Not all pericytes are born equal: Pericytes from human adult tissues present different differentiation properties

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Not all pericytes are born equal: pericytes from human adult tissues present different differentiation properties

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Abstract

Pericytes (PCs) have been recognized for a long time only as structural cells of the blood vessels. The identification of tight contacts with endothelial cells and the ability to interact with surrounding cells through paracrine signalling, revealed additional functions of PCs in maintaining the homeostasis of the perivascular environment. PCs got the front page, in the late nineties, following the identification and characterization of a new embryonic cell population, the mesoangioblasts, from which PCs present in the adult organism are thought to derive. From these studies it was clear that PCs were also endowed with multipotent mesodermal abilities. Furthermore, their ability to cross the vascular wall and to reconstitute skeletal muscle tissue following systemic injection opened the way to a number of studies aimed to develop therapeutic protocols for a cell therapy of muscular dystrophy. This has resulted in a major effort to characterize pericytic cell populations from skeletal muscle and other adult tissues. Additional studies addressed also their relationship with other cells of the perivascular compartment and with mesenchymal stem cells. These data have provided initial evidence that PCs from different adult tissues might be endowed with distinctive differentiation abilities. This would suggest that the multipotent mesenchymal ability of PCs might be restrained within different tissues, likely depending on the specific cell renewal and repair requirements of each tissue. This review will present current knowledge on human PCs and will highlight recent data on the differentiation properties of PCs isolated from different adult tissues.

1- A historical introduction

Pericytes (PCs) were initially described by the French scientist Charles-Marie Benjamin Rouget as a population of perivascular contractile cells surrounding the endothelial cells of small blood vessels. They were named Rouget cells after their discoverer, but later the term "pericytes", which refers to their location around small vessels, was introduced [1, 2]. PCs, surrounded by the basal membrane, establish intimate contacts, called peg-and-sockets, with endothelial cells (ECs). These contacts are composed by cytoplasmic elongations of PCs (pegs), invaginated into the endothelial cells membrane (sockets) [3-5]. Interactions between PCs and ECs take place through tight and gap junctions rich in connexin 43 that are present at the contact sites [6–8] and are stabilized by adhesion plaques between these cells and fibronectin in the extracellular matrix [8–10]. These multiple interactions have the role of reinforcing the communication between PCs and ECs and contribute to the correct distribution of the mechanical contractile force generated by vascular smooth muscle cells [11–14]. In the past years, different names, like mural cells, pericytes and vascular smooth muscle cells (VSMCs), have been used interchangeably to refer to all the perivascular cells that support microvasculature and establish intimate cell-to-cell contacts [15]. However, more recently, differences between PCs and VSMCs have been recognized, indicating that in spite of sharing common markers, they represent two distinct cell populations that can be distinguished depending on their specific localization and functional role [2, 11, 15, 16]. Indeed, PCs are predominantly localized around small vessels, while VSMCs are mainly positioned around larger vessels. In this latter anatomical location, VSMCs are wrapped in multiple layers perpendicularly distributed along the axis of vessels, thus providing structural support in regulating blood flow and in controlling, especially in arterioles, the diameter of the vessels. In contrast, PCs are longitudinally oriented with respect to the axis of vessels and are

generally organized in a single cell layer [11, 16, 17]. Another distinctive feature of PCs and VSMCs is their relative abundance around the vasculature. In fact, the number of VSMCs and PCs may vary depending on of the caliber of the vessel and among different tissues. For instance, in retinal capillaries, VSMCs and PCs are present in a 1:1 ratio, while in other tissues VSMCs are usually more represented than PCs [18, 19]. Moreover, VSMCs, at variance with PCs, do not establish direct contacts with ECs, as they are separated from the endothelium by the basal membrane or, in case of larger arteries, by the intima layer [11]. Based on this evidence, PCs, although considered for many years the microvascular counterpart of VSMCs, are now recognized as a well-defined perivascular cell population that plays a role in regulating homeostatic processes between the endothelial cells and surrounding tissue [20–22]. This function is of increased significance in tissues, like the central nervous system, where PCs contribute to form a functional barrier with the blood stream [23].

