REVIEW

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Transdifferentiations and heterogeneity in the stromal niches of uterine leiomyomas

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Abstract

Uterine leiomyomas, also known as uterine fibroids (UFs), are benign smooth muscle cells tumors, the most frequent tumors in women. Even though UFs are monoclonal tumors, they contain a heterogeneous and versatile cells population. There are scarce proofs about the processes of transdifferentiation that might occur in UFs, modify the tumor microenvironment and support blood and lymph vessels formation. The stromal niches of the UFs harbor cells with angiogenic/lymphangiogenic, as well as with vasculogenic/lymphvasculogenic potential, which belong to a phenotypic continuum between the endothelial and mesenchymal lineages. Within these niches, the expressions of CD44 and podoplanin were less investigated and regarded as markers of such processes of transdifferentiation.

Keywords: uterine leiomyomas, CD44, podoplanin, vasculogenesis, telocytes, lymphangiogenesis.

Introduction

Uterine leiomyomas, also known as uterine fibroids (UFs), are benign smooth-muscle cells tumors and represent the most frequent tumors in women [1–3]. They are regarded as a major public health problem [4], but the degree to which UFs contribute to infertility is controversial [5, 6]. Even though UFs are monoclonal tumors [1, 2], they contain a heterogeneous cells population, with smooth muscle cells and fibroblasts being the major constituents [7]. Although the etiology of UFs is unknown, it was hypothesized their development from myometrial cells transitioning into a fibrotic tissue with myofibroblastic characteristics [3]. The cell composition is different in small and large UFs [7]. The microvascular bed in UFs is reduced compared to the normal myometrium [8] in larger, but not seedling fibroids [7].

There are scarce proofs about the processes of transdifferentiation that might occur in UFs, modify the tumor microenvironment and support blood and lymph vessels formation. However, the stromal niches of the UFs harbor cells with angiogenic/lymphangiogenic, as well as with vasculogenic/lymphvasculogenic potential, which belong to a phenotypic continuum between the endothelial and mesenchymal lineages.

Tumor-associated stromal cells

The tumor niches are inhabited by a heterogeneous population of cells, the bulk tumoral cells and supporting cells; the latter [endothelial cells (ECs), pericytes, adipocytes, fibroblasts, mesenchymal stem cells (MSCs)] are recruited from the nearby stroma and are involved in tumor angiogenesis, proliferation, invasion, metastasis and therapeutic resistance [9].

CD44, podoplanin, tumor-initiating cells

A subpopulation of cells in leukemia and several solid tumors of epithelial origin have been termed cancer stem cells (CSCs), or tumor-initiating cells (TICs), or cancer-initiating cells (CICs) [10, 11]. TICs are characterized by high clonal expansion capacity and are responsible for generating and sustaining tumors, being resistant to chemo- and radiotherapy [12–15]. Moreover, TICs are potentially the cause of tumors that reiterate the histology of primary tumor at remote locations, which they reach through the lymphatic or blood vessels [14]. TICs grow on serial transplantation in xenogeneic models [11]. Podoplanin, CD15, CD44, CD49f, CD105, CD133 and p63 are TICs-specific markers [10, 15–20]. p53 was shown to be a TIC-suppressor that binds to the promoter of CD44 and represses its expression [21]. However, p53 represses the expression of more than 20 target genes that may contribute to the maintenance of the pool of TICs [21].

Podoplanin is a TIC-specific marker for the cell line A431 found in human squamous cell carcinoma [13].

Podoplanin-expressing A431 cells share the expression of sonic hedgehog and CD44 with stem cells in normal squamous epithelium [13]. Podoplanin-expressing TICs have a high clonal expansion capacity resulted from a decreased cell death through podoplanin-mediated signaling [12].

As TICs frequently express CD44, the question on what are the effects of this protein was raised but, as the association of them is a recent finding, most information regarding the function of CD44 is rather circumstantial [11].

