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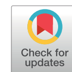
Telocytes and lymphatic endothelial cells: Two immunophenotypically distinct and spatially close cell entities



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ABSTRACT

Telocytes (TCs) have recently emerged as a peculiar type of stromal cells located in both perivascular and interstitial compartments of multiple anatomical sites in humans, other mammals and vertebrates. Pioneer electron microscopy studies have ultrastructurally defined TCs as “stromal cells with telopodes” (*i.e.* very long and thin cell processes with a moniliform morphology conferred by the irregular alternation of slender segments and small, bead-like, dilated portions), whereupon it has become apparent that TCs largely correspond to the CD34+ stromal/interstitial cells detectable by immunohistochemical assays. Besides CD34, TCs are also characterized by the expression of platelet-derived growth factor receptor (PDGFR)α. Interestingly, recent works recommended that lymphatic endothelial cell (LEC) markers should be routinely assessed to discriminate with certainty TCs from LECs, because these two cell types may exhibit similar morphological traits, especially when initial lymphatics are sectioned longitudinally and appear as vascular profiles with no obvious lumen. Furthermore, it has been argued that lymphatic microvessels immunostained for the small mucin-type transmembrane glycoprotein podoplanin (PDPN), which is widely used as lymphatic endothelial marker, can be easily misidentified as TCs. Nevertheless, surprisingly these assumptions were not based on double tissue immunostaining for TC and LEC markers. Therefore, the present morphological study was undertaken to precisely investigate the mutual spatial organization and putative relationships of TCs and lymphatic vessels in tissues from different human organs. For this purpose, we carried out a series of double immunofluorescence analyses simultaneously detecting the CD34 or PDGFRα antigen and a marker of LECs, either PDPN or lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1). In the connective tissue compartment of different organs, TCs were CD34+/PDGFRα+/PDPN−/LYVE-1− while LECs were CD34−/PDGFRα−/PDPN+/LYVE-1+, thus representing two definitely distinct, though spatially close, cell entities. The arrangement of telopodes to intimately surround the abluminal side of LECs suggests a possible role of TCs in the regulation of lymphatic capillary functionality, which is worth investigating further.

1. Introduction

Over the last decade, we have witnessed a thorough reappraisal of our knowledge of the microscopic anatomical configuration of a variety of organs in humans, other mammals and vertebrates, following the characterization of a long-neglected, peculiar type of stromal cells, named telocytes (TCs) (Faussone-Pellegrini and Popescu, 2011; Cretoiu et al., 2012, 2017; Cretoiu and Popescu, 2014; Diaz-Flores et al., 2014; Marini et al., 2018b, c; Rosa et al., 2018, 2019; Manetti et al., 2019). Based on their distinctive ultrastructural traits revealed through in-depth transmission electron microscopy investigations, TCs have been primarily defined as “stromal cells with telopodes”, which are very long and thin cytoplasmic prolongations with a characteristic moniliform morphology conferred by the irregular alternation of slender segments (termed podomers) and small, bead-like, dilated portions (termed podoms) that accommodate mitochondria, endoplasmic reticulum and caveolae (Faussone-Pellegrini and Popescu, 2011; Cretoiu et al., 2012; Cretoiu and Popescu, 2014; Marini et al., 2017b). Within the stromal/interstitial compartment of different organs, the telopodes of TCs are characteristically interconnected or connected with other cell types to establish complex reticular networks, whose peculiar three-dimensional morphology has been further revealed through FIB-SEM tomography analyses (Cretoiu and Popescu, 2014; Cretoiu, 2016). Owing to their exceptional ability to establish multiple cell-cell contacts and to release

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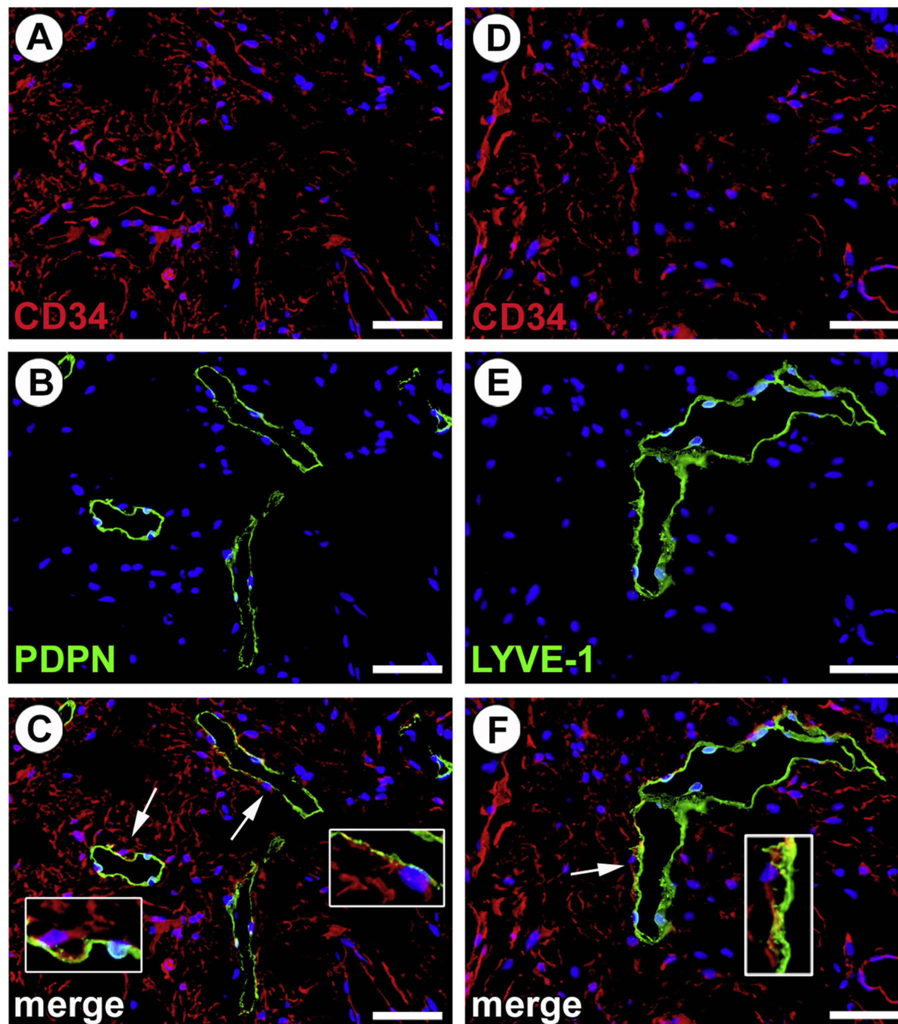

