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Identification of telocytes in the upper lamina propria of the human urinary tract

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Abstract

The upper lamina propria (ULP) area of interstitial cells (IC) has been studied extensively in bladder, but is rather unexplored in the rest of the urinary tract. This cell layer is intriguing because of the localization directly underneath the urothelium, the intercellular contacts and the close relationship with nerve endings and capillaries.In this study, we examine the ULP layer of IC in human renal pelvis, ureter and urethra, and we make a comparison with ULP IC in bladder. Tissue was obtained from normal areas in nephrectomy, cystectomy and prostatectomy specimens, and processed for morphology, immunohistochemistry and electron microscopy. A morphological and immunohistochemical phenotype for the ULP IC was assessed and region-dependent differences were looked for.The ULP IC in renal pelvis, ureter and urethra had a similar ultrastructural phenotype, which differed somehow from that of bladder IC, that is, thinner and longer cytoplasmic processes, no peripheral actin filaments and presence of dense core granules and microtubules. Together with their immunohistochemical profile, these features are most compatible with the phenotype of telocytes, a recently discovered group of stromal cells. Based on their global ultrastructural and immunohistochemical phenotype, ULP IC in human bladder should also be classified as telocytes. The most striking immunohistochemical finding was the variable expression of oestrogen receptor (ER) and progesterone receptor (PR). The functional relevance of ULP telocytes in the urinary tract remains to be elucidated, and ER and PR might therefore be promising pharmacological research targets.

Keywords: urinary tract • bladder • ureter • renal pelvis • urethra • interstitial cell • telocyte • lamina propria

Introduction

The urinary tract extends from the renal pelvis towards the urethra, and is integrally lined by urothelium, which is a specialized transitional type of epithelium. The area between urothelium and detrusor is called lamina propria. Several authors have reported on the presence of IC in this area, especially in the bladder [1–4]. A particular and dense layer of IC has been described in bladder ULP, which was found in both humans [3–6] and animals [2, 7]. These ULP IC have been categorized heterogeneously as interstitial cells of Cajal (ICC) [1], interstitial Cajal-like cells (ICLC) [2, 5] or myofibroblasts [3, 8, 9]. Reasons for this diverse nomenclature might be the use of different experimental methods (electron microscopy *versus* light microscopy and immunohistochemistry) and the use of different tissue hosts (human *versus* animals).

In renal pelvis, ureter and urethra the presence of ULP IC is less explored. Interstitial cells have been reported in the renal pelvis lamina propria of different species, where they are thought to play a role in conducting and amplifying pacemaker signals [2]. In human renal pelvis, IC have been reported in the muscular layer [10]. In human ureter, IC have been described in between the smooth muscle fibres [11], whilst in guinea pig, IC were not found in the ureter ULP [2]. Urethral IC are well known to possess pacemaker properties [12]. These IC are localized between urethral smooth muscle cells. Interstitial cells have also been reported in the lamina propria of human urethra [4].

Because of their particular organization just underneath the urothelium, ULP IC have attracted interest of many investigators as they could embody a structural and functional link between urothelial cells and sensory nerves and/or between urothelial cells and smooth muscle cells. Moreover, these cells might be involved in the pathophysiology of urinary tract disorders. Particularly in bladder disease, functional and morphological alterations in ULP IC have been

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reported [5]. In the present study, we explored the morphology and immunohistochemical phenotype of IC in the ULP areas in human renal pelvis, ureter and urethra, and compared their phenotypes with that of the ULP IC in human bladder.

Materials and methods

Patient selection

The study protocol was in accordance with the EU guidelines and approved by the institution's ethical committee. All patients received information about the study and signed an informed consent file. To avoid gender bias, only male patients were included, all aged between 40 and 60 years. Each experimental group consisted of tissue samples from seven different patients.

Tissue sampling and processing

Renal pelvis and ureter tissues were obtained from radical nephrectomy specimens. Bladder tissue was obtained from cystectomy specimens and urethra tissue was obtained from radical prostatectomy specimens. All tissues were taken by an experienced pathologist from the resection specimens immediately after surgery. All biopsies came from normal (non-neoplastic) areas and were examined microscopically. One part of each biopsy was immediately fixed in 6% formalin and subsequently embedded in paraffin; the other part was fixed in glutaraldehyde and prepared for electron microscopy.