A novel interest in PCs was stimulated by studies that revealed that PCs are capable of differentiating into several mesodermal cell lineages, thus revealing additional and unexpected properties of these cells that have opened a new area of research oriented toward a possible use of PCs in the regenerative medicine field [21, 24–26]. These properties of PCs should not surprise, as we are aware now of the contribution provided by a number of stem/progenitor cells, including multipotent or more lineage-restricted ones, associated with blood vessels in maintaining the homeostasis of tissues [27]. Furthermore, the cellular dynamics of blood vessels have attracted increasing interest over the past years since, in addition to their physiological role, they are also involved in pathological conditions such as atherosclerosis and cancer [19, 28, 29]. Recognition of the ability of PCs to differentiate into different mesodermal lineages has also opened new questions about their relationships with others stem/progenitor cells associated to the vascular

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compartment, and more in general, with mesenchymal stem cells (MSCs) derived from connective tissue [30].

2- PCs: from the developing embryo to the perivascular compartment of adult tissues

Studies based on chimeras and cell-fate mapping indicated that VSMCs arise from distinct embryonic compartments. In fact, lineage-tracing experiments of VSMCs progenitors in the embryo and in the adult organism revealed that VSMCs might derive from neural crest, pro-epicardium, mesothelium, the secondary heart field and somitic and splanchnic mesoderm [17, 31, 32]. In a similar fashion, PCs found in the head and thymus are apparently also derived from the cranial neural crest, while PCs residing in others anatomical regions of the adult body are likely derived from the mesoderm [2, 16, 31]. In 1999, Cossu and collaborators reported the characterization of a novel cell population following in vitro culture of E9.5 mouse dorsal aorta explants. These cells co-expressed early endothelial and myogenic markers. Following the initial association of mesoangioblasts to the embryonic dorsal aorta, additional experiments based on lineage tracing indicated that these cells can originate from the hemogenic endothelium [33]. In addition, these cells were capable of giving rise to multiple differentiated cells of mesodermal derivatives, following both *in vivo* transplantation and *in vitro* culture [24]. Due to their capacity to originate both vascular and extravascular mesodermal derivatives, this cell population of embryonic aorta-associated multipotent progenitors was named mesoangioblasts [34]. However, much of the interest on mesoangioblasts, and later on adult PCs, was triggered by the initial evidence that transplant of murine mesoangioblasts contributed to the growth and regeneration of muscle fibers *in vivo* [24]. Additional experiments elegantly demonstrated also the ability of systemically transplanted mesoangioblasts to reach the developing skeletal muscle fibers through

the circulation [35]. Notably, the *in vivo* potential of mesoangioblasts were not limited only to skeletal myogenesis. Experiments on chimeric embryos, where embryonic aorta derived-cells from quail or mouse were grafted into chick embryo, revealed that mesoangioblasts initially integrated into the vasculature of the host, actively concurring to the generation of chimeric microvasculature in different tissues. Grafted mesoangioblasts were finally found as fully differentiated cell component in a broad range of mesodermal tissues [34]. Mesoangioblasts ability to travel through the bloodstream led to the hypothesis that, during embryonic development, they could migrate from the dorsal aorta along the forming blood vessels, contributing to the perivascular cell compartment of post-natal tissues [36]. On the basis, it is evident that adult PCs have some of the functional properties observed in mesoangioblasts, including the mesenchymal, endothelial and vasculogenic abilities [21, 37].

3- Stem and progenitor cells associated to the blood vessels wall

Regardless of their likely relationship with the embryonic mesoangioblasts, PCs present in adult tissues represent a population of cells of the perivascular compartment that can differentiate into different mesodermal cell types [37, 38]. A well-studied cell population that shares several properties with adult PCs is represented by the adventitial reticular cells present in the wall of the sinusoids of bone marrow tissue. Adventitial reticular cells form a layer on the abluminal side of ECs of the sinusoids, which is therefore not different from the relationship that PCs establish with EC in capillaries and small vessels [39]. A number of studies have associated adventitial reticular cells with the stromal cells of bone marrow from which the so-called “bone marrow mesenchymal/stromal stem cells” are derived [40]. The ability of these cells to generate progenitors that can differentiate in cartilage, bone and adipocytes closely resembles the properties of pericytes. Actually, both cell types express a set of common markers [41].