The CD44 molecule and uterine leiomyomas

CD44 is a polygamic cell adhesion glycoprotein molecule with the main ligands being hyaluronic acid (HA) and osteopontin [22–28]. CD44 is a well-known marker for stem cells and cancer stem cells [23, 24, 29–33]. The smallest CD44s isoform (standard or hematopoietic) is ubiquitously expressed on the membrane of most vertebrate cells of mesodermal and hemopoietic origin and is composed of 341 amino acids with a molecular mass of 85–90 kD [11, 34, 35]. Between the extracellular domains 5 and 6, can be inserted, through alternative splicing, up to 10 variant exon products, which can generate CD44 variant (CD44v) isoforms with distinct molecular weights [11, 34, 36, 37].

CD44 expression in uterine tumors

Altered CD44 expression was associated with tumorigenesis, carcinogenesis, and prognosis of tumors [38]. Its expression is decreased in leiomyosarcomas compared to leiomyomas and normal myometrium, which was considered with prognostic significance [38]. Moreover, CD44v3 is expressed in normal tissues and UFs, but the expression is lost in leiomyosarcomas, which might serve as diagnostic clue [5].

The expressions of CD44s, CD44v3, and CD44v6 are cyclic in the normal menstrual cycle, being downregulated in the proliferative phase and upregulated in the secretory phase [39]. Thus, the expression of CD44 is related to differentiation or maturation of endometrial glandular cells during the menstrual cycle [39]. Saegusa *et al.* showed a reverse correlation between the expression of CD44 and hormone receptor status potentially implying a link with ovarian hormones [39].

However, we could not identify in that report if, or how, the endothelial expression of CD44 was distinguished from the tumor cells expression of that marker. This is important for diagnostic purposes because CD44v3 is expressed in human immortalized and *in vivo* ECs, in which is mainly located in the cytoplasm, and only has a limited, filopodial, surface expression, being suggested to have a role in tumor-induced angiogenesis [40]. A CD44v3 blocker could prevent leukocyte extravasation by blocking CD44v3 on ECs [41].

Therefore, a reduced or absent expression of CD44 isoforms in leiomyosarcomas could equally indicate a decreased vascular or angiogenic potential in these tissues. In these regards, it is to be noted that different studies found a decrease of the CD31 expression and microvessels density in large UFs compared to incipient UFs [7]. The vascular area and microvessels density are reduced in UFs compared to normal myometrium [8], and in uterine smooth muscletumorscompared to normal myometrium [42].

CD44 in the stem niche of uterine fibroids

CD44 is a typical MSC marker, as are CD73, CD90 and CD105 [43]. A study performed on samples of human myometrium and UFs evaluated the presence of Stro-1+/CD44+ isolated myometrial/fibroid stem cells that were ABCG2+/Oct4+/Nanog+/GDB3+ undifferentiated cells, corresponding to the minimal standards of identification for MSCs: they expressed CD73, CD90 and CD105, did not express hematopoietic stem cells (HSCs) markers, and were able to differentiate towards the adipogenic, chondrogenic and osteogenic lines [1]. Such Stro-1+/CD44+ cells formed fibroid-like lesions when xenografted [1]. Therefore, CD44 and Stro-1 were assumed as putative MSCs markers in UFs [1].

However, the expression of Stro-1 in stromal cells, which is commonly regarded as a MSCs marker, is an induced event, as Stro-1 is intrinsically an endothelial antigen [44–46]. Therefore, UFs cells identified as Stro-1+/CD44+ could be either endothelial descendants or ascendants.

CD44, an endothelial and vasculogenic molecule

Endothelial expression of CD44

CD44 has a specific role in the normal functioning of ECs [47]. Endothelial cells have specialized plasma membrane microdomains containing the non-specific protein caveolin-1 [48]; HA recruits CD44 into these caveolin-enriched microdomains and CD44 interacts in these situses with the underlying actin cytoskeleton [49].