Immunohistochemistry

From a series of consecutive sections, the first slide from each biopsy was routinely stained with haematoxylin and eosin to check for the presence of urothelium, lamina propria and at least some muscular layers of the detrusor. For immunohistochemistry, 5-μm thick sections were deparaffinized in xylene, followed by immersion in alcohol and rehydration. Before staining, heat-induced epitope retrieval was performed by incubating the sections in Tris-EDTA buffer (pH 9.0) for 30 min. in a hot water bath at 98.5°C. Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide in methanol for 20 min. Sections were incubated with primary antibodies for 30 min. at room temperature, followed by incubation with a peroxidase-labelled polymer (Envision; DakoCytomation, Glostrup, Denmark) for 30 min. and a subsequent incubation with a substrate-chromogen for another 15 min. In between each step, the sections were thoroughly rinsed in PBS (pH 7.2). Nuclear counterstaining was performed with haematoxylin. The primary antibodies used are listed in Table 1. The panel of antibodies was chosen to phenotype IC: vimentin for mesenchymal properties, α-smooth muscle actin (sma) and desmin for smooth muscle properties, c-kit and CD34 for ICC properties, CD10 for properties of activated fibroblasts, neurofilament and S100 for neural properties and synaptophysin for neuro-endocrine properties. The titres of the primary and secondary antibodies were determined during use for daily clinical immunohistochemistry in our laboratory. Negative controls consisted of omission of the primary antibody, resulting in absence of immunoreactivity. For most antibodies, internal positive controls in bladder tissue were present. To compare histological staining patterns, serial sections were stained with different antibodies. Images were acquired using a Leica DM LB microscope equipped with a DC300FX camera (Leica Microsystems, Aartselaar, Belgium).

Table 1 Antibodies used in the present study

Antibody	Manufacturer	Host	Clone	Titre
Vimentin	Dako, Glostrup, Denmark	Mouse	V9	1/500
α-smooth muscle actin	Dako, Glostrup, Denmark	Mouse	1A4	1/200
Desmin	Dako, Glostrup, Denmark	Mouse	D33	1/50
CD34	Dako, Glostrup, Denmark	Mouse	Qbend 10	1/100
CD10	Dako, Glostrup, Denmark	Mouse	56C6	1/50
c-kit	Dako, Glostrup, Denmark	Rabbit	/	1/750
Oestrogen receptor	Dako, Glostrup, Denmark	Mouse	1D5	1/200
Progesterone receptor	Dako, Glostrup, Denmark	Mouse	PgR636	1/400
Synaptophysin	Dako, Glostrup, Denmark	Mouse	SY38	1/50
VEGF	Dako, Glostrup, Denmark	Rabbit	/	1/100
S100	Dako, Glostrup, Denmark	Rabbit	/	1/400
Caveolin-1	Santa-Cruz, Santa-Cruz, USA	Rabbit	/	1/100
Neurofilament	Dako, Glostrup, Denmark	Mouse	2F11	1/40

Electron microscopy

A small sample of each biopsy was fixed in 2.5% glutaraldehyde and 0.1 mol/l phosphate buffer at 4°C overnight. After 1 hr post-fixation in 1% osmium tetroxide and 0.1 mol/l phosphate buffer at 4°C, the samples were dehydrated in graded series of alcohol and embedded in epoxy resin. First, semi-thin sections were made to check the presence of urothelium and lamina propria. Then, ultra-thin sections (50–60 nm) were cut from representative samples, stained with uranyl acetate and lead citrate and examined at 50 kV with a Zeiss EM 900 electron microscope (Carl Zeiss, Oberkochen, Germany). The images were recorded digitally with a Jenoptik Progress C14 camera system (Jenoptik, Jena, Germany) operated with Image-Pro express software (Media Cybernetics, Bethesda, MD, USA). Analysis was performed by an experienced ultrastructural morphologist.

Semi-quantitative analysis

The percentages of caveolin-1$^+$, ER$^+$ and PR$^+$ ULP telocytes were assessed. Therefore, counts were made of caveolin-1$^+$ telocytes, ER$^+$ telocytes and PR$^+$ telocytes in the ULP region/high-power field (HPF). These counts are expressed as a percentage of the vimentin$^+$ telocytes in the same HPF. Therefore, immunohistochemical staining was performed and analysed on four serial sections. Counts were performed on seven tissue samples per region. For each sample, six HPFs were counted and averaged.

Results

Phenotype of ULP IC in renal pelvis

Ultrastructurally, ULP IC contained 2–3 layers of long cells with thin long cytoplasmic processes (at least 20–30 μm; Figs 1 and 2). These processes could occasionally present synapse-like thickenings. The cell body was rather small: the cytoplasm presented a moderate amount of organelles as rough endoplasmic reticulum cisternae and some mitochondria. Almost no cytoplasmic bundles of actin filaments with densifications were found. Some IC could contain microtubular structures and dense core vesicles. At the peripheral cell membrane, caveolae were seen; plasmalemmal thickenings and fibronexus were not obvious (Fig. 3). Intercellular connections consisted mostly of intermediate and gap-like junctions. Only occasional interlaying axonal nerve endings were observed. Close relationship with superficial capillaries was observed (Figs 1 and 2). The ULP IC population was intermingled with sparse fibroblasts (see Table 2 for an overview).

Immunohistochemical characterization of renal pelvis ULP IC revealed the expression of vimentin (Fig. 4). The majority of ULP IC expressed ER (67%), PR (70%) and caveolin-1 (70%; Table 3 and Fig. 4). There was no expression of α-sma, CD34 (Fig. 4), desmin, CD10, VEGF, synaptophysin, c-kit, neurofilament and S100 (not shown).