Fig. 1. Representative images of normal human tongue lamina propria double immunostained for (A–C) CD34 (red)/podoplanin (PDPN; green) and (D–F) CD34 (red)/lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1; green). CD34+ telocytes form an extensive reticular network distributed throughout the tongue lamina propria. PDPN and LYVE-1 immunofluorescent signal is detectable only in the endothelium of initial lymphatic vessels. The prolongations of CD34+ telocytes intimately surround the abluminal side of lymphatic capillaries with discrete regions of colocalization of the red and green fluorescent signals (yellow staining). Nuclei are counterstained in blue with 4′,6-diamidino-2-phenylindole (DAPI). Tissue areas pointed by arrows are shown at higher magnifications in the insets. Scale bar: 50 μm (A–F). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

microvesicles and exosomes containing bioactive molecules, TCs are, indeed, regarded as cells principally engaged in juxtacrine and paracrine intercellular signaling processes, a function that is thought to be important in the coordination of tissue morphogenesis and maintenance of postnatal homeostasis, and whose impairment may be associated with a variety of pathologies, including heart and lung disease, autoimmune disorders, chronic inflammation and multiorgan fibrosis (Cretoiu and Popescu, 2014; Cretoiu et al., 2016, 2017, 2019; Faussone-Pellegrini and Gherghiceanu, 2016; Ibba-Manneschi et al., 2016; Song et al., 2016; Marini et al., 2017a, 2018a, c, Rusu and Hostiuc, 2019; Varga et al., 2019).

Although electron microscopy is still considered the gold-standard methodology to study TCs for which specific antigenic markers have yet to be identified, it is widely accepted that in organs of different species these cells largely correspond to the CD34+ stromal/interstitial cells detectable by immunohistochemical assays and, hence, they are also commonly referred to as TCs/CD34+ stromal cells by several authors (Diaz-Flores et al., 2014; Marini et al., 2017b, 2018c; Cretoiu et al., 2019). Another suitable marker to immunolocalize TCs within tissues by light microscopy is platelet-derived growth factor receptor (PDGFR)α, which has steadily been demonstrated to be co-expressed with CD34 in TCs from different organs (Cretoiu et al., 2019). Moreover, since a marked expression of the cell surface glycoprotein CD34 is shared by TCs and vascular endothelial cells, several studies have proven the usefulness of CD34/CD31 double immunostaining to distinguish unequivocally between CD34+/CD31− TCs and CD34+/CD31+ blood capillaries, especially when the latter are captured within tissue sections as elongated vascular profiles (Manetti et al., 2013, 2015, 2019; Marini et al., 2018b, c; Rosa et al., 2018, 2019). In that context, recent works have also recommended that lymphatic endothelial cell (LEC) markers should be routinely assessed to discriminate with certainty TCs from LECs, because these two cell types may exhibit similar morphological traits, particularly when blind-ended and thin-walled initial lymphatics (*i.e.* lymphatic capillaries) are sectioned longitudinally with no obvious lumen (Manta et al., 2018; Rusu et al., 2019a, b; Toader et al., 2019). In addition, it has been argued that microlymphatics immunostained for the small mucin-type transmembrane O-glycoprotein podoplanin (PDPN; also known as gp38), which is widely used as a marker of LECs (Florez-Vargas et al., 2008; Hasselhof et al., 2016; Kong et al., 2017; Petrova and Koh, 2018), can be easily misidentified as TCs (Manta et al., 2018; Toader et al., 2019). Nevertheless, surprisingly the aforementioned assumptions were not solidly based on double immunostaining of tissue sections by combining the currently best available and most widely used TC markers, namely CD34 and PDGFRα, and markers for LECs.

On these premises, the present morphological study was designed to precisely investigate the mutual spatial organization and putative relationships of TCs and lymphatic vessels in tissues from various human organs. For this purpose, tissue sections were subjected to double fluorescence immunohistochemistry to simultaneously reveal the

