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The molecular phenotypes of ureteral telocytes are layer-specific

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ABSTRACT

Telocytes (TC) are the delicate interstitial (stromal) cells defined by their long, thin and moniliform processes termed telopodes. Numerous studies determined that different subsets of telocytes populate almost all tissues and attempted to relate these subsets to various functions, from cell signaling to tissue repair and regeneration. Extremely few studies addressed the urinary tract though few data on the molecular pattern of the urinary TCs actually exist. We therefore hypothesized that subsets of urinary TCs co-localize within the human ureter and we aimed at performing an immunohistochemical study to evaluate the tissue-specific molecular pattern of TCs. On sample tissues of proximal ureter drawn from ten human adult patients during surgery were applied primary antibodies against CD34, CD105, von Willebrand Factor, the heavy chain of smooth muscle myosin (SMM) and c-erbB-2. The molecular pattern indicated three different subsets of ureteral TCs which are neither endothelial nor epithelial in nature: (a) type I: the CD34-/CD105+ TCs of the superficial layer of lamina propria; (b) type II: the CD34+/CD105 ± myoid TCs of the deep layer of lamina propria and (c) type III: the CD34+/CD105+ perivascular TCs. Although apparently different, all these subsets of TCs could belong to the stem/progenitor niche of the ureter.

1. Introduction

Telocytes (TCs) were defined in 2010 by Popescu and Faussone-Pelegrini who indicated a peculiar feature of these, the cell processes they termed "telopodes" (Popescu and Faussone-Pellegrini, 2010). Telopodes are long and extremely long moniliform prolongations with thin segments termed podomeres and dilations termed podoms (Rusu, 2014; Rusu et al., 2014b, 2012a). There are studies indicating that different subsets of TCs with various functional roles populate different tissues (Grigoriu et al., 2016; Petre et al., 2016; Rusu et al., 2016, 2017, 2014b, 2012b, 2012c; Vannucchi and Faussone-Pellegrini, 2016; Vrapciu et al., 2014a) and usually build stromal networks (Cretoiu and Popescu, 2014; Rusu et al., 2014b; Sanches et al., 2016). The lack of uniformity in TCs description is mostly due to the fact that these cells are defined on a morphological basis, their molecular phenotype being extremely versatile in publications. Nevertheless, in adult tissues TCs may be considered as mesenchymal stromal cells (MSCs) able of giving rise to various cell types of differentiated resident stromal cells (Vannucchi et al., 2016). A reliable marker which points to TCs, although also indicative for other cell types (Ilie et al., 2015; Rusu et al., 2013a, 2013b, 2015; Stanescu et al., 2012; Vrapciu et al., 2014a), is CD34 (Urban et al., 2016; Vannucchi and Faussone-Pellegrini, 2016; Zhang et al., 2016; Zhao et al., 2016; Zhaofu and Dongqing, 2016; Zheng and Wang, 2016). However, TCs show tissue-related immunohistochemical differences (Vannucchi and Faussone-Pellegrini, 2016).

Few authors studied or reviewed the TCs of the kidney and the urinary tract (Balikci et al., 2015; Li et al., 2014; Povysil et al., 2014; Qi et al., 2012; Rusu et al., 2014a; Vannucchi et al., 2014; Wolnicki et al., 2016; Zheng et al., 2012). Of these, some attempted to evaluate the urinary TCs in immunohistochemistry (Gevaert et al., 2012; Povysil et al., 2014; Rusu et al., 2014a) but although myoid suburothelial interstitial cells were found, the CD34 expression in such cells is controversial, either positive (Rusu et al., 2014a), or negative (Povysil et al., 2014). Nor the bilaminar structure of ureteral lamina propria was related with different subsets of TCs of the urinary tract (Gevaert et al., 2012). We therefore hypothesized that subsets of urinary TCs co-localize within the human ureter and we aimed at performing an immunohistochemical study to test this hypothesis.

2. Material and method

Sample tissues of the proximal 3–4 cm of ureter were obtained from human adult patients (6 male and 4 female) after nephrectomies.

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Informed consent for research use of tissues was obtained prior to surgery. Approval for the present study was granted by the institutional Committee. All experiments on human subjects were conducted in accordance with the Declaration of Helsinki http://www.wma.net/en/30publications/10policies/b3/index

The samples of ureter were oriented and paraffin-embedded. These were further were fixed for 24 h in buffered formalin (8%) and 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 hematoxylin and eosin.

We used primary antibodies for the smooth muscle myosin heavy chain (SMM, mouse monoclonal, clone S131, Novocastra-Leica, Leica Biosystems Newcastle Ltd, Newcastle Upon Tyne, UK, 1:100, code NCL-MHC-Sm), CD34 (mouse monoclonal, clone QBEnd/10, Biocare Medical, Concord, CA, USA, 1:50, code CM 084 A, B, C), endoglin/CD105 (mouse monoclonal, clone SN6 h, Thermo Scientific, Pierce Biotechnology, Rockford, USA, 1:100, code MA5-11854), von Willebrand Factor (rabbit polyclonal, Abcam, Cambridge, UK, 1:100, code ab6994) and c-erbB-2 (mouse monoclonal, clone CB11, Biocare Medical, Concord, CA, USA, 1:50, code ACA 076A,C).