Phenotype of ULP IC in ureter

Ultrastructurally, the ULP IC showed a similar phenotype as the ULP IC population in the renal pelvis (see above, Table 2 and Figs 1–3). Immunohistochemical characterization of ureter ULP IC revealed the expression of vimentin (Fig. 5). A subset of the ULP IC expressed ER (48%); a minor subset of these IC expressed PR (23%; Fig. 5 and Table 3). Only partial expression of caveolin-1 was found (55%; Fig. 5 and Table 3). There was no expression of α-sma, CD34 (Fig. 5), desmin, CD10, VEGF, synaptophysin, c-kit, neurofilament and S100 (not shown).

Phenotype of ULP IC in bladder

This phenotype was discussed recently [5]; therefore, we summarize the original findings and include new data from the present study. Ultrastructurally, the ULP IC contained caveolae in the peripheral cell membrane and well-developed rough endoplasmic reticulum, mitochondria and peripheral actin filaments (mostly organized in bundles as illustrated by the frequently observed densifications) in the cyto-

Table 2 Characteristic ultrastructural features of upper lamina propria interstitial cells in renal pelvis, ureter, bladder and urethra

	Renal pelvis	Ureter	Bladder	Urethra
Layers	2–3	2–3	10–20	2–3
RER	+	+	+++	+
Actin filaments	No bundles	No bundles	Many bundles	No bundles
Caveolae	+++	+++	+++	+++
Mitochondria	++	++	++	++
Dense granules	+	+	–	+
Microtubules	+	+	–	+

RER: rough endoplasmic reticulum.

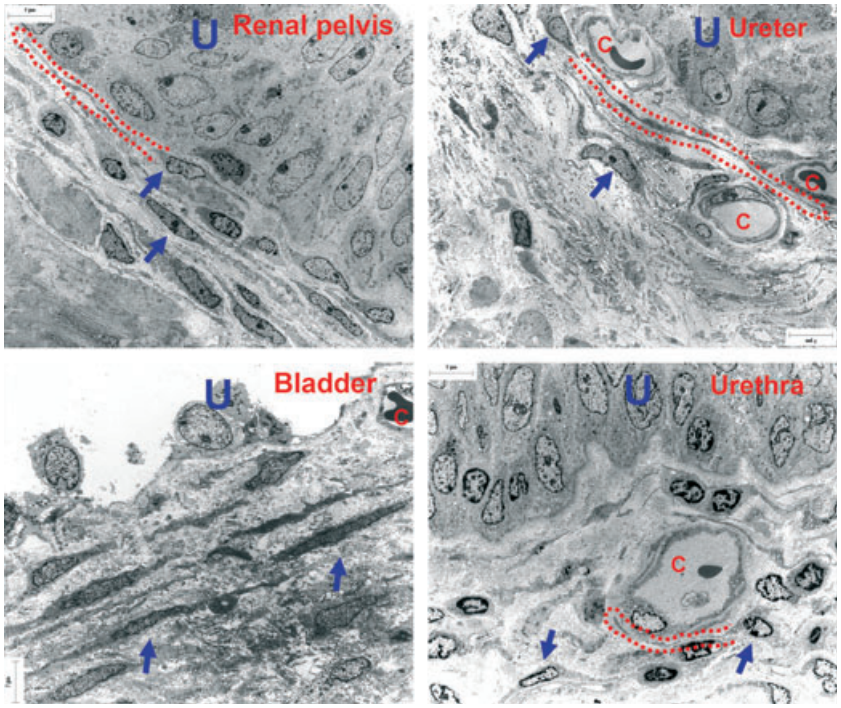

Fig. 1 Electron micrograph of upper lamina propria (ULP) interstitial cells (IC) in renal pelvis, ureter, bladder and urethra. ULP IC in renal pelvis, ureter and urethra are mainly telocytes (arrows), characterized by long cytoplasmic prolongations, and called telopodes (red dashed lines). In bladder, ULP IC have a different phenotype, with shorter cytoplasmic prolongations, but are also identified as telocytes (arrows). In bladder, there are around 10–20 layers of ULP telocytes, while in the other urinary tract regions, ULP telocytes are only 2–3 rows thick. Scale bars = 7 μm. U: urothelial area.