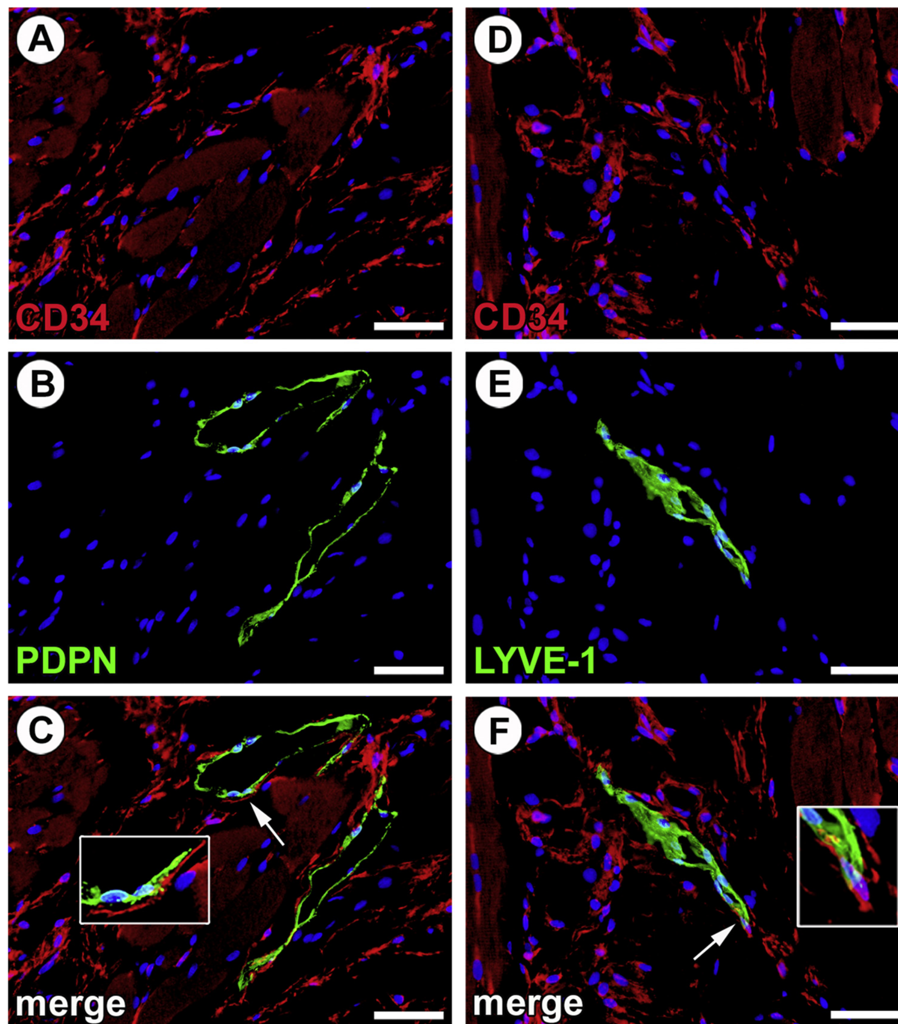

Fig. 2. Representative images of normal human tongue skeletal muscle tissue double immunostained for (A–C) CD34 (red)/podoplanin (PDPN; green) and (D–F) CD34 (red)/lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1; green). A diffuse reticular network of CD34+ telocytes is evident in the tongue skeletal muscle interstitium. PDPN and LYVE-1 immunoreactivity is detectable only in the endothelium of initial lymphatics. The abluminal side of lymphatic capillaries is closely surrounded by CD34+ telocyte prolongations with discrete regions of colocalization of the red and green fluorescent signals (yellow staining). Nuclei are counterstained in blue with 4′,6-diamidino-2-phenylindole (DAPI). Tissue areas pointed by arrows are shown at higher magnifications in the insets. Scale bar: 50 μm (A–F). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

localization of the CD34 or PDGFRα antigen and two of the most commonly used markers for LECs of initial lymphatics, namely PDPN and lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) (Podgrabinska et al., 2002; Aspelund et al., 2016; Hasselhof et al., 2016; Kong et al., 2017; Petrova and Koh, 2018).

2. Materials and methods

2.1. Archival tissue slides

Slides with paraffin-embedded sections (3–5 μm thick) of histologically normal human tongue, colon and skin tissues were retrieved from the archives of the Section of Anatomy and Histology, Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy, as described elsewhere (Manetti et al., 2013, 2015; Rosa et al., 2019). Archival tissue specimens had been routinely processed to paraffin blocks from April 2008 to December 2017. The investigation was conducted in accordance with the ethical principles for medical research involving human subjects of the Declaration of Helsinki and the approval of the Committee for Investigation in Humans of the Careggi University Hospital, Florence, Italy.

2.2. Double immunofluorescence staining

Paraffin-embedded tissue sections were subjected to double immunofluorescence staining combining anti-CD34 antibodies with anti-PDPN, anti-LYVE-1 or anti-CD31 antibodies. Other tissue sections were double immunostained for PDGFRα and either PDPN or LYVE-1. Tissue sections were routinely deparaffinized and rehydrated before being boiled in sodium citrate buffer (10 mM, pH 6.0) for antigen unmasking. Slides were then rinsed in phosphate-buffered saline (PBS), exposed to a 2 mg/ml glycine solution for 10 min to quench autofluorescence, and subsequently blocked for 1 h at room temperature with PBS containing 1% bovine serum albumin (BSA). After blocking of non-specific antibody binding sites, a mixture of two primary antibodies diluted in PBS with 1% BSA was applied to tissue sections overnight at 4 °C. The combinations of primary antibodies used were as follows: i) rabbit monoclonal anti-CD34 (1:100; clone EP373Y, catalog no. ab81289; Abcam, Cambridge, UK) and mouse monoclonal anti-PDPN (1:50; clone D2-40, catalog no. M3619; Dako, Glostrup, Denmark) IgG; ii) mouse monoclonal anti-CD34 (1:50; clone QBEnd-10, catalog no. M7165; Dako) and rabbit polyclonal anti-LYVE-1 (1:100; catalog no. ab33682; Abcam) IgG; iii) mouse monoclonal anti-CD34 (1:50; Dako) and rabbit polyclonal anti-CD31/platelet-endothelial cell adhesion molecule-1 (1:50; catalog no. ab28364; Abcam) IgG; iv) goat polyclonal anti-PDGFRα (1:100; catalog no. AF-307-NA; R&D Systems, Minneapolis, MN, USA) and mouse monoclonal anti-PDPN (1:50; Dako) IgG; and v) goat polyclonal anti-PDGFRα (1:100; R&D Systems) and rabbit polyclonal anti-LYVE-1 (1:100; Abcam) IgG. Isotype-matched and concentration-matched irrelevant IgG from mouse, rabbit and goat