Adventitial reticular cells and the stromal cells of bone marrow have been shown to represent a specific cell population of skeletal stem cells devoted to bone growth and renewal as well as to participate in the establishment of the hematopoietic stem cell niche [39, 42].

Over the years, a number of studies have indicated that, in addition to PCs and to adventitial reticular cells/stroma cells of bone marrow, a number of multipotent or lineage-restricted progenitor cell populations are present in the wall of postnatal vessels, where they are essentially mainly associated with the function of maintaining the structural integrity of the vascular system. Lineage-specific progenitors mainly dedicated to the replacement of endothelial cells or to the generation of new smooth muscle cells have been identified in the arteries of adult tissues [19, 43, 44]. In addition to lineage-restricted progenitors, a number of studies have also identified multipotent stem/progenitor cells present in the wall of blood vessels. Most of these cell populations are generically defined as MSCs, yet their characterization is still lagging behind given the inherited difficulties to distinguish MSCs from PCs [29, 37, 45–48]. Other studies have resulted in the initial characterization of additional multipotent cell populations of the vascular wall that bear some relationships with PCs: adventitial cells and myogenic endothelial cells. Of note, these two cell populations share with PCs both the ability to differentiate into some mesodermal lineages and the expression of several MSC markers [28]. Adventitial cells, which are different from the “adventitial reticular cells” of bone marrow, were firstly isolated from adipose stromal vascular fraction as CD34+ CD31- CD45- CD146- expressing cells [49, 50] with a lower efficiency to differentiate into adipocytes when compared to isogenic PCs [50]. Their localization in the adventitia can easily distinguish them from typical CD34+ endothelial cells [47]. Intriguingly, adult adipose-derived adventitial cells appear to represent a source of MSCs clearly distinct from PCs [29]. Myogenic endothelial cells have been identified in vessels from skeletal muscle where they can be identified by the expression of different myogenic markers,

such as CD56 and Pax7, but also of endothelial markers, such as CD34, CD144 and vWF [51]. Myogenic endothelial cells are currently defined as CD56+ CD34+ CD144+ CD45- cells and are able to differentiate into skeletal muscle cells, chondrocytes, osteocytes and adipocytes [52, 53]. Altogether, adventitial cells and myogenic endothelial cells have the capacity to proliferate and the potential to give rise to endothelial, smooth muscle and additional mesenchymal cell types. Accordingly, the vascular wall appears to possess the characteristic of a niche-like environment, a concept of high relevance considering the involvement of the vascular compartment in physiological and pathological conditions [2, 15, 45, 54, 55].

4-PCs, MSCs and the perivascular compartment

The term MSCs was introduced by Caplan to define cells derived from bone marrow that were able to differentiate in different mesenchymal lineages including adipocytes, osteocytes and chondrocytes [56]. Following the original isolation from human bone marrow [57], MSCs have been also isolated from a wide variety of adult human organs and tissues by exploiting an isolation procedure based on the enzymatic digestion of the stromal vascular fraction of bioptic specimens [58–63]. In analogy to other adult stem cells, we still need to identify unique markers for recognizing MSCs, since all surface antigens routinely used to characterize MSCs are also expressed by a variety of unrelated cells (see ref. 41 for a compelling list of MSCs and perivascular cells markers). Accordingly, MSCs are currently defined based on both operational and functional criteria: MSCs must be adherent to plastic, must possess self-renewing ability and must be able to differentiate into at least adipogenic, osteogenic and chondrogenic lineages, to express CD105, CD73 and CD90 while being negative for the expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules [64].