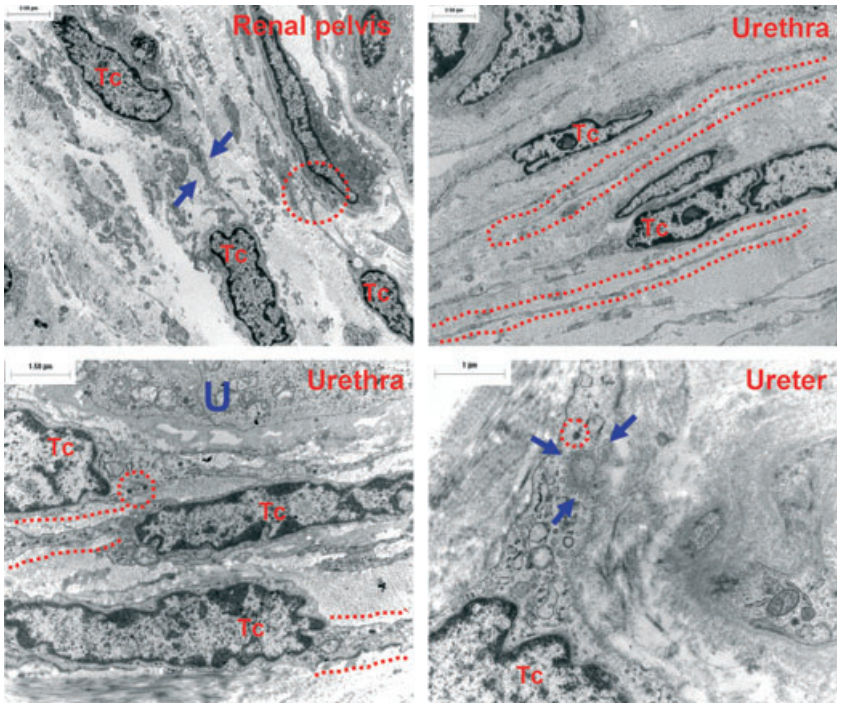

Fig. 2 Electron micrographs showing upper lamina propria (ULP) telocytes in more detail in renal pelvis, urethra and ureter. The close relationship with other cells/structures is shown (renal pelvis): contacts between two telocytes (blue arrows) and the close relationship between a telocyte and a blood capillary (red dashed circle). Dashed red lines illustrate the sometimes very long cytoplasmic prolongations (telopodes; urethra). Details of the perinuclear cytoplasm are given (ureter), with abundant caveolae (blue arrows) and a dense core granule (dashed red circle). In between the blue arrows, a protrusion of a neighbouring cell is seen. Dense core granules are also noticed in urethra telocytes (lower left, dashed red circle). Scale bars = 2.5 μm (upper panels); 1.5 μm (lower left); 1 μm (lower right).

plasm (Figs 1–3). The cells also showed several discontinuous plasmalemmal thickenings, but no clear fibronexus was seen. Intercellular connections consisted preferentially of intermediate junctions although occasional gap junctions were also observed (data not shown). Many interlaying axonal nerve endings were observed (see Table 2 for an overview).

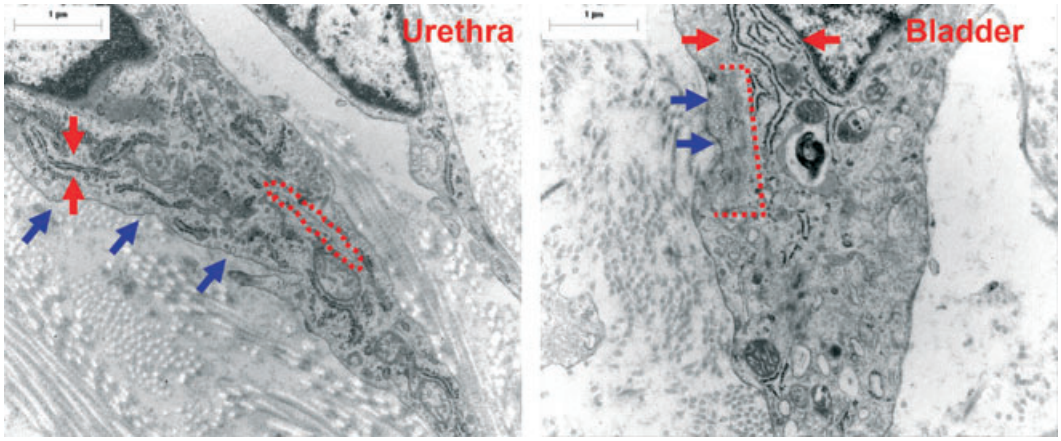

Fig. 3 Electron micrographs illustrating the ultrastructural differences between a typical upper lamina propria (ULP) telocyte in urethra (which is similar to those in renal pelvis and ureter) and a typical bladder ULP telocyte. ULP telocytes in urethra often have microtubular structures (inside dashed red line) and dense core vesicles (not visible; see Fig. 2, urethra and ureter). Instead, almost no cytoplasmic bundles of actin filaments with densifications were found. ULP telocytes in bladder typically have many bundles of actin filaments with densifications (inside dashed red line), but no microtubules or dense core vesicles. Both telocyte populations share the presence of rough endoplasmic reticulum cisternae (red arrows) and caveolae at the peripheral cell membrane (blue arrows). Scale bars = 1 μm.