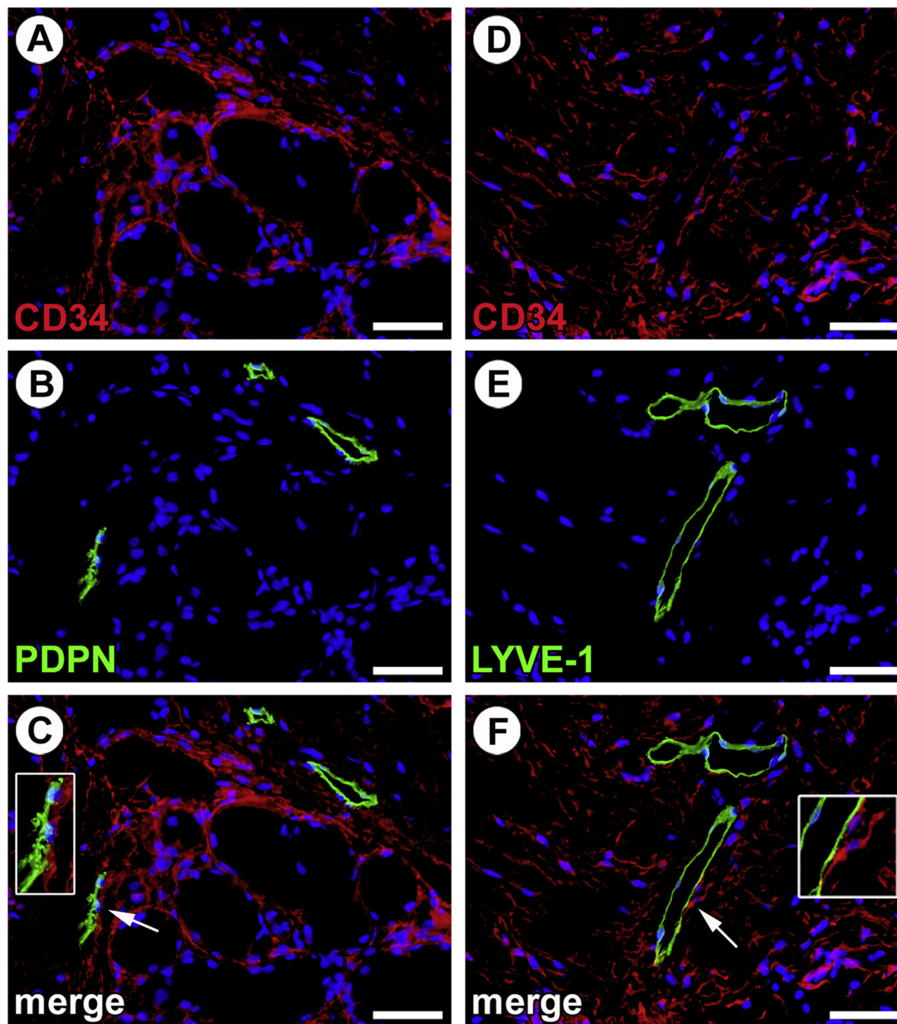

Fig. 3. Representative images of normal human colon submucosal layer double immunostained for (A–C) CD34 (red)/podoplanin (PDPN; green) and (D–F) CD34 (red)/lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1; green). CD34+ telocytes are arranged in a complex network throughout the colonic submucosa. PDPN and LYVE-1 immunoreactivity is restricted to initial lymphatics appearing either as a continuous layer of lymphatic endothelial cells delimiting a lumen or as spindle-shaped vascular profiles with unidentifiable lumen. Discrete regions of colocalization of the red and green fluorescent signals (yellow staining) arise from the juxtaposition of CD34+ telocyte prolongations and the abluminal side of the lymphatic endothelium. Nuclei are counterstained in blue with 4′,6-diamidino-2-phenylindole (DAPI). Tissue areas pointed by arrows are shown at higher magnifications in the insets. Scale bar: 50 μm (A–F). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

serum (catalog nos. I8765, I8140 and I9140, respectively; Sigma-Aldrich, St. Louis, MO, USA) were applied to serial tissue sections as negative controls. Alexa Fluor-488-conjugated and Rhodamine Red-X-conjugated goat or donkey anti-mouse and anti-rabbit IgG and donkey anti-goat IgG (Invitrogen, San Diego, CA, USA) diluted 1:200 in PBS with 1% BSA were used as secondary antibodies. Tissue sections were finally incubated with 4′,6-diamidino-2-phenylindole (DAPI; Chemicon International, Temecula, CA, USA) for nuclear counterstaining and mounted using an antifade aqueous mounting medium. Fluorescence images were captured with a Leica DFC310 FX 1.4-megapixel digital color camera under a Leica DM4000 B microscope (Leica Microsystems, Mannheim, Germany).