In spite of extensive studies aimed to better define the in vitro properties of MSCs, once isolated from tissues, their localization and physiological role in vivo still remain to be clarified. Based on their nearly ubiquitous presence, it has been proposed that *in vivo* MSCs might reside within the perivascular compartment of adult connective tissues [36, 65, 66]. Indeed, the identification of the multipotent properties of PCs supported the hypothesis that also MSCs might have a perivascular origin [30, 65]. Studies of lineage tracing, based on NG2, PPARγ, AP and Gli1 expression, indicated that progenitors capable of differentiating *in vivo* into either osteogenic, adipogenic or myogenic cells (i.e. mesodermal progenitors) are localized in the perivascular niche of incisors, fat and skeletal muscle, respectively [67-70]. However, the relationship between these progenitors from the perivascular niche and PCs is not yet completely determined.

Evidence supporting the association of *in vivo* multipotent MSCs with the perivascular compartment has been provided in bone marrow. Here, the adventitial reticular cells that surround bone marrow sinusoids are considered, along with bone marrow stromal cells, the endogenous progenitor of MSCs, given their ability to self-renew and to fully organize the complete hematopoietic environment of bone marrow [40, 43]. Culture of adherent cells derived from adventitial reticular and stromal cells are referred to as bone marrow stromal cells or bone marrow mesenchymal stem cells.

Results from several laboratories have reported that MSCs isolated from different tissues displayed similar properties to bone marrow MSCs in terms of surface expression markers and growth rate. However, the multipotent and clonogenic abilities of MSCs from other tissues are less efficient with respect to those derived from bone marrow [71]. In this context, Peault's group reported that in vivo PCs, identified as such by the co-expression of known perivascular markers such as NG2, CD146 and PDGF-Rβ, when purified from various adult tissues and cultured *in*

vitro, retain the expression of perivascular and mesenchymal markers and also express additional MSCs markers, suggesting that the intimate connection between PCs and MSCs appears to be more than a hypothesis [37, 46, 72]. A comparison between MSCs from various sources, including bone marrow, and retinal cultured-PCs, initially indicated that also MSCs and PCs were similar in terms of immunophenotype and differentiation abilities, although they reported that CD146 was expressed only by PCs [73]. Other studies have shown that CD146 is expressed also by MSCs isolated from tissues other than bone marrow, although at variable levels with respect to PCs [74, 75], as also discussed later. A more recent compelling comparison between bone marrow MSCs and PCs has revealed that MSCs and PCs share several immunophenotypic markers and are both able to differentiate towards adipogenic, osteogenic and chondrogenic lineages, although their multipotent abilities were differentially dependent on the culture conditions [74]. Additional differences between MSCs and PCs were also observed in the analysis of the transcriptome of MSCs and PCs [74]. Evidence of differences between MSCs and PCs should not surprise, considering that MSCs are obtained without a prospective isolation and therefore represent a highly heterogeneous cell population containing progenitors with distinct differentiation abilities that vary depending on the tissue of origin [58, 76-79]. Further differences between PCs and MSCs are also supported by the evidence that cultured PCs from skeletal muscle, but not MSCs from the same tissue, were able to fuse and form myotubes in vitro, suggesting that PCs from skeletal muscle present a broader range of differentiation abilities than MSCs. In line with this finding, a more recent comparison between isogenic MSCs and PCs from adipose tissue revealed that PCs are endowed with more efficient differentiation abilities than their MSCs counterparts, although both cell populations were nearly identical in term of surface marker expression [75]. Opposite evidence in terms of differentiation abilities have been obtained in the myometrium, where PCs can only differentiate toward smooth muscle cells [80],

while MSCs are able to differentiate towards adipogenic, osteogenic and chondrogenic lineages [81]. Experiments with MSCs isolated from dental pulp and adipose tissue indicate that not all MSCs isolated from those tissues are derived from PCs [29, 68]. Based on this evidence, one can conclude that, although the perivascular compartment contains mesodermal precursors like PCs and MSCs, a direct relationship between PCs and MSCs, although possible, still remains to be formally proved in most tissues.