Positive controls were used, as follows: (1) for CD34–external: choroid plexuses, endothelial cells, internal: blood vessels, endothelial cells; (2) for CD105–external: myocardium, endothelial cells, internal: blood vessels, endothelial cells; (3) for SMM – external: breast, myoepithelial cells; (4) for von Willebrand Factor – external: human tongue, endothelial cells; (5) for c-erbB-2–external: breast. Internal negative controls resulted when the primary antibodies were not applied on slides. Tissues were deparaffinized and rehydrated, then endogenous peroxidase was blocked using Peroxidased 1 (Biocare Medical, Concord, CA, USA). For the heat induced epitope retrieval was used the Decloaking Chamber (Biocare Medical, Concord, CA, USA) and retrieval solution pH 6 (Biocare Medical, Concord, CA, USA). Background Blocker (Biocare Medical, Concord, CA, USA) was used to reduce non-specific background staining. The primary antibody was then applied. Different HRP-based detection systems were used: for CD34, CD105 and von Willebrand factor two-steps detection used a 4plus detection system and for c-erbB-2 and SMM was used MACH 4 (Biocare Medical, Concord, CA, USA). A HRP-compatible chromogen (DAB) was applied. Sections were counterstained with hematoxylin and rinsed with deionized water. For washing steps was used TBS solution, pH 7.6.

3. Results

On transverse cuts the ureteral wall consisted of an internal mucosa, a middle muscular coat and an external adventitia. The mucosa consisted of urothelium and lamina propria, this later being subdivided into a superficial (suburothelial) layer or upper lamina propria, and a deep one. The deep layer of lamina propria was a myoid layer as it consisted of SMM-expressing smooth muscle cells and telocytes (Fig. 1). Such myoid telocytes were not found elsewhere in the ureteral wall.

Within the lamina propria endoglin labeled, preferentially but not exclusively, spindle-shaped telocytes (type I) with long telopodes (30–50 µm), with a concentrically layered disposition within the superficial layer (Fig. 2), while CD34 was exclusively expressed by network-building dichotomously branching telocytes (type II) within the deep layer of the lamina propria and the muscular coat interstitia (Fig. 3). Within the muscular coat the networks of telocytes did not express endoglin or SMM. Expression of endoglin (CD105) and of CD34 were positive in microvascular endothelia (Figs. 2 and 3) as well as in perivascular telocytes (type III) of the ureteral wall. The ureteral telocytes did not express neither the von Willebrand factor (Fig. 4), nor the epithelial marker c-erbB-2 (Fig. 5). These two markers were both expressed in endothelial cells. Epithelial cells, as well as non-spindle shaped stromal cells, also expressed c-erbB-2, which was also scarcely positive in type III perivascular telocytes.

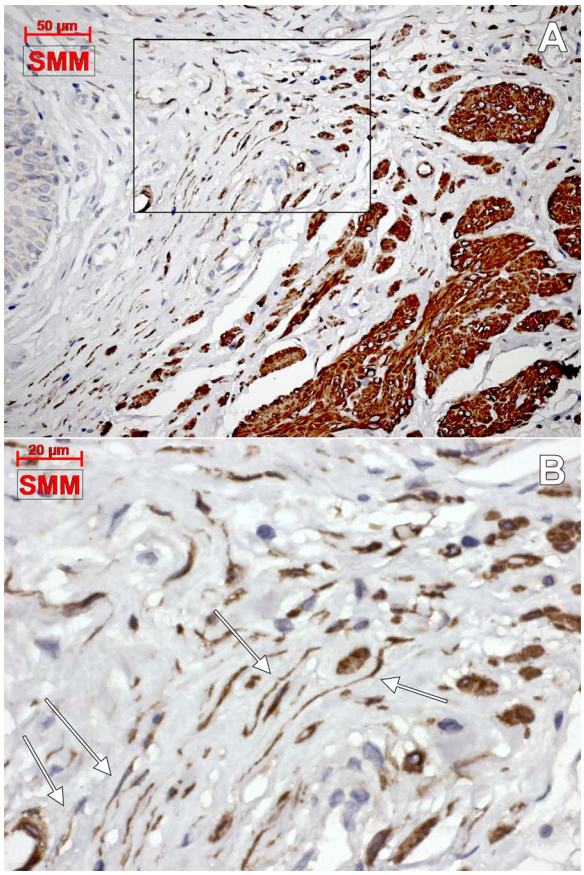

Fig. 1. Human adult ureter. The heavy chain of smooth muscle myosin is expressed in the deep layer of lamina propria (inset in A, detailed in B at higher microscopic resolution) in isolated smooth muscle cells and in myoid telocytes (arrows).

4. Discussion

There were gathered here proofs for different subsets of urinary TCs located within the ureteral wall. We therefore defined three distinctive subsets of ureteral TCs: (a) type I: the CD34-/CD105+ TCs of the superficial layer of lamina propria; (b) type II: the CD34+/CD105 ± myoid TCs of the deep layer of lamina propria and (c) type III: the CD34+/CD105+ perivascular TCs.

As the specific endothelial marker, the von Willebrand factor, was not expressed in stromal cells, being exclusively expressed by endothelial cells, we considered the subsets of telocytes we found did not belong to the endothelial lineage. Moreover, although c-erbB-2 was expressed in urothelial, as well as in isolated stromal cells lacking prolongations, it was not expressed in spindle-shaped stromal cells, which excluded an epithelial phenotype, or origin, of ureteral TCs. The urothelial expression of c-erbB-2 is rather normal, being previously described as moderate strong in all cells throughout the layers of the urothelium (Gullick et al., 1987). Urothelial expression of c-erbB-2 is useless for cancer staging or prognosis, the regulatory mechanisms and properties of this protein in urothelium needing a better understanding (Lee et al., 1994).

A previous study found c-kit-expressing TCs which were separated, cultured and expanded, further exhibiting high proliferation capacity as proven by expression of endoglin (60%) and Ki67 (80%) (Bojin et al., 2011). It is therefore reasonably to speculate a precursor role of the suburothelial type I of ureteral TCs we found in the superficial layer of lamina propria, the CD105-expressing ones. Also the type II ureteral