CD44 mediates several of its effects on endothelial through the modulation of adhesion protein expression [25]. It mediates the vascular barrier integrity by regulating CD31 expression and mediates apoptosis and the proliferation of microvascular ECs by modulating CD31 and vascular endothelial (VE)-cadherin expression, and the Hippo pathway [25].

The interaction between CD44 and its ligand HA enhances the pro-apoptotic effect of transforming growth factor-beta 1 (TGF-β1) but does not enhance the effect of the anti-angiogenic thrombospondin-1 on ECs, contributing to the degeneration of the capillary network [28].

Although there are discrepancies regarding the expression of CD44 on ECs, which is variably reported as either negative or positive, it was demonstrated that CD44 is an activation antigen on human ECs and has an enhanced expression in solid tumors, the ECs being efficiently killed by targeting a specific immunotoxin to CD44 [50]. A strong endothelial expression was found for variant isoforms v5, v7–8, and v10, while the expression was weaker for v3 and v6 [51].

CD44 contributes to the organization and stabilization of the endothelia of forming or newly formed vessels, and the loss of its endothelial expression impairs *in vivo* angiogenesis [47].

Neovessels of uterine fibroids

Neovascular structures are a common finding in UFs

[52]. In adults, endothelial regeneration neovessels formation occurs either by angiogenesis from preexisting resident endothelia, or by vasculogenesis from endothelial precursor cells [53]. Angiogenesis occurs by sprouting and non-sprouting (intussusception or elongation) mechanisms [54].

Mature hematopoietic cells, such as are myeloid cells, derive from HSCs and hematopoietic progenitor cells (HPCs) from the bone marrow (BM); monocytes, granulocytes, platelets and even HSCs/HPCs have been shown to be involved in vascular repair, is thus difficult to discriminate from circulating endothelial progenitor cells (EPCs) [53]. Although CD45 is a common marker for hematopoietic cells, it was also found expressed in EPCs [53].

Vascular endothelial growth factor (VEGF) facilitates both vasculogenesis and angiogenesis [55–57], controls lymphatics' growth [57], and is produced in many solid tumors [57]. Vascular endothelial growth factor receptor-3 (VEGFR-3) is an angiogenetic mediator for lymphatics [57, 58]. Even though a strong VEGF reaction was detected in the cytoplasm of uterine smooth muscle cells and UF, the VEGF expression was not correlated with the clinical/pathological parameters in UF or myometrium, and no relation between VEGF expression and the vascular area, microvessel density and vascular luminal area was found [8].

The expressions of VEGF-C, its receptor VEGFR-3, and CD44 were researched using feline mammary samples and between the expression of CD44, and the number of lymphatic vessels with VEGFR-3a, was identified an inversely proportional correlation in malignant infiltrating tumors [57]. It was, therefore, suggested limited biological importance of the intratumoral lymphatics [59].

CD44, matrix metalloproteinases (MMPs) and neovessels formation

Pericellular proteases, such as the MMPs, play important roles during the processes of neovessels formation [60]. MMP-9 stimulates angiogenesis and localizes to the membranes by binding to the CD44 [60]. MMP-9 acts as a processing enzyme that cleaves CD44; this event quickens cell motility, and inhibition of either CD44 or MMP-9 inhibits cell migration [61]. During fibrosis, the pool of myofibroblasts is supplied by epithelial-to-mesenchymal transition (EMT) processes, in which structural changes are regulated by the receptor for advanced glycation end-products (RAGE) [62]. The RAGE expression is decreased after cytokine stimulation, with the release of its soluble isoform *via* an MMP-9-dependent mechanism; in pulmonary fibrosis, RAGE is markedly decreased, while the levels of CD44 are enhanced [62].

The CD44 expression is associated with vasculogenic structures

The functions of CD44 include the modulation of the cell to cell and cell to matrix interactions, the activation of cell survival responses, the induction of cell motility and the activation of lymphocytes and monocytes [25, 36, 63, 64]. CD44 mediates an HA-dependent cell adhesion that promotes invasion, but also increases neoangiogenesis, and secondarily stimulates tumor cell proliferation [57].