5- PCs: surface markers expression and procedures for isolation and in vitro culture

PCs are currently isolated from human adult tissues using two different procedures: selection of perivascular cell populations by fluorescent activated cell sorting (FACS) or selection of weakly adhering cells that emerge from culture of explants of biopsies enriched in small blood vessels. In some cases, these two approaches have been combined. Several markers associated to cells present in the perivascular compartment are currently known (see ref. 41 for a compelling list of perivascular cells markers). Nevertheless, markers that can be univocally associated to PCs are still missing. Studies performed on tissue biopsies have shown that PCs in vivo can be identified by three main markers. A widely used markers for characterization of PCs is the cell adhesion molecule CD146, which also recognize adventitial reticular cells of the bone marrow [40] and is also present on cell surface of additional cell populations such as endothelial progenitors of the VSMC lineage [82], but also mature endothelial cells [83]. A second marker is Neural-glial-2 (NG2), a proteoglycan also expressed by progenitors of oligodendrocytes and chondrocyte [84, 85] and that is present on mural cells during vascular morphogenesis [86]. The last marker used for PCs identification is the platelet derived growth factor receptor-beta (PDGFRß), the receptor for a powerful mitogen of cells of mesenchymal origin that also plays a pivotal role in vessel formation, being involved in differentiation and proliferation of both PCs and VSMCs [87, 88].

In situ PCs, also express α-smooth muscle actin and desmin, in addition to other mesodermal markers such as CD73, CD90 and CD105. Finally, *in vivo* PCs can be identified as they do not express endothelial and hematopoietic markers such as CD31, von Willebrand Factor, CD34 and CD45 [37, 38]. PCs also express alkaline phosphatase (AP), although its expression has been formally observed *in vivo* only in skeletal muscle PCs [88]. Based on the combination of markers expressed by PCs in vivo, these cells have been purified from the stromal vascular fraction of adipose tissue as a population of cells positive for CD146 and negative for CD34, CD45 and CD31 by using multicolor FACS. Following expansion *in vitro,* purified pericytes also express NG2, PDGFRß and other mesodermal markers leading to the definition of a compelling PCs molecular signature based on the expression of CD146, NG2, PDGFRβ, AP and the absence of CD34, CD45,vWF and CD144 [37]. Cell sorting based on these markers has been also exploited to isolate PCs from human fetal tissues, endometrium, heart and also from skeletal muscle [37, 89].

PCs from adult human skeletal muscle tissue are usually isolated following the technique of culture of explants starting from tissue biopsies. Nearly two weeks after the initial plating, small round-refractile cells start to emerge from the cultured explants. These cells are then harvested and expanded to obtain cultured PCs [38]. PCs derived by explant-culture from skeletal muscle or from other adult tissues share the known surface marker profile identified *in vivo* and on FACS-purified cells [75, 80, 90]. A combination of the explant-culture protocol and FACS selection has been more recently introduced to isolate skeletal muscle PCs. This approach is based on the selection of AP-expressing cells from the population of weakly adhering cells emerging from skeletal muscle explant-culture. Of note, this isolation procedure generates a

population of cultured PCs again expressing the same molecular signature reported above [88, 91].

6- PCs from skeletal muscle

The increased attention on the multipotent properties of PCs stems from the seminal work on embryonic mesoangioblasts [24, 34]. The characterization of mouse embryo mesoangioblasts and later of postnatal adult PCs, provided solid evidence that these cells, even when delivered systemically, are able to colonize skeletal muscle and to differentiate in healthy muscle fibers resulting in a significant functional recovery in dystrophic mice [92]. Mesoangioblasts, in fact, express a subset of integrins and receptors that efficiently allow both migration through the blood stream and vascular wall crossing [35, 93]. In the following years, cell populations endowed of myogenic potential have been isolated also from canine's post-natal skeletal muscle biopsies. In analogy with previous results obtained in dystrophic mice, systemic delivery of healthy donor dog mesoangioblasts resulted in the improvement of muscle function in dystrophic dogs [94]. Finally, Cossu and coworkers used explant cultures of adult skeletal muscle biopsies to isolate human adult PCs [90]. Cultured PCs from human skeletal muscle, in line with mouse and dog adult cells, displayed a strong spontaneous myogenic potential *in vitro* and were also able to fuse with mouse muscle fibers once injected in the femoral artery of scid-mdx immunodeficient mice. In addition, cultured PCs differentiated *in vitro* also toward adipogenic and osteogenic lineages, thus combining biological properties of original mesoangioblasts [90].

