD117, HLA-DR, and stem cell-associated markers, such as CD34 and CD271, and low levels of stromal cell markers, such as CD29, CD90, and CD105. In general, as MSCs are serially passaged, expression levels of CD34 and CD271 fall, while levels of the above-mentioned stromal markers rise [1]. Interestingly, it has been reported that CD34 expression is typically lost after short time when the cells are cultured in classical DMEM/FBS medium and regained after culture with endothelial basal medium [21]. Thus, it will be very important to develop common criteria and universal standards for the preparation and the culture of MSCs in order to facilitate the comparison of results from different laboratories.

Further studies are needed to understand the reversible expression of some markers, and more importantly, to understand the relationship between the populations found in vitro with those localized in vivo.

Time to Update the ISCT Criteria for Defining MSCs?

In 2006, the ISCT proposed three minimal criteria to define MSCs [7] in order to help the researchers to find standardized rules. Now after 5 years, we believe that these criteria should be updated due to the amount of new and important knowledge acquired. We think that the 2006 rules had general character and were derived mainly from studies performed on in vitro expanded adult bone marrow-derived MSCs and did not take into account the now well-established sources of variability. It is now clear, for instance, that most if not all of the surface markers used to characterized expanded MSCs are highly modulated by many factors in vitro, like culture conditions (e.g., use of fetal calf or human serum or serum-free growth medium [72, 73]), the number of passages [74], and the cell density of the cultures [3]. Furthermore, the ISCT definition of MSCs states that these cells do not express hematopoietic antigens [7]. However, we now know that adipose tissue-derived MSCs are positive for CD34 [18, 21]. In addition, since 2006 several new MSCs markers were identified such as CD56, CD146, CD200 [75], CD271, NG2, and GD2 [76], which is consistently expressed at high level in freshly isolated as well as in cultured MSCs. Dynamic markers expression and newly discovered markers should hence be included in an updated ISCT criteria but also the tissue origin of MSCs should be take into account. It has been established in fact that MSCs isolated from different tissues, although similar for many aspects, exhibit significant differences in the global gene expression patterns [77] and also distinct sensitivities to inductive bioactive molecules in culture [78]. For example, the inductive conditions for marrow MSCs are quite different from those required by fat-derived MSCs [79]. This different reactivity may macroscopically reflect the diverse niches occupied by the MSCs in vivo.

In conclusion, we think that for the definition of new ISCT criteria, it could be more suitable to define a cocktail of markers appropriate for the characterization of SVF cells freshly extracted from a given tissue under well-defined conditions, prior to in vitro culture.

Recently, a mesoderm-derived precursor for mesenchymal stem and endothelial cells has been identified and named mesenchymoangioblast [52]. Hopefully, this cell and its characteristic marker apelin receptor (APLNR) will offer the opportunity to investigate the cellular and molecular pathways for the development of mesodermal lineage cells. So, soon it may be possible to work out a stem and progenitor cells hierarchy tree also for MSCs. This would enormously facilitate the work of the Mesenchymal and Tissue Stem Cell Committee of the ISCT to propose the criteria to define human MSCs.

Conclusions and Future Challenges

Not long ago, the major interest in studying adipose tissue was confined to a single cell, namely, the adipocyte. Nowadays, the complexity of adipose tissues is emerging, and the plasticity of adipose-derived progenitor cells, initially “naively” thought as a single population entity, is bringing new hopes in regenerative medicine. So far, when all data are carefully put together, it clearly appears that different types of progenitor cells are present in adipose tissue and located within the walls of blood vessels. Since

all organs and tissues are supplied by blood vessels, this should also explain why MSCs have been found almost everywhere in the human body. Furthermore, this ubiquitous mixture of multipotent progenitor cells with different developmental origin may now explain experiments done 10 years ago where miraculous "transdifferentiations" of adult stem/progenitor cells were reported to happen in vivo (reviewed in [80]).

MSCs have already found their way into the clinic and may represent a potential chance of cure for some degenerative disorders and for immune modulation [81]. The lack of common criteria and universal standards for preparation of MSCs has greatly hampered further progress and comparisons of results from different laboratories. Therefore, there is an urgent need for a comprehensive view of the identity, the developmental origin, the in vivo location, and the characteristics of the multipotent cells found within the vessels. It would be also extremely interesting to understand the hierarchy and the relationships of these cells. All this knowledge could be used in the future to define a specific technology which will allow the exploitation of the SVF extracted from fat tissue as a resource for multipotent cells for safer and more effective cell therapies.

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