The role of HSCs and BM-derived EPCs is largely accepted [65]. BM-derived EPCs are critical regulators of the angiogenic switch and are recruited luminally to the neovessels in metastases during the progression from micrometastases to macrometastases [66, 67].

In tumors, neovessels equally result from the pre-existing vasculature and by *de novo* luminal recruitment of BM-derived EPCs [68]. Early tumors recruit in their periphery BM-derived EPCs that further differentiate in ECs and incorporate into angiogenic sprouts; in late tumors, BM-derived vessels are diluted with non-BM-derived vessels from the periphery [68]. These BM-derived EPCs express VE-cadherin, $CD31_{low}$, CD105 and CD133 [68]. A different study identified, but without testing whether they are of extratumoral origin, non-tumorigenic CD133-expressing endothelial stem cells (ESCs) in human ovarian cancer cells, which are attracted by TICs and thus augment tumor development through their capacity of establishing an entire endothelial cell hierarchy [66].

Several subsets of monocytes-derived circulating progenitor cells were classified and include early and late EPCs [69]. ECs derived from cultured monocytes simultaneously express macrophage/monocyte markers (CD68, CD80, CD45, CD36) and EC markers (CD31, von Willebrand factor, Tie-2) [70]. VEGF and angiopoietin-1 could be regarded as critical inducers of monocyte differentiation toward the endothelial cell lineage [70].

Aggressive tumor cells can transdifferentiate into cells with endothelial features and are able to generate vasculogenic networks by vasculogenic mimicry, which may increase tumor malignancy and lead to a poorer prognostic [24]. The CD44/c-Met cascade is heavily relevant for the vasculogenic mimicry, CD44 being over-expressed in aggressive tumors [24]. The standard isoform CD44s and the splice variant CD44v6 were related to the increased aggressiveness in vascular mimicry, being also observed that CD44 expression is associated with vasculogenic structures [24]. Tumor cells increase their chances of survival by adopting mechanisms appertaining to angiogenic endothelial cells [24].

Transdifferentiation processes make the stem niches versatile

MSCs are versatile players that generate variable molecular and morphological phenotypes within the stem niches.

The mesenchymal–endothelial transition contributes to neovascularization

Postischemic fibroblasts were shown to adopt an endothelial fate by gaining the anatomical and functional phenotype of endothelial cells through mesenchymal-to-endothelial transition (MEndT), which contributes to neovascularization [71]. In these regards, telocytes (TCs), which were previously suggested to belong to the endothelial lineage [72–74], could be intermediate stages during such a MEndT. It is, however, difficult to assess the phenotypic switch involved, as an endothelial-to-mesenchymal transition (EndMT) could occur instead of a MEndT [75]. Moreover, the endothelial differentiation potential of MSCs is debatable and may be contingent on the origin of the tissue [43]. *In vitro* experiments indicate a mesodermal origin of MSCs from precursors with angiogenic potential, namely

mesenchymoangioblasts, which should be further tested *in vivo* conditions [76].

BM-derived MSCs promote angiogenesis as well as lymphangiogenesis

BM-derived MSCs promote angiogenesis through the recruitment of EPCs, the differentiation into ECs and pericyte-like cells, the secretion of soluble angiogenic factors, such as VEGF-A or basic fibroblast growth factor (bFGF), and the release of exosomes [77].

BM-derived MSCs stimulate lymphangiogenesis through a direct effect on lymphatic endothelial cells (LECs) *via* the secretion of VEGF-A [77]. As they are important sources of VEGF-A, MSCs and macrophages exert synergistic effects on *in vivo* lymphangiogenesis [77]. MSCs also express other lymphangiogenic factors, such as epidermal growth factor (EGF), fibroblast growth factor-2 (FGF-2), hepatocyte growth factor (HGF), and insulin-like growth factor-1 (IGF-1) [78].