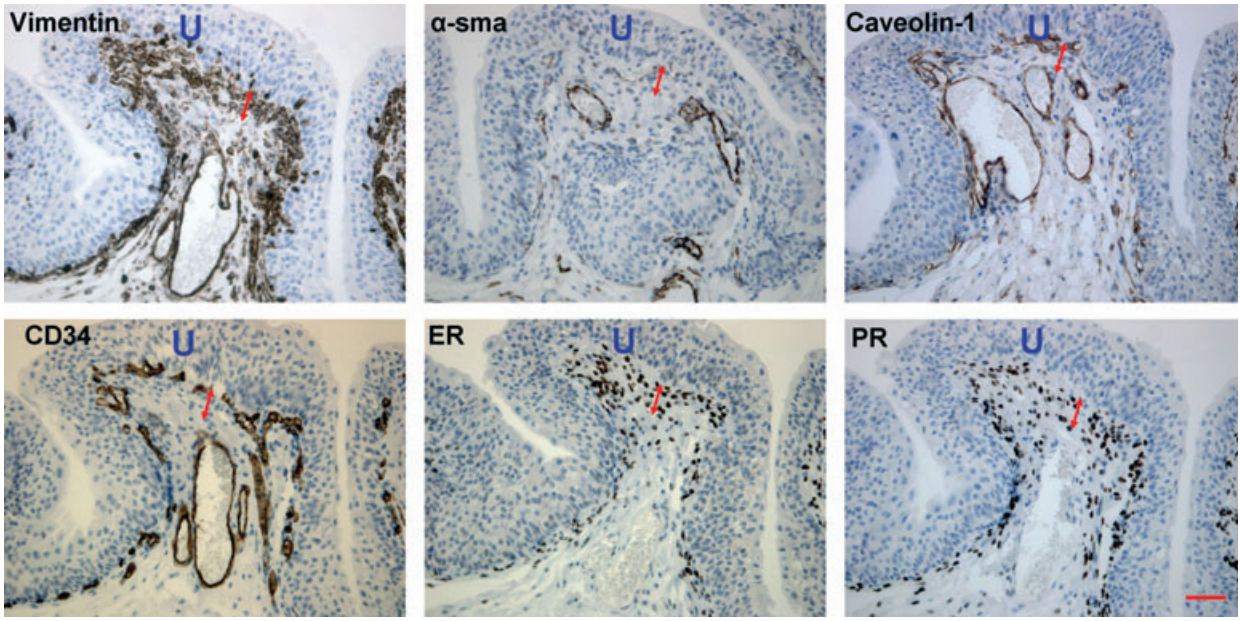

Fig. 4 The immunohistochemical phenotype of upper lamina propria (ULP) telocytes in human renal pelvis: immunoreactivity for vimentin, oestrogen receptor (ER; 67%), progesterone receptor (70%) and caveolin-1 (70%); negativity for α-smooth muscle actin (sma), CD34 and caveolin-1. Vimentin is also expressed on blood vessels. α-sma stains the perivascular smooth muscle cells. Caveolin-1 and CD34 are expressed on blood vessel endothelium. Note the organization of telocytes in 2–3 densely packed cell layers, as illustrated by ER stains. Double arrows mark the ULP telocyte layer. Scale bars = 50 μm. U: urothelial area.

Immunohistochemical characterization of ULP IC revealed the expression of vimentin, α-sma and CD10 (Fig. 6). We also found expression of caveolin-1 (90%) and PR (50%) in this study (Fig. 6 and Table 3). Upper lamina propria IC were negative for CD34 (Fig. 6), desmin, c-kit and neurofilament (not shown). There was also no expression of ER (Fig. 6), VEGF, S100 and synaptophysin (not shown).

Table 3 Semi-quantitative listing of the immunohistochemical phenotypes of upper lamina propria telocytes in the different regions of the urinary tract. Values are means and expressed as percentages of vimentin$^+$ telocytes

	Renal pelvis	Ureter	Bladder	Urethra
Caveolin-1	70	55	90	100
Oestrogen receptor	67	48	0	0
Progesterone receptor	70	23	50	10

Phenotype of ULP IC in urethra

Ultrastructurally, the ULP IC showed a similar phenotype as the ULP IC population in the renal pelvis and ureter (see above, Table 2 and Figs 1–3). Immunohistochemical characterization of urethra ULP IC revealed the expression of vimentin and caveolin-1 (100%) (Fig. 7 and Table 3). We found no expression of ER (0%) and only sparse expression of PR (10%) in ULP IC, while PR was strongly expressed in the deeper stromal cells (Fig. 7 and Table 3). There was no expression of α-sma, CD34 (Fig. 7), desmin, CD10, VEGF, synaptophysin, c-kit, neurofilament and S100 (not shown).

Discussion

For many years, IC in urinary tract, especially in bladder, have attracted the interest of investigators. Interstitial cells in the ULP of bladder have been well studied, while ULP IC in renal pelvis, ureter and urethra are relatively underexplored. In human bladder, ULP IC have recently been characterized in normal and pathological conditions [5]. In this study, we identified IC in ULP of renal pelvis, ureter and urethra. Remarkably, IC in those different anatomic regions of the urinary tract showed a similar ultrastructural phenotype, which was different from that of bladder IC: more slender cytoplasmic processes and almost no densifications, several microtubules and often dense core vesicles. Immunohistochemically, we encountered a somewhat different phenotype for those three groups: renal pelvis IC were vimentin$^+$/α-sma$^-$/ER$^+$ (67%)/PR$^+$ (70%)/caveolin-1$^+$ (70%); ureter IC were vimentin$^+$/α-sma$^-$/ER$^+$ (48%)/PR$^+$ (23%)/caveolin-1$^+$ (50%) and urethra IC were vimentin$^+$/α-sma$^-$/ER$^-$/PR$^+$ (10%)/caveolin-1$^+$ (100%).

