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What podoplanin tells us about cells with telopodes☆

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ABSTRACT

Telocytes (TCs) are stromal cells with telopodes, which represent long, thin, moniliform cell processes; however, this morphological feature alone is insufficient to define a cell type. Specific markers of lymphatic endothelial cells (LECs), such as Prox-1, podoplanin (D2-40) or LYVE-1, are not usually tested in TCs. We thus aimed at performing a study in light microscopy to evaluate whether or not LECs could be mistaken for TCs. Therefore we used CD34, α-smooth muscle actin and D2-40 for an immunohistochemical study on archived paraffin-embedded samples of uterine leiomyoma. Lymphatic vessels were identified by the expression of D2-40, but on the microscopic slides, false spindle-shaped TCs appearances either corresponded to collapsed lymphatic lumina or were determined by grazing longitudinal cuts of lymphatics. It is therefore mandatory to check the expression of lymphatic markers in telocyte-like cells and, moreover, to carefully examine the bidimensional cuts in order to avoid false results.

1. Introduction

Telocytes (TCs) are defined as stromal/interstitial cells with telopodes (Cretoiu, 2016; Dobra et al., 2018; Faussone-Pellegrini and Popescu, 2011; Popescu and Faussone-Pellegrini, 2010; Rusu et al., 2017a; Rusu et al., 2012a; Rusu et al., 2012b), which are long, thin, moniliform cell processes, a feature whose presence alone is not enough to define a cell type (Petrea et al., 2018).

Most markers shown to identify TCs, including CD34, PDGFR-α, PDGFR-β, vimentin, EGFR and c-kit are also expressed in endothelial cells, vascular (VECs) and/or lymphatic (LECs) (Cimini et al., 2017; Janas et al., 2018; Marino et al., 2013; Rusu et al., 2017b; Vrapciu et al., 2014). Initially used to identify ICLCs, those markers were further employed to identify TCs, and the results were presented in numerous papers (Cretoiu and Popescu, 2014; Hinescu and Popescu, 2005; Li et al., 2016; Liu et al., 2016; Manole et al., 2015; Milia et al., 2013; Popescu et al., 2006; Rusu et al., 2017a; Sheng et al., 2014; Vannucchi and Faussone-Pellegrini, 2016; Vannucchi et al., 2014; Vannucchi et al., 2013). However, positive proofs of these markers were often presented as cropped areas of micrographs, which did not accurately indicate the general microscopic anatomy of the tissue.

Specific markers of lymphatic endothelial cells (LECs), such as Prox-1, podoplanin (D2-40) or LYVE-1, were not tested in TCs before these were proposed as a new cell type.

Telocytes were explored mostly on bidimensional cuts, in light and/or electron microscopy. Although SEM 3D patterning was attempted on single samples in few instances (Cretoiu et al., 2015; Cretoiu et al., 2014; Kostin and Popescu, 2009), the results are conflicting and could not indicate a peculiar cell type. The possible ribbon-like 3D appearance of TCs (Cretoiu et al., 2014) led us to observe that bidimensional cuts could cause misdiagnoses when vascular and lymphatic vessels are cut tangentially, or their lumina are collapsed. While such events can be clearly demonstrated in light microscopy, on ultrathin cuts they can generate misinterpretations.

We therefore designed an immunohistochemical study to test the hypothesis that in light microscopy, the lymphatic marker podoplanin (D2-40) demonstrates false cells with telopodes, especially if the area of interest is cropped.

2. Material and method

We performed the immunohistochemical study retrospectively on archived paraffin-embedded samples of uterine leiomyoma (five

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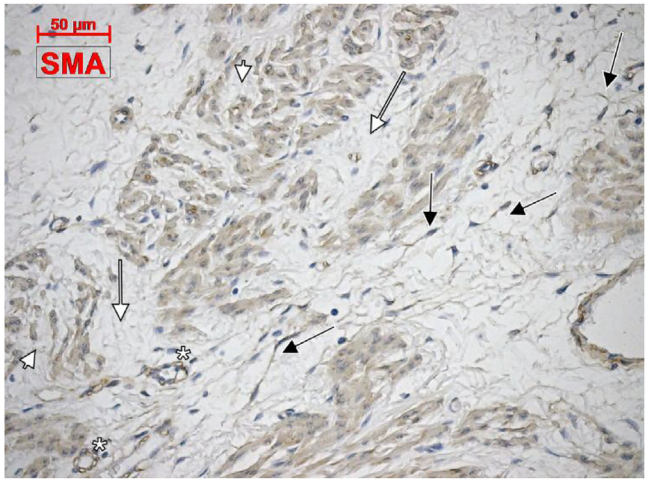

Fig. 1. Expression of α-SMA in uterine leiomyoma. Myometrial cords are separated by interfascicular collagenous stroma (white arrows) and also present intrafascicular fibrosis (arrowheads). Microvascular pericytes (*) and stromal myofibroblasts (black arrows) also express α-SMA.