MSCs acquire a lymphendothelial phenotype

Adult lymphangiogenesis is limited to sites of chronic inflammation, tissue injury or remodeling and cancer, and is initiated by the activation of LECs' VEGFR-3 by its ligands, VEGF-C or VEGF-D [79]. These VEGFR-3 ligands can be produced by immune cells, stromal cells, epithelial cells or malignant cells [80]. Tumor-induced lymphangiogenesis is essential for metastasis, as the spread of the cancerous cells typically begin with lymphatic-assisted cell passage to lymphatic nodes [81].

Conflicting evidence indicates that tumor-associated lymphatics may be primarily formed from foregoing lymphatic networks through a process of vessel sprouting [82].

MSCs may express a lymphatic phenotype when put in contact with VEGF-C and lymph-inductive media [82]. Adult lymphatic endothelial progenitor cells (LEPCs) derive not only from MSCs, but also from other precursors, such as HSCs, adipose-derived stem cells (ADSCs), and myeloid stem/progenitor cells (EPCs, myeloid-derived LEPCs) [79]. The LEPCs coexpress: (*a*) markers of their parent lineages (mesenchymal or myeloid), (*b*) stem/progenitor markers and (*c*) lymphatic-specific markers, such as are podoplanin or lymphatic vessel endothelial receptor-1 (LYVE-1) [79]. Myeloid LEPCs thus express the common HSC markers CD133 (prominin) and CD34, but the expression of CD117/c-kit and Sca-1 was also reported [65, 79, 83]. It is interesting to note in this context that TCs, stromal cells with a peculiar morphology, are consistently reported to have CD34+ and c-kit+ and Sca-1 phenotypes [72, 84–97].

The CD68+ osteoclast-like giant cells (OLGCs) in uterine leiomyoma

OLGCs are rarely encountered in the uterus, but described in leiomyosarcomas, and also uterine leiomyoma, when they expressed CD68 [98]. Such tumor-associated OLGCs have a controversial origin, either from osteoclasts, epithelial cells, or macrophages/monocytes/histiocytes [99]. It was demonstrated that OLGCs differentiated from BM-derived macrophages promote tumor growth and lymphangiogenesis by secreting VEGF-C [100].

The endothelial–mesenchymal transition

The EndMT regulates pathological processes, such as cancer and fibrosis [101–103]. Most commonly, TGFs-β and bone morphogenetic proteins (BMPs) stimulate the processes of EndMT, which, in turn, are inhibited by VEGF-A, fibroblast growth factor receptor 1 (FGFR1), and various microRNAs (miR-15a, miR-23b, and miR-199a) [101]. The EndMT can generate fibroblasts, cancer-associated fibroblasts (CAFs) and CSCs [101, 104]. The EndMT is not the only mechanism generating fibroblasts and fibrosis, as they could equally derive from resident fibroblasts, or from BM-derived circulating progenitor cells, monocytes and fibrocytes [105]. Such CD34-expressing fibrocytes are predominantly bipolar in uterus [106], their phenotype being mostly identical to CD34+ TCs [93].

Endometrial miR-200c regulates the expression of angiogenic factors such as VEGF-A, FLT1 (VEGFR-1), and fibulin 5 (FBLN5), and it undergoes dynamic changes during the transition from normal into cancerous states [107]. It was assessed that miR-200c, which plays central roles in EMT and mesenchymal-to-epithelial transition (MET) [108], is aberrantly expressed in UFs [3]. miR-200c induces the senescence and apoptosis of ECs [109]. In turn, ECs apoptosis can cause EndMT through the upregulation of TGF-β1 in both apoptotic and viable ECs [101, 110].

The mesothelial–epithelial transition

Among the targets of the miR-200 family are the Zn-finger transcriptional repressors ZEB1 and ZEB2, and it was demonstrated that a miR-200/ZEB double-negative feedback loop regulates the processes of mesothelial-to-epithelial transition (MesoET) [108]. On the other hand, the mesothelial cells co-express mesenchymal and epithelial markers [108], being thus able to supply tissues with mesenchymal cells resulted from EMT processes.