The determination of the phenotype of IC in bladder (and the urinary tract in general) is not an easy process, which is reflected by the heterogeneous nomenclature for these cells (see the 'Introduction' section). The use of electron microscopy is still indispensable to study the ultrastructure of these cells. Upper lamina propria IC in renal pelvis, ureter and urethra have typical thin and long cytoplasmic processes. Furthermore, these IC have almost no peripheral actin filaments, but instead have several microtubules and dispersed dense

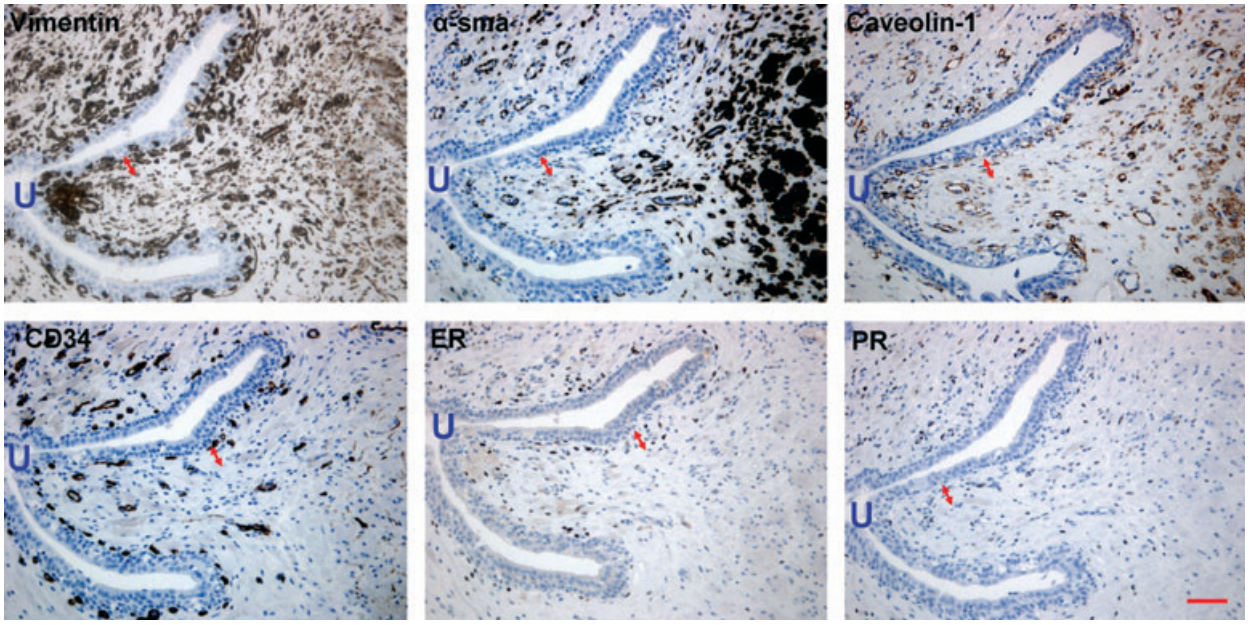

Fig. 5 The immunohistochemical phenotype of upper lamina propria (ULP) telocytes in ureter: immunoreactivity for vimentin, oestrogen receptor (ER; 48%), progesterone receptor (23%) and caveolin-1 (55%); negativity for α-smooth muscle actin (sma) and CD34. Vimentin is also expressed on blood vessels. α-sma stains the smooth muscle cells around the blood vessels and in the contractile coat. Caveolin-1 and CD34 are expressed on blood vessel endothelium. Note the organization of ULP telocytes in 2–3 densely packed cell layers, as illustrated by ER stains. Double arrows mark the ULP telocyte layer. Scale bars = 50 μm. U: urothelial area.