3. Results

Tissue samples of normal human tongue (Figs. 1 and 2 A–F), colon (Figs. 3A–F and 4A–F) and skin (Fig. 5A–F), in which the distribution of TCs/CD34+ stromal cells had previously been investigated in detail (Manetti et al., 2013, 2015; Rosa et al., 2019), underwent double immunofluorescence staining to simultaneously localize the expression of the CD34 antigen and either PDPN or LYVE-1 lymphatic endothelial markers. In agreement with previous observations (Manetti et al., 2013, 2015; Rosa et al., 2019), TCs/CD34+ stromal cells with an elongated, spindle-shaped morphology and very long and thin prolongations were found arranged in extensive and complex reticular networks in both the lamina propria and the skeletal muscle interstitium of the tongue (Figs. 1A,D and 2 A,D), in the different layers of the colonic wall (Figs. 3 and 4A,D), and in the dermis (Fig. 5A,D). In all tissue sections analyzed, both anti-PDPN and anti-LYVE-1 antibodies selectively immunolabeled thin-walled initial lymphatic vessels, which appeared as a continuous layer of LECs delimiting a lumen, and lymphatic pre-collectors, as well as occasional spindle-shaped structures that were presumably lymphatic vascular profiles with unidentifiable lumen because of the section plane (Figs. 1B,E, 2 B,E, 3 B,E, 4 B,E and 5 B,E). In every organ, the overlay of CD34 (red) and PDPN or LYVE-1 (green) fluorescent images clearly revealed that CD34+ TCs were negative for both lymphatic endothelial markers, and that PDPN+ and LYVE-1+ lymphatic capillaries lacked CD34 immunoreactivity (Figs. 1C,F, 2 C,F, 3 C,F, 4 C,F and 5 C,F). Nearby the lymphatic capillaries, CD34+ TCs were often arranged to intimately surround or even contact with their prolongations the abluminal side of LECs, as testified by discrete regions of colocalization of the red and green fluorescence signals (Figs. 1C,F, 2 C,F, 3 C,F, 4 C,F and 5 C,F insets).

As shown in Fig. 6A–F, the aforedescribed findings were fully replicated when using PDGFRα as an additional marker of TCs. In fact, PDGFRα+ TCs were negative for both PDPN and LYVE-1 LEC markers, while PDPN+ and LYVE-1+ lymphatic microvessels lacked PDGFRα expression (Fig. 6 A–F). Finally, as displayed in Fig. 7A–C, CD34/CD31 double immunofluorescence staining allowed in clearly discriminating among three different stromal cell populations, namely CD34+/

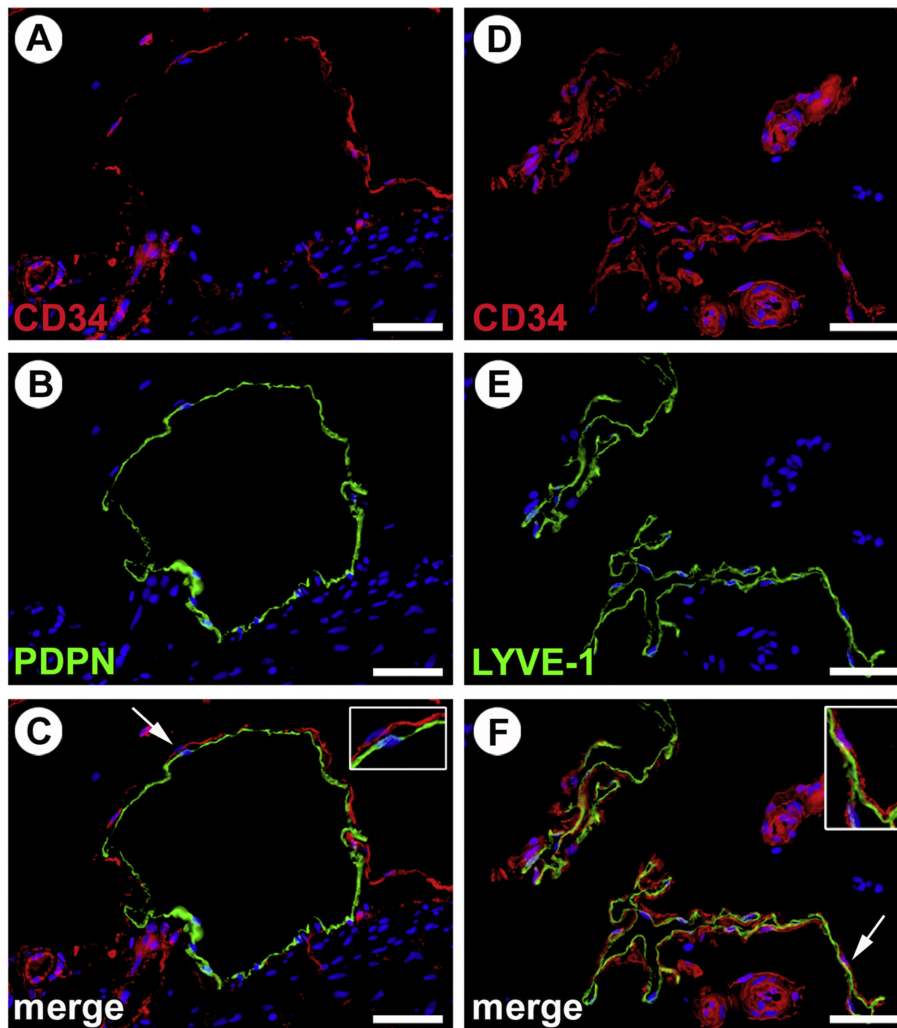

Fig. 4. Representative images of normal human colon subserosal layer double immunostained for (A–C) CD34 (red)/podoplanin (PDPN; green) and (D–F) CD34 (red)/lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1; green). In the colonic subserosa, CD34+ telocytes intimately encircle the abluminal side of a PDPN+ lymphatic pre-collector (A–C) and LYVE-1+ endothelial cells of lymphatic capillaries (D–F). Discrete regions of intercellular contacts are evident as yellow fluorescent signal. Nuclei are counterstained in blue with 4′,6-diamidino-2-phenylindole (DAPI). Tissue areas pointed by arrows are shown at higher magnifications in the insets. Scale bar: 50 μm (A–F). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CD31− TCs, CD34+/CD31+ endothelial cells of blood vessels, and CD34−/CD31+ LECs.