The therapeutic potentials of PCs in contributing to skeletal muscle tissue regeneration when injected in preclinical models of muscular dystrophy reflect a physiological role of these cells. In fact, recent work by several groups has shown that in skeletal muscle tissue PCs are part of a pool

of cells that, in addition to the "proper" myogenic precursors (i.e. satellite cells), can directly [88, 95] or indirectly [96, 97] contribute to the process of muscle repair and regeneration. Indeed, lineage tracing of skeletal muscle PCs showed that these cells are able to enter the skeletal muscle niche compartment in the early stages of postnatal growth and to contribute to the formation of the adult satellite cell compartment. These PC-derived satellite cells can fuse into developing skeletal muscle fibers and contribute to normal muscle growth in unperturbed conditions during adult life and, even more, participate to regeneration of skeletal muscle tissue following injury [88]. Altogether these data indicate that the observed ability of isolated and cultured PCs to enter the myogenic cell-fate, when used for therapeutic protocols, reflect a tissue-specific commitment of these cells. Based on the results obtained in murine and canine preclinical models of muscular dystrophy, a phase I/IIa clinical trial using cultured PCs was recently started. This trial aimed to treat pediatric patients affected by Duchenne muscular dystrophy with the intra-arterial infusion of HLA-matched allogeneic PCs obtained from adult skeletal muscle of healthy donors. Unfortunately, the engraftment of the transplanted PCs was minimal and the overall efficacy of this treatment was negligible in terms of functional recovery, as compared with results obtained in preclinical models. Nevertheless, this study indicated that a cell therapy approach based on infusion to each patient of hundreds of millions PCs can be considered safe, providing an encouraging outcome for future improvements of the procedure [98].

7- PCs from other adult human tissues

The identification of multipotent PCs in skeletal muscle, the encouraging outcomes obtained in preclinical animal models and the emerging role of pericytes in tissue regeneration in physiological and pathological conditions, inspired additional studies aimed to the isolation of

PCs from other human adult tissues. PCs have been isolated by FACS as CD146+ CD34- CD56- CD45- cells from the stromal vascular fraction of adipose tissue from where a large number of PCs can be obtained [37, 72]. Adipose tissue PCs-derived cells are clonal cells able to give rise to adipocytes, osteocytes and chondrocytes. When injected in skeletal muscle of cardiotoxin-treated mice, they are also able to contribute to fiber regeneration, although evidence for spontaneous skeletal muscle differentiation has not been provided. A more compelling analysis on adipogenic potential of distinct stromal cell populations revealed that pericyte-derived cells display a stronger tendency to differentiate into multilocular adipocytes than endothelial progenitors, supra adventitial-cells and mature endothelial cells [50]. More recently, Lauvrud et al. further confirmed that adipose PCs-derived cells expressing CD146 are endowed with a more robust adipogenic potential than their CD146 negative counterpart [99]. Interestingly, FACS-sorted adipose PCs-derived cells improved fracture healing when percutaneously injected in an atrophic non-union rat model [100], giving promising results also for future clinical exploitation of the osteogenic potential of adipose PCs. In this context, the sorting procedure proposed by Crisan et al., which is considered the most accurate approach to isolate adipose PCs by FACS [46], has been slightly improved to obtain a clinically relevant number of cells without the need of expansion in culture. PCs from adipose tissue have been also isolated as floating cells outgrowing from explant-culture of fat tissue biopsies, in strict analogy with the procedure used to isolate PCs from skeletal muscle. These cultured PCs express the classical pericytic markers, including CD146, AP and NG2, while do not express the endothelial markers CD31 and CD34. They are able to differentiate into adipocytes, osteocytes and smooth muscle cells, while they are unable to spontaneously differentiate into skeletal muscle cells unless when co-cultured with an established myogenic cell line [75]. As a matter of fact, PCs from adipose tissue either isolated through FACS selection or explant-culture appear to possess comparable characteristics.