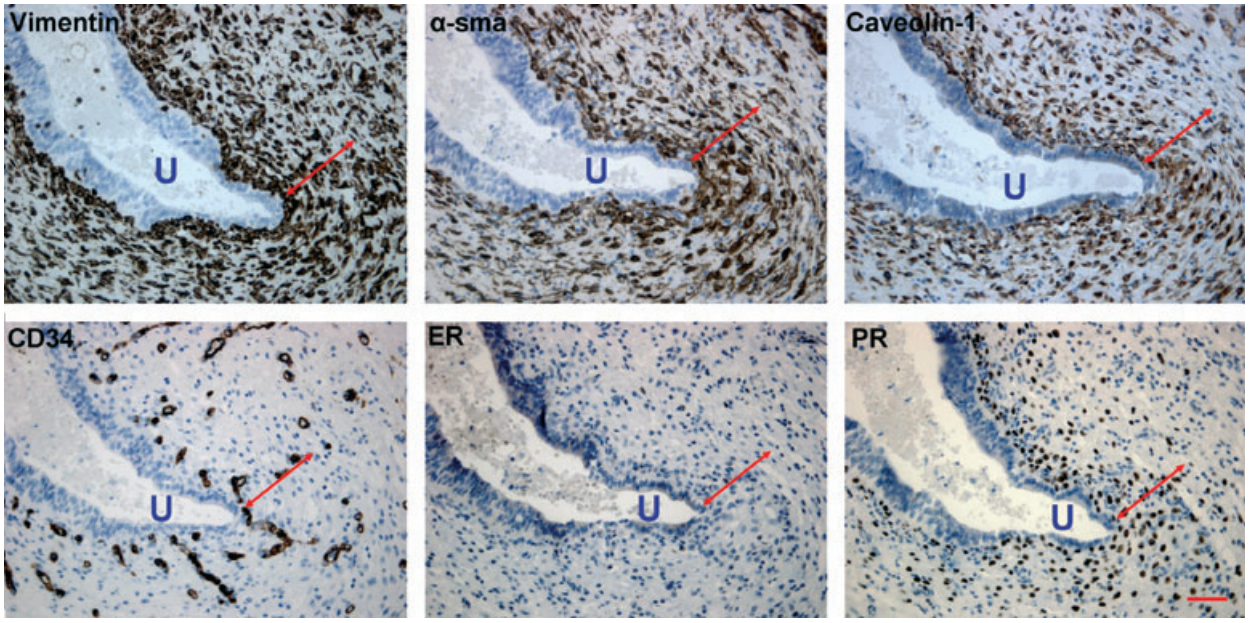

Fig. 6 The immunohistochemical phenotype of upper lamina propria (ULP) telocytes in human bladder: immunoreactivity for vimentin, α-smooth muscle actin (sma), caveolin-1 (90%) and progesterone receptor (50%); negativity for CD34 and oestrogen receptor. Vimentin is also expressed on blood vessels. α-sma additionally stains the perivascular smooth muscle cells. Caveolin-1 is also expressed on blood vessel endothelium. CD34 stains the ULP blood vessel endothelium. Note the organization of ULP telocytes in 10–20 densely packed cell layers, as illustrated by vimentin, α-sma and caveolin-1 stains. Double arrows mark the ULP telocyte layer. Scale bars = 50 μm. U: urothelial area.

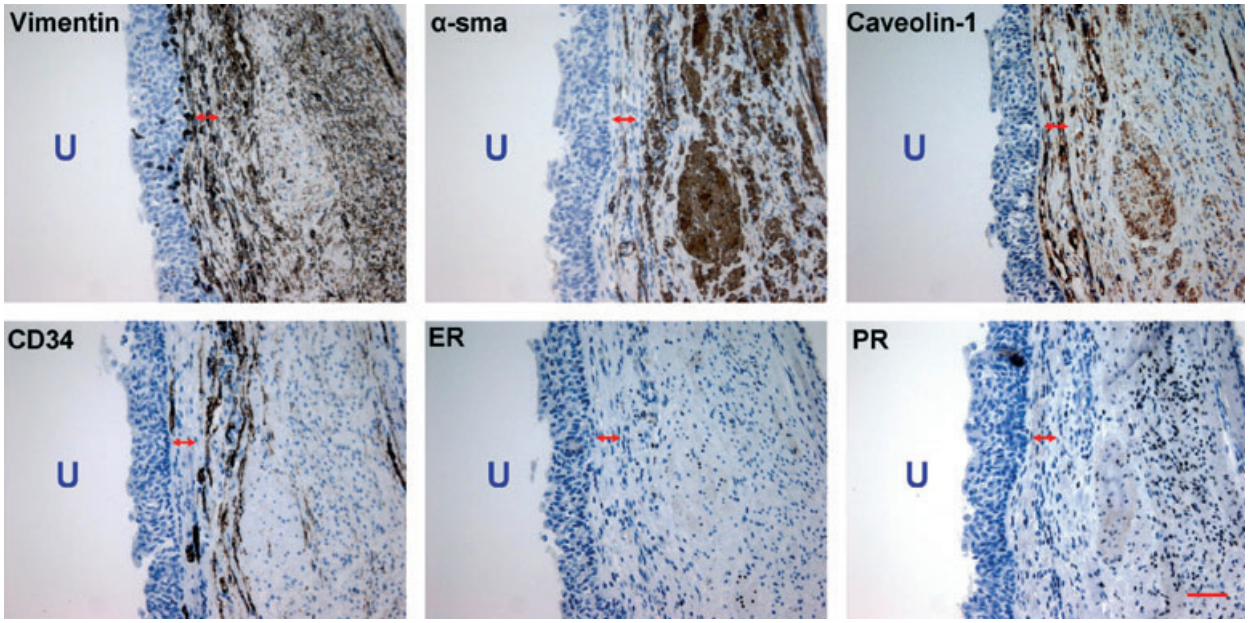

Fig. 7 The immunohistochemical phenotype of upper lamina propria (ULP) telocytes in human urethra: immunoreactivity for vimentin and caveolin-1 (100%); negativity for α-smooth muscle actin (sma), CD34 and oestrogen receptor. A small subpopulation expressed progesterone receptor (10%). Vimentin is also expressed on blood vessels. α-sma additionally stains the smooth muscle cells around the blood vessels and in the periurethral contractile coat. Caveolin-1 is additionally expressed on blood vessel endothelium, as is CD34. Note the organization of telocytes in 2–3 densely packed cell layers, as illustrated by caveolin-1 stain. Double arrows mark the ULP telocyte layer. Scale bars = 50 μm. U: urothelial area.