4. Discussion

In recent years, a growing interest has been focused on TCs, a unique stromal/interstitial cell population that has been discovered in numerous anatomical locations throughout the human body, and for which some intriguing changes have been reported in a number of diseases (Faussone-Pellegrini and Popescu, 2011; Cretoiu and Popescu, 2014; Diaz-Flores et al., 2014; Ibba-Manneschi et al., 2016; Cretoiu et al., 2017, 2019). For instance, it has been demonstrated that a severe impairment of the TC interstitial networks may occur in different disorders characterized by chronic inflammation and/or abnormal remodeling of the extracellular matrix, such as inflammatory bowel diseases, myocardial and liver fibrosis, keratoconus and systemic sclerosis (Bei et al., 2015; Ibba-Manneschi et al., 2016; Marini et al., 2017b; Cretoiu et al., 2019). Indeed, these cells are believed to be implicated in various intercellular communication processes that may be essential in the guidance of tissue arrangement during development and, afterward, in the preservation of the physiologic structure of organs, which currently makes them very interesting candidates for possible applications in the field of regenerative medicine (Cretoiu and Popescu, 2014; Bei et al., 2015; Horch et al., 2016; Cretoiu et al., 2017, 2019; Marini et al., 2017a, 2018a, c; Manetti et al., 2019). However, at present, the majority of studies on TCs are mainly descriptive and, hence, their cell identity and proposed functions are still matter of debate (Rusu et al., 2019; Varga et al., 2019). The current lack of specific antigenic markers can sometimes make trouble a proper identification of TCs and, therefore, a combination of multiple markers is preferable when studying these cells by light microscopy. In particular, it has been demonstrated that TCs from a multitude of tissues co-express CD34 and PDGFRα, and that it is extremely helpful to discriminate TCs as CD34+/CD31− cells with respect to the often spatially close CD34+/CD31+ blood microvessels (Cretoiu et al., 2017, 2019; Marini et al., 2018c). Recently, some authors have also suggested the use of LEC markers, because TCs and LECs of initial lymphatics could exhibit similar morphologies and, in particular, PDPN+ microlymphatics could be easily misidentified as TCs (Manta et al., 2018; Rusu et al., 2019a, b; Toader et al., 2019). In such a context, we believe that the results of the present study contribute to advance our still limited knowledge of the TC antigenic profile. In fact, we herein clearly demonstrated for the first time that, in the connective tissue compartment of healthy human organs, there is no immunophenotypic overlap between CD34+/PDGFRα+/PDPN−/LYVE-1− TCs and CD34−/PDGFRα−/PDPN+/LYVE-1+ LECs. Therefore, when labeling TCs with CD34 or PDGFRα, definitely there is no possibility of mistaking longitudinally cut lymphatic capillaries for TCs. Moreover, it is obvious that thin-walled PDPN+ microlymphatics may be misidentified as TCs only when using a single immunostaining approach, as done in recent works (Manta et al., 2018; Toader et al.,