Interest in identifying cardiac stem cells also triggered studies on the isolation of human PCs from heart. Resident microvascular heart PCs were identified as cells surrounding microvessels and capillaries, which co-expressed CD146, NG2, PDGFRß and were negative for endothelial markers. These cells were further isolated by FACS starting from the myocardium using a positive selection for CD146 and a negative selection for CD34, CD45, CD56, CD117, in strict analogy with PCs from adipose tissue. Cultured heart PCs displayed null skeletal myogenic potential and limited cardiomyogenic capacity, being only committed to an immature cardiomyocitic phenotype. In contrast, heart PCs could be robustly differentiated towards adipogenic, osteogenic and chondrogenic lineages [101]. Murine heart cell populations, endowed with strong cardiomyogenic ability, have been isolated by FACS as CD34 and NG2 positive cells or, following colony-forming assay, as CFU-Fs. Of note, CD34+ NG2+ cardiomyogenic cells failed to differentiate towards additional mesenchymal lineages, while cardiac CFU-Fs displayed adipogenic, osteogenic and chondrogenic potentials [102, 103]. These data indicate that, although homologous cell populations have not been isolated yet from human heart, distinct progenitors with different differentiation potentials can be found in the perivascular compartment of the heart. Intriguingly, heart cell populations that expressed pericytic markers appear to be selectively committed towards the cardiac phenotype.

A tissue characterized by the presence of a predominant smooth muscle tissue component is the uterus. The presence of PCs around microvessels in biopsies of endometrium has been revealed by immunostaining for CD146 and PDGFRß. Accordingly, these cells have been isolated by FACS, using CD146 and PDGFRß as selection markers, starting from the enzymatic digestion of endometrial biopsies. Endometrial PCs were capable to differentiate into multiple mesenchymal lineages, including smooth muscle. A comparative transcriptomic analysis between endometrial

PCs, endometrial fibroblasts and endometrial endothelial cells revealed that freshly isolated PCs express genes involved in smooth muscle differentiation at higher levels with respect to the other cell populations [104]. PCs from uterus have been more recently isolated by explant culture of myometrial biopsies. These PCs from myometrium are similar to the ones isolated from the endometrium in terms of morphology and surface marker expression [80, 104]. Nevertheless, myometrial PCs can readily differentiate only into smooth muscle cells, while they were totally incapable to differentiate in other mesenchymal lineages, including skeletal muscle differentiation. Both myometrial and endometrial PCs express Notch receptors [80, 105], whose activation is known to be involved in the development of endometrium as well as in PCs differentiation [21, 106, 107]. However, inhibition of notch signaling had no effect on the properties of myometrial PCs while it affected both gene expression and proliferation of PCs from the endometrium [80, 108].

Based on the reported evidence, it appears that PCs from distinct tissues are endowed with different mesodermal potentials, although they are virtually indistinguishable in terms of morphology and marker expression. In addition, when considering adipose tissue, skeletal muscle, heart and uterus, PCs appear to retain a preferential commitment towards the main cell type of the tissue where they reside. Intriguingly, this aspect has also been observed in bone marrow, where in situ adventitial reticular cells, which can be considered the bone marrow equivalent of PCs, showed a preferential tendency to differentiate into bone [40]. Very recently, Sacchetti et al. performed a compelling comparison between CD146+/CD34-/CD45- cells isolated from different human tissues [109]. These authors reported that, in addition to marked differences between the cell populations analyzed in terms of transcriptomic signature, bone marrow-derived cells were skeletogenic but neither myogenic nor chondrogenic, while skeletal muscle-derived cells were myogenic, but not skeletogenic. Additional analysis of the