core granules. These features are at first sight compatible with an archetypical ICC phenotype. However, we found no immunohistochemical expression of c-kit and CD34, which is rather unusual for ICC. Recently, a novel stromal cell type was discovered and termed telocytes [13]. These cells are unique because of the presence of telopodes. These are very thin and very long cytoplasmic processes, which have a small emergence from the cell body [13]. Telocytes are organized into networks and interconnected with gap junctions. Furthermore, these cells have a close relationship with nerve endings, capillaries and inflammatory cells [14]. Given the ultrastructural and immunohistochemical criteria for telocytes, we think that ULP IC in renal pelvis, ureter and urethra (except from the sparse fibroblasts) can be classified as telocytes.

One could argue whether bladder ULP IC should not better be classified as telocytes. As discussed before, ULP IC in bladder are no archetypical ICC, nor classic myofibroblasts, and were therefore classified as ICLC [5]. Indeed, Popescu *et al.* found after thorough research that ICLC are in fact phenotypically clearly different from ICC, and therefore the new name 'telocytes' was proposed [13, 15, 16]. Upper lamina propria IC in bladder definitely have phenotypical features of telocytes, but we did not find the typical telopodes (long cytoplasmic processes) in these cells, which are one of the essential features of telocytes. Nevertheless, to reduce confusion with all acronyms used for bladder interstitial cells, we believe that ULP IC in bladder should also be considered as telocytes, based on the overall ultrastructural and immunohistochemical phenotype. Future research on telocytes is expected to increase knowledge of the possible heterogeneity and plasticity of this new cell population, which might refine the actual nomenclature.

One of the interesting findings in the present study was the expression of the steroid hormone receptors ER and PR on ULP telocytes. Previous publications have reported the presence of ER and PR on telocytes in human fallopian tube [17] and myometrium [17, 18]. Several studies have explored the expression of ER and PR in the urinary tract, but only few have used topographical methods. Both steroid hormone receptors are expressed in male and female urinary tracts [19–21]. Oestrogen receptor immunoreactivity has been reported in human urethra [19, 20], but not in human bladder [19, 20]. Progesterone receptor immunoreactivity has been described in human bladder mucosa [21]. The expression of one or both of these receptors indicates that the function of ULP telocytes is at least partially influenced by steroid hormones. Telocytes are considered as signal modulators and transducers because of their organization into networks and their close affinity with nerve endings, capillaries and other cell types [10, 13]. It was therefore suggested that telocytes in fallopian tube and myometrium could act as steroid sensors, possibly *via* a gap junction (connexin 43)-mediated mechanism [16, 18]. Furthermore, a study in ER knock-out mice suggested a role for ER in afferent signalling in bladder [22]. In the urinary tract, modulation of signal transduction by steroid receptors might then also be hypothesized, especially given the particular organization of telocytes directly underneath the urothelium.

It is clear that there are obvious phenotypical differences between ULP telocytes in the different regions of the urinary tract. Bladder ULP telocytes are the most distinct population because of ultrastructural properties, which are not found in the other regions. Immunohistochemistry reveals differences between all regional telocyte populations with caveolin-1, ER and PR as the most striking markers (Table 3). The often partial expression of these antigens in ULP telocytes suggests that each region itself might contain subpopulations of telocytes. One of the main functional hypotheses for bladder ULP telocytes is a role in signal transduction between the urothelium and the underlying nerve endings during bladder filling and bladder emptying [5]. A signal transduction role might then also be suggested for the ULP telocyte networks in the entire urinary tract, that is, during collection of urine in the renal pelvis, during propulsion of urine in the ureter and during the barrier function of the urethra. These regional differences in function might then be reflected by the different (immunohistochemical) phenotypes between ULP telocytes. The most striking immunohistochemical feature is the different regional expression pattern of both ER and PR in ULP telocytes, which indicates regional differences in a steroid-driven function. A role in signal transduction and modulation is further supported by the close relationship of ULP telocytes with suburothelial capillaries, which are found throughout the urinary tract. Furthermore, we previously showed a close relationship between bladder telocytes and lymphocytes, especially in pathological conditions [5], which could equal the 'stromal synapse' as proposed by Popescu *et al.* [16].

In conclusion, we identified the ULP IC in human renal pelvis, ureter, bladder and urethra as telocytes. Upper lamina propria telocytes in bladder are ultrastructurally different from ULP IC in the other urinary tract regions, and telocytes have a region-dependent immunohistochemical profile. The role in urinary tract physiology remains unclear and should be elucidated with functional studies. Previous reports of a changed phenotype of ULP telocytes in bladder disease support a functional relevance of these cells, while the expression of ER and/or PR indicates a steroid hormone-driven function. Future studies should focus on specific receptor and ion channel expression of these cells to understand their role in urinary tract physiology and pathophysiology. Oestrogen receptor and PR might therefore be interesting pharmacological research targets.

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Conflicts of Interest

The authors confirm that there are no conflicts of interest.

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