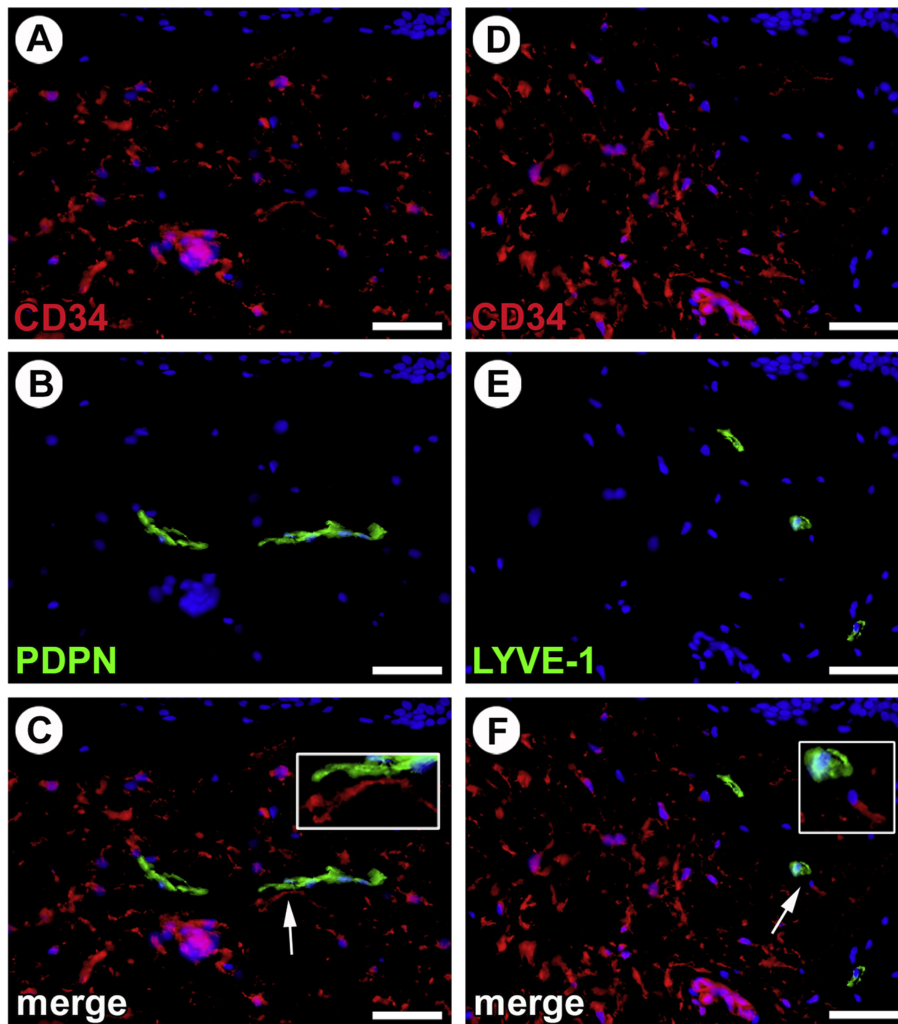

Fig. 5. Representative images of normal human forearm skin tissue double immunostained for (A–C) CD34 (red)/podoplanin (PDPN; green) and (D–F) CD34 (red)/lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1; green). Note the presence of numerous CD34+ telocytes within the dermis. PDPN and LYVE-1 immunofluorescent signal is detectable only in the endothelium of initial lymphatic vessels often appearing as spindle-shaped vascular profiles with unidentifiable lumen. Nuclei are counterstained in blue with 4′,6-diamidino-2-phenylindole (DAPI). Tissue areas pointed by arrows are shown at higher magnifications in the insets. Scale bar: 50 μm (A–F). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2019). Of note, our observations are also in agreement with a previous report showing that CD34 is exclusively expressed by PDPN+/LYVE-1+ LECs in human tumors but not in corresponding normal tissues (Fiedler et al., 2006). In addition, our immunohistological findings even highlighted that CD34/CD31 double staining may allow the simultaneous identification of three different connective tissue components, that is CD34+/CD31− TC networks, CD34+/CD31+ blood vessels, and CD34−/CD31+ lymphatics.

Besides the demonstration that TCs and LECs definitely represent two immunophenotypically distinct cell entities, our double immunofluorescence findings have also highlighted interesting spatial relationships between the CD34+/PDGFRα+/PDPN−/LYVE-1− TCs and CD34−/PDGFRα−/PDPN+/LYVE-1+ LECs. Specifically, we observed that the TC stromal network is closely distributed around the abluminal side of lymphatic capillaries, where LECs are surrounded by a thin, discontinuous basement membrane and are connected with the surrounding extracellular matrix *via* specialized anchoring filaments that are essential for their functionality (Petrova and Koh, 2018). Indeed, LECs are capable of readily sensing any increase in interstitial pressure by means of these anchoring filaments, which can modulate the opening of “button-like” inter-LEC junctions to allow the entry of fluid and immune cells (Petrova and Koh, 2018). Moreover, we also observed the presence of TCs around putative lymphatic pre-collectors. Although further in-depth studies will be required, the observation of CD34+/PDGFRα+ TCs in the close vicinity of the abluminal side of LECs allows speculating that these cells might somehow participate in the regulation of microlymphatic function. On the basis of our current knowledge of the possible roles exerted by TCs (Cretoiu and Popescu, 2014; Cretoiu et al., 2016, 2017, 2019; Marini et al., 2017a), this could be either by providing a kind of structural support to initial lymphatics, which have a very thin wall and, unlike blood capillaries, lack pericytes, or by releasing mediators, such as cytokines, growth factors and microRNAs, acting on LECs. In this regard, it is important to note that several lines of evidence arising from *in vivo* and *in vitro* studies have suggested that TCs may exert a number of positive effects on blood vascular endothelial cells and promote angiogenesis, such as *via* the release of exosomes and the secretion of vascular endothelial growth factor and pro-angiogenic microRNAs (Manole et al., 2011; Zheng et al., 2014; Nour et al., 2017; Yang et al., 2017; Zhou et al., 2019). Therefore, future investigations should address whether, in a similar way, TCs might also influence multiple LEC functions and modulate the process of lymphangiogenesis, which is important during development and is implicated in a variety of diseases (Alitalo and Carmeliet, 2002). Finally, besides the TCs surrounding lymphatic capillaries and pre-collectors, since these cells have been identified in the tunica media and adventitia of arteries and veins (Zhang, 2016), it will be interesting to verify whether they are also finely distributed in the structured wall of lymphatic collecting vessels, where they might exhibit important connections with smooth muscle cells. Interestingly, the presence of CD34+ cells in the tunica adventitia and in the outer part of the tunica