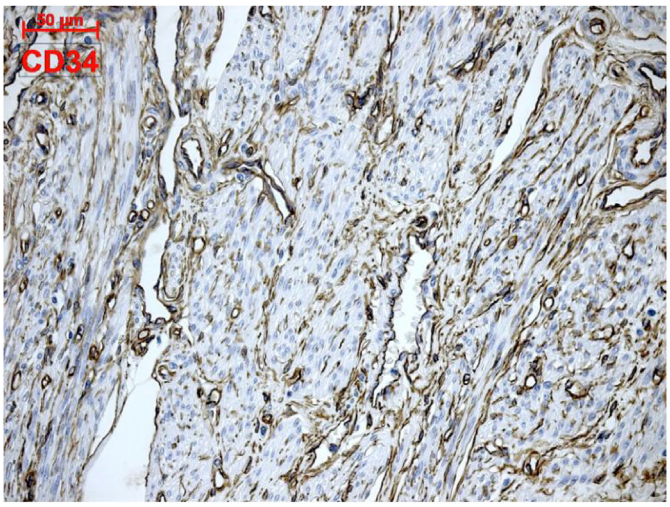

Fig. 2. CD34 expression in uterine leiomyoma samples is equally stromal and endothelial and could not distinguish in light microscopy any peculiar type of stromal cell.

cases). The ages of patients ranged from 47 to 54 years. We obtained the patients' written informed consent for all medical data to be used for research purposes, provided the protection of their identity is maintained. The study was tacitly approved by the responsible authorities where the work was carried out, and it was conducted in accordance with the general principles of medical research, as stated in the Declaration of Helsinki.

The paraffin-embedded samples were processed with an automatic histoprocessor (Diapath, Martinengo, BG, Italy) with paraffin embedding. Sections were cut manually at 3 µm and mounted on SuperFrost® electrostatic slides for immunohistochemistry (Thermo Scientific, Menzel-Gläser, Braunschweig, Germany). Histological evaluations used 3 µm-thick sections stained with haematoxylin and eosin. Internal negative controls resulted when the primary antibodies were not applied on slides.

We used primary antibodies for CD34 (clone QBEnd/10, Biocare Medical, Concord, CA, USA, 1:50), D2-40 (clone D2-40, Biocare Medical, Concord, CA, USA, 1:100) and for alpha-smooth muscle actin (α-SMA, mouse monoclonal, clone D33, Biocare Medical, Concord, CA, USA, 1:50).

Tissues were deparaffinized and rehydrated; then endogenous peroxidase was blocked using Peroxidazed 1 (Biocare Medical, Concord, CA, USA). For the heat-induced epitope retrieval, we used the Decloaking Chamber (Biocare Medical, Concord, CA, USA) and retrieval solution pH 6 (Biocare Medical, Concord, CA, USA). We used Background Blocker (Biocare Medical, Concord, CA, USA) to reduce nonspecific background staining. The primary antibody was then applied. We used different HRP-based detection systems: for CD34 the two-steps detection used a 4plus detection system, and for α-SMA and D2-40 we used MACH 4 (Biocare Medical, Concord, CA, USA). A HRP-compatible chromogen (DAB) was applied. Sections were counterstained with haematoxylin and rinsed with deionized water. For the washing steps, we used TBS solution, pH 7.6.

3. Results

All tissue samples were evaluated histopathologically as leiomyoma. Myometrial intrafascicular and interfascicular fibrosis was evident; microvessels were provided with α-SMA+ pericytes or vascular smooth muscle cells and had RBC content. The collagenous stroma was populated with α-SMA+ myofibroblasts, which were either spindle-shaped or multipolar (Fig. 1).

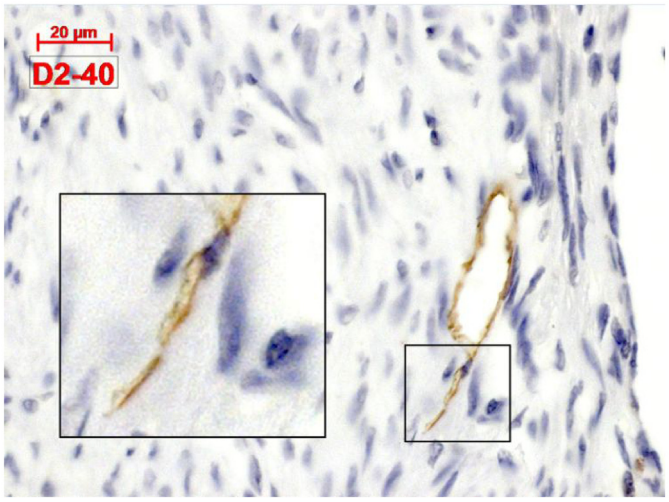

Fig. 3. D2-40 expression in uterine leiomyoma samples indicates lymphatic endothelial cells. A large lymphatic capillary expresses D2-40. A grazing cut generated a detail (inset, digitally magnified detail) which could be misinterpreted as telocyte.

On slides labelled with the CD34 antibody, the positive expression of that marker was pan-endothelial and stromal and could not determine any peculiar cell type (Fig. 2).

Lymphatic vessels lacking pericytes and with clear lumina were accurately identified by positive expression of D2-40 (Figs. 3–6). We found that LECs expressing D2-40 could correspond on bidimensional cuts to the morphology assigned to TCs, as they appear as cells with extremely long and thin prolongations (Fig. 5). Grazing longitudinal cuts of the D2-40-expressing lymphatics created false evidence of spindle-shaped stromal cells with telopodial prolongations, which, if cropped, could be interpreted as TCs (Figs. 3–6). Also, longitudinally cut collapsed lymphatic lumina can lead to a false spindle-shaped cell appearance.

4. Discussion

4.1. LECs are false TCs

Our results prove that caution should be taken when performing a histological diagnosis of telocytes. Longitudinal grazing cuts