Mesothelial cells, as well as ECs, are mesodermally-derived simple squamous epithelial cells [111]. In these regards, a direct influence of miR-200c on the EndMT processes in UFs is suggested, which is supported by the miR-200c positive influence on cancer ECs migration and tumor angiogenesis [112].

Interestingly, miR-200c was found equally involved in the biology of cancer, as well as normal stem cells: miR-200c-141, miR-200b-200a-429, and miR-183-96-182 were found to be downregulated in normal mammary stem cells, in human breast CSCs, and in embryonal carcinoma cells; moreover, miR-200c modulates the expression of BMI1, a known regulator of stem cell self-renewal [113].

The endothelial–hematopoietic transition

In zebrafish embryos was shown that HSCs emerge from the endothelial tube through a stereotyped process, which encompasses a strong twisting followed by egress of single endothelial cells from the ventral vascular wall into the perivascular space, and their simultaneous conversion toward HSCs [114]. A distinct perivascular niche of HSCs and HPCs was suggested recently, being indicated that human CD146+ perivascular cells could be the equivalents of the perivascular hematopoietic niche [115]. On the other hand, CD146, as well as Stro-1, are well-recognized markers of MSCs [73, 116–120], which,

in turn, could derive either from ECs or pericytes (PCs) [121–124].

A subset of pericytes could derive from endothelial cells

Weibel–Palade bodies (WPBs) are characteristic to ECs [74]. However, dense bodies similar to the ones found in endothelial cells were also identified in pericytes within the dental pulp stem niche [124]. As also partly mural and partly perivascular transitional cells were found harboring such WPBs-like [124], an endothelial origin of these WPBs-containing non-ECs is suggested. As processes of pericyte–(myo)fibroblast transition (PFT) occur in various tissues [125–129], a complete multistage continuum from ECs to stromal cells is pictured. PFT significantly contributes to tumor invasion and metastasis [130].

The endothelial–mesenchymal–endothelial cycle

A two-steps cycle of cell-fate transition, in which EndMT leads to the formation of MSCs, which, in turn, differentiate back into endothelial cells through a MEndT, was proposed as a circuit controlling the reversibility in cell-fate determination [59].

Uterine telocytes at a glance

Most ultrastructural features of TCs, formerly known as interstitial Cajal-like cells (ICLCs) [131], are similar to those of the LECs.

Creţoiu *et al.* found evidence of myometrial TCs [132], but the authors failed to indicate why these TCs are not LECs; when that paper [132], as well as other papers dealing with uterine TCs [94, 133] were searched for the keyword "lymphatic", nothing was returned. The evidence of myometrial TCs in transmission electron microscopy could equally demonstrate initial lymphatics with large collagen-free lumina (Figure 1) [132], or WBCs-containing lumina [134]. This because the telopodes (Tps), which are the long, thin, and moniliform prolongations of TCs, are not enough to characterize these cells as a distinctive cell type [74, 135].

Cultured myometrial TCs were found expressing CD34, which is usually regarded as an HSC marker, c-kit, commonly regarded as a stem/progenitor marker, platelet-derived growth factor receptor-alpha (PDGFR-α), which labels a plethora of cell types, and vimentin, which is equally expressed in mesenchymal and endothelial cells [94, 133, 134, 136, 137]. The expressions of CD34, vimentin, and connexin-43 were detected in endometrial TCs [138].

The expression of c-kit in TCs was found in normal myometrium but not in leiomyomas [139], which is intriguing because they are constantly populated by c-kit expressing cells [135]. The c-kit expression is, apparently, not enough to distinguish between a TC, a stem/progenitor cell, or an EPC.

Podoplanin, not just a lymphatic marker

Podoplanin is a 38-kD transmembrane mucin-type glycoprotein that is highly expressed in podocytes, keratinocytes, the cells of the choroid plexus, alveolar lung cells, LECs, and is involved in lymphatic function, EMT processes and tumor progression [140, 141]. Although it is a lymphatic endothelial and epithelial marker [142], podoplanin was also found in intratumoral stromal cells, which can function as normal stromal cells [141].