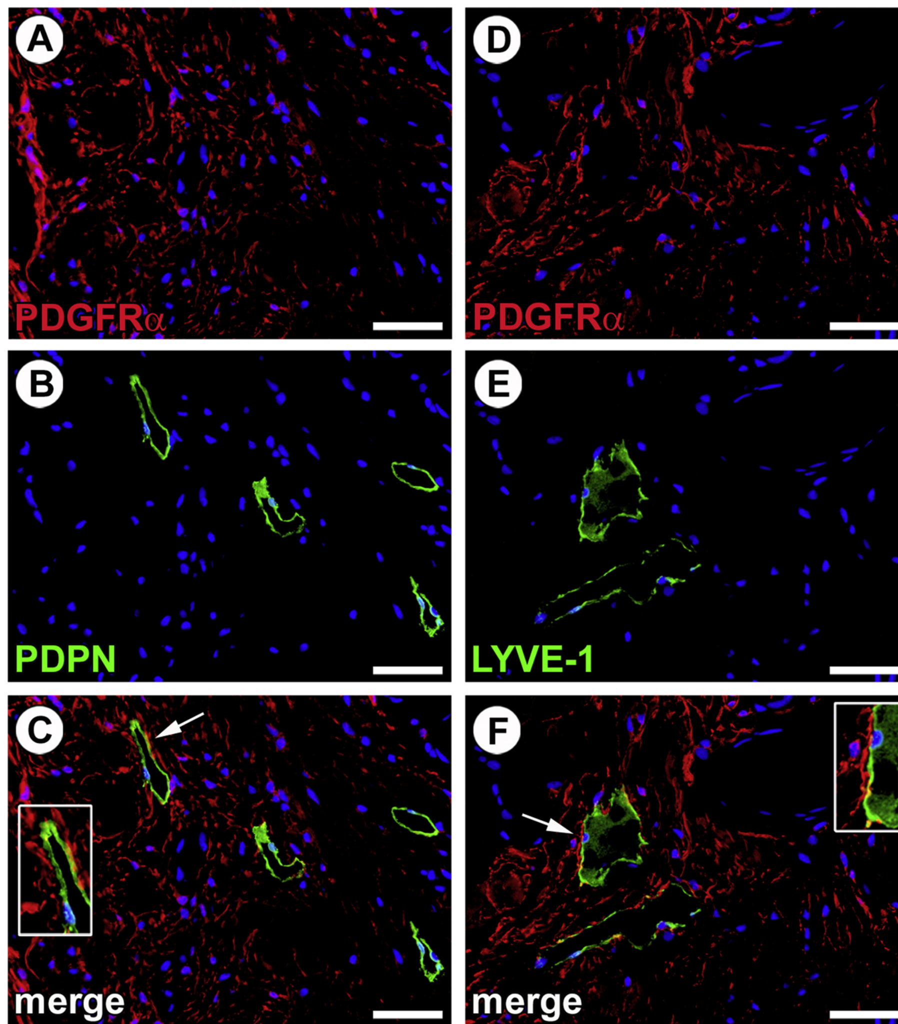

Fig. 6. Representative images of normal human tongue lamina propria double immunostained for (A–C) PDGFRα (red)/podoplanin (PDPN; green) and (D–F) PDGFRα (red)/lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1; green). Note the presence of numerous PDGFRα+ telocytes arranged to form a diffuse stromal network within the tongue lamina propria. PDPN and LYVE-1 immunoreactivity is found only in the endothelium of lymphatic microvessels. The prolongations of PDGFRα+ telocytes closely surround lymphatic capillaries at their abluminal side, as testified by discrete regions of red and green fluorescent signal colocalization (yellow staining). Nuclei are counterstained in blue with 4′,6-diamidino-2-phenylindole (DAPI). Tissue areas pointed by arrows are shown at higher magnifications in the insets. Scale bar: 50 μm (A–F). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

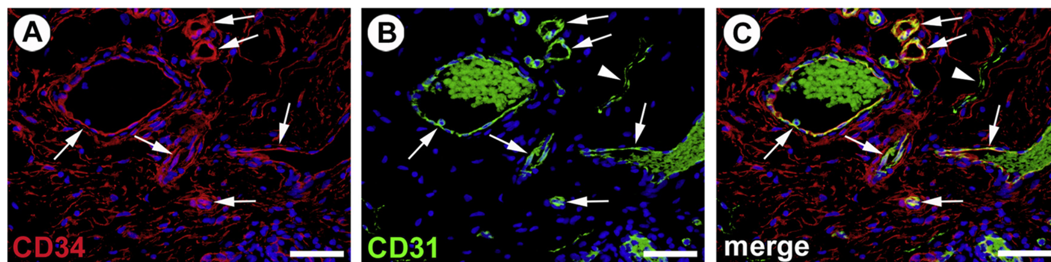

Fig. 7. Representative images of normal human colon submucosal layer double immunostained for CD34 (red)/CD31 (green) (A–C). The extensive stromal network of telocytes is CD34+/CD31–. Endothelial cells of blood microvessels are CD34+/CD31+ (arrows; yellow staining), while endothelial cells of microlymphatics are CD34–/CD31+ (arrowhead). Note the presence of CD34+/CD31– telocytes that follow from the outside the profile of a CD34–/CD31+ lymphatic capillary. Green autofluorescent erythrocytes are evident within the lumen of blood vessels. Nuclei are counterstained in blue with 4′,6-diamidino-2-phenylindole (DAPI). Scale bar: 50 μm (A–C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

media of lymphatic collectors has been previously noticed (Hasselhof et al., 2016). As a general consideration, whenever possible, future studies dealing on TCs should try to implement an immunoelectron microscopy approach in order to identify these cells on the basis of both their peculiar ultrastructural traits and their CD34+/PDGFRα+ immunophenotype.

In conclusion, we are confident that, by shedding light on a previously neglected close relationship between TCs and LECs, our findings lay the foundation for further functional studies that will certainly advance our knowledge of the biology of both TCs and lymphatic vessels.

CRediT authorship contribution statement

Irene Rosa: Investigation, Methodology, Visualization, Writing - review & editing. **Mirca Marini:** Investigation, Validation,

Visualization, Writing - review & editing. **Eleonora Sgambati:** Validation, Visualization, Writing - review & editing. **Lidia Ibba-Manneschi:** Resources, Validation, Visualization, Writing - review & editing. **Mirko Manetti:** Conceptualization, Data curation, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interests.

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