differentiative properties of perinatal cord blood-derived cells indicated that these cells were chondro-osteogenic but not myogenic, further supporting the idea that perivascular/MSCs progenitors can be endowed with differentiation abilities closely related to the tissue of origin. However, it should be noted that cell populations used by Bianco and collaborators were obtained using a prospective isolation protocol slightly different from the one described by Peault's group [37, 46]. In addition, previous studies based on different isolation strategies, culture conditions and differentiation assays have provided results that differs from those reported by Sacchetti et al. [109-111]. Indeed, one should always be aware that even minimal changes in isolation and culture protocols might induce small variations in the cell populations obtained, which might then cause differences in the observed properties of the resulting cells. Nonetheless, even when using the same prospective isolation protocol, Sacchetti et al. [109] noted that only PCs from skeletal muscle were capable to spontaneously differentiate into skeletal muscle cells. In this context, it is worth noting that iPSCs obtained by reprogramming skeletal muscle PCs are endowed with better skeletal muscle differentiation efficiency than iPSCs obtained by fibroblasts reprogramming [112, 113]. On the other hand, contrasting evidence on the ability of pericytes and related cell populations from brain to spontaneously differentiate into neuronal lineages have been reported [114- 117]. Altogether, currently available data on PCs isolated from adipose and cardiac tissues, endometrium, myometrium, bone marrow and skeletal muscle appear to be more prone to differentiate toward the cell type specific of the tissue from where they were isolated.

8- Concluding remarks

In this review we attempted to describe the state of the art on PCs from adult human tissues with respect to their identification and their differentiation properties. Cells capable of differentiating

in different lineages of mesodermal origin have been isolated from several tissues of mice and humans. Among these cells, PCs have recently attracted the interest of several research teams because of their potential applications in cell therapy protocols and also for representing a potential source for MSCs. However, in spite of the high interest and of intensive investigation, we are still far from having attained a unique idea on the overall identity of PCs. In spite of the lack of specific surface markers that would allow to unambiguously label homogeneous populations of PCs, these cells can be identified *in vivo* as non-endothelial cells ($CD34^-$ and $CD31^-$ cells) that natively express perivascular and mesenchymal markers (NG2, AP, PDGFR-β, CD44, CD73, CD90, CD105 and CD146) [37, 38, 72, 91]. However, these markers are not always all present on PCs from adult human tissues [41, 72, 118]. Prospective isolation of PCs can be obtained selecting for $CD146^+$ $CD34^-$ $CD45^-$ $CD56^-$ cells [37, 46]. Once in culture, these cells retain the above-mentioned markers, but also express additional mesenchymal markers [37, 67]. Nevertheless, these markers do not allow establishing a clear relationship between PCs and MSCs, and between PCs and the numerous progenitor cell populations found in the perivascular compartment or in the connective tissue of the different organs of the adult organism. Initial results indeed claimed that PCs isolated from various tissues might share common traits including growth properties, surface marker expression and differentiation potencies. This initial enthusiasm has been tempered by more recent data obtained by different groups, which while contributing to expand our knowledge on PCs' biology, also have added additional information suggesting that PCs may significantly differ from MSCs [29, 68]. Furthermore evidence has been provided indicating additional variability among PCs populations. In this context, recent data suggest that even within the same tissue pericytes may differ on the basis of specific properties that include the type and size of blood vessels of residence and the interactions that they establish

with other the cells [28, 72, 118]. In addition, pericytes within a given tissue may functionally behave differently under pathological conditions [119-121].

In conclusion, work form several laboratories during the last few years has provided a large body of information on the properties of PCs. Noteworthy, converging evidence derived from studies mainly performed on PCs isolated from skeletal muscle, bone marrow and adipose tissue, and new results obtained from additional tissues including endometrium, myometrium and cardiac tissue, suggest that PCs isolated from different tissues, even if might present a varied range of differentiation properties, appear to have a characteristic preference to differentiate toward the specific cell type of the tissue from where they have been isolated. This is likely due to the local cues imposed by the environment where PCs reside, which might reflect the cell renewal and repair processes required for maintenance of the "tissue homeostasis" of each specific tissue.

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