Several anti-podoplanin antibodies are available, including NZ-1, D2-40, AB3 and 18H5 [143]; the epitope of NZ-1 is the platelet aggregation-stimulating (PLAG) domain 2/3; D2-40, AB3, and 18H5 have a common epitope, namely PLAG1/2 [143].

Lymphangiogenesis and lymph metastasis are induced by the cell expression of podoplanin

Lymphatic neovascularization is attained through two interdependent ways: lymphangiogenesis (the formation of new lymphatic vessels from preexisting lymphatic vasculature) and lymphvasculogenesis (the *de novo* generation of the lymphatic vessel through stem or progenitor cells) [65].

Both angiogenesis and lymphangiogenesis occur in response to tissue injury or cancer, in which macrophages are activated, and their number increased [144].

Lymphangiogenesis is a very early step in the lymphatic metastasis and is influenced by the niche constituents of the tumors, namely tumor cells, CAFs, MSCs, dendritic cells, macrophages, the extracellular matrix, cytokines and growth factors [145].

The lymphatic vessels play roles in human cancers and is known that the cell lymphatic invasion of a tumor may influence the prognosis in a significant manner. It is not acknowledged yet if pre-existing lymphatics are sufficient for tumor dissemination or it is needed a *de novo* development [57].

Podoplanin is included in shedded vesicles and exosomes, where it colocalizes with CD63; these podoplanin-expressing exosomes are known to increase lymphangiogenesis; CD63+ LECs could have a TC-like morphology [135].

It was demonstrated that the expression of podoplanin in tumor cells induces tumor lymphangiogenesis without determining an increase in the volume of the primary tumor [146]. A role for VEGF-A in tumor-mediated lymphangiogenesis has also been reported, being involved the recruitment of BM-derived LEPCs [147].

Endothelin-1, villin-1, and tenascin-C appear as potential mediators of podoplanin-induced tumor lymphangiogenesis [146]. Podoplanin upregulates the expression of the endothelial-derived vasoconstrictor 21-amino acid peptide endothelin-1 *in vivo*, which, in turn, is a major pro-angiogenic factor [146, 148–152]. Endothelin-1 and VEGF-A promote tumor-progression through an angiogenic-independent EMT mechanism [148], and fibrosis through an EndMT mechanism [153–156]. Endothelin acts as an *in vivo* and *in vitro* activator of macrophages [157], and it can also stimulate platelets [158]. In the human leiomyoma, endothelin-1 mRNA expression is upregulated, this peptide being involved in normal myometrial and leiomyoma cell proliferation and survival [152]. Endothelin-1 acts through the endothelin receptors A and B, but the potential role of these receptors in the pathology of uterine leiomyoma tumors remains to be established *in vivo* [152].

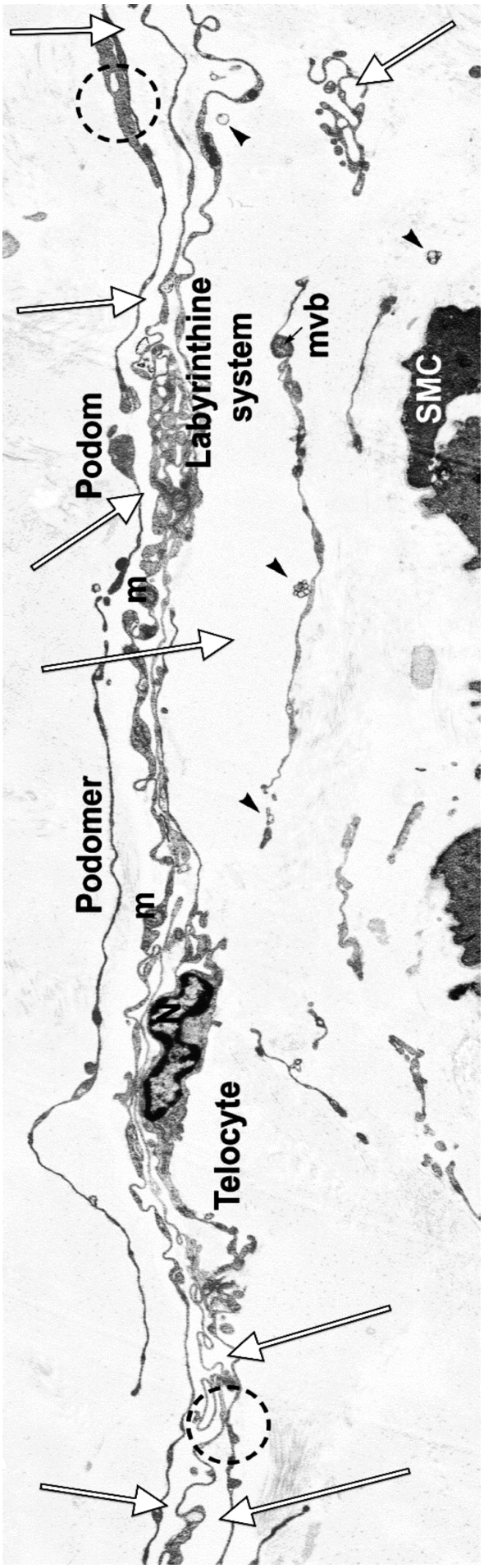

Figure 1 – ***Cropped and modified in Adobe Photoshop CC to grayscale colors from Figure 1 from [132] and reprinted from John Wiley and Sons, which is licensed under CC BY 4.0. The original caption details are: "Representative ultrathin section of the human non-pregnant myometrium. Two-dimensional sequenced concatenation from eight serial electron micrographs depicting the 3D network of telocytes. Oblique section through smooth myocytes (brown) (SMC) are bordered by numerous Tps (blue) interconnected by homocellular junctions (dotted circles) forming a 3D network. The inset illustrates the diagram of the interstitial network built-up by TCs and Tps with uneven calibres: podoms and podomers. Exosomes and shedding vesicles (arrowheads) are digitally coloured in purple. coll: collagen; m: mitochondria; mvb: multivesicular bodies; N: nucleus." However, TCs and Tps limit collagen-free lymphatic lumina-like spaces (we indicated these with white arrows).***

The myeloid–lymphatic transition

Heterogeneous subpopulations of monocytes and different macrophages form the mononuclear phagocyte system [159]. Macrophages represent the end-stage of differentiation of circulating monocytes [160]. Antibodies to CD68 recognize a 110-kD glycoprotein on tissue macrophages and blood monocytes [160, 161].

In a variety of tissues, there are close interconnections between macrophages with endothelia, epithelia and parenchymal cells [159]. Myeloid-derived lymphatic endothelial cell progenitors (LECPs) are induced through processes of inflammation and have important functions in adult lymphangiogenesis [81]. This process was indicated as myeloid–lymphatic transition (MLT) [81]. Macrophage-derived or myeloid/monocyte-derived LECPs (M-LECPs) were demonstrated to contribute to new lymphatic vessel formation [80]. LECPs exist in humans and significantly impact cancer pathology [81]. Moreover, the tumor-associated macrophages release a series of lymphangiogenic molecules, including VEGF-A, VEGF-C, VEGF-D, platelet-derived growth factor (PDGF), or FGF-2 [162, 163].

TC-like macrophages, the phagocytic properties of some TCs and CD68 expression in TC-like cells [73] would fit within the hypothesis of a MLT through intermediate stages of TC-like cells. Such cells with mixed myeloid and lymphatic identity can integrate into pre-existing lymphatic vessels before sprouting [81, 162].

Conclusions

The significance of CD44 and podoplanin expression in uterine leiomyomas was mostly overlooked, which makes the evaluation of these transdifferentiation processes difficult to assess.

Conflict of interests

The authors declare that they have no conflict of interests.

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