Identification of Different Phenotypes of Interstitial Cells in the Upper and Deep Lamina Propria of the Human Bladder Dome

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Purpose: There is increasing evidence for an important role of the lamina propria in bladder physiology and interstitial cells seem to have a key role in this area. Interstitial cells in the upper lamina propria have been studied most frequently. We characterized interstitial cells in the deeper lamina propria and hypothesized that the 2 interstitial cell populations have different phenotypes based on their ultrastructural and immunohistochemical properties.

Materials and Methods: Tissue samples were obtained from macroscopically and microscopically normal areas of radical cystectomy specimens. A panel of immunohistochemical markers was used to characterize lamina propria interstitial cells. Single/double immunohistochemistry/immunofluorescence was performed. At a second phase electron microscopy was used to compare upper and deeper lamina propria interstitial cells.

Results: Overall the phenotype of upper lamina propria interstitial cells was vimentin, α-smooth muscle actin, caveolin-1 and 2, PDGFRα, and nonphosphorylated and phosphorylated connexin 43 positive, and CD34 and c-kit negative. The overall phenotype of deeper lamina propria interstitial cells was vimentin, CD34 and nonphosphorylated connexin 43 positive, and α-smooth actin, caveolin-1 and 2, PDGFRα, phosphorylated connexin 43 and c-kit negative. Based on ultrastructural findings upper lamina propria interstitial cells were fibroblasts with myoid features and sparse myofibroblasts while deeper lamina propria interstitial cells were interstitial cell of Cajal-like cells.

Conclusions: To our knowledge this is the first study of 2 main interstitial cell populations in the upper and deeper lamina propria of the human bladder with distinct ultrastructural and immunohistochemical phenotypes. Future research is needed to elucidate whether these morphological findings reflect different roles for upper and deeper lamina propria interstitial cells in bladder physiology.

Key Words: urinary bladder, mucous membrane, phenotype, fibroblasts, interstitial cells of Cajal

Abbreviations and Acronyms

α-SMA = α-smooth muscle actin
ANO-1 = anoctamin-1
Cav = caveolin
Cx43 = connexin 43
DAPI = diaminobenzidine
IC = interstitial cell
ICC = interstitial cells of Cajal
ICLC = IC of Cajal-like cell
LP = lamina propria
NP = nonphosphorylated
P = phosphorylated
PDGFRα = platelet derived growth factor receptor-α
RER = rough endoplasmic reticulum
Tryp = tryptase
Vim = vimentin

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In the literature there are reports of 2 populations of stromal cells or ICs in the bladder, including ICs in the LP[1–3] (area between the urothelium and detrusor smooth muscle) and ICs in the detrusor[4] (intermuscular and intramuscular). These 2 IC populations have been reported in the bladder of different species, including the rat,[5] guinea pig,[4,6] mouse[7] and human.[2,8] Therefore, they seem to be a constant species independent

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finding. Functional data on bladder ICs are still limited. The general hypothesis is that LP ICs could be involved in nonneural sensory signal transduction from urothelium to detrusor while detrusor ICs might have a role in pacemaking and/or transduction of pacemaking signals in the detrusor area.[1]

Increasing knowledge shows that bladder IC organization and function seem to be more complex than the basic division between LP and detrusor ICs. There has been great interest in LP ICs directly beneath the urothelium, which are organized as several densely packed cell layers (suburothelial or upper LP ICs). These upper LP ICs have attracted the interest of many researchers due to their vicinity to the urothelium and they are a constant finding in several species, including humans.[2,4–6,8] Much morphological and functional data on upper LP ICs have appeared in the literature. Some frequently reported findings are myoid differentiation[2,8] with ongoing debate on whether these cells are myofibroblasts or ICs with myoid differentiation,[9,10] close contacts with afferent nerve endings,[11] and a role in cholinergic[12,13] and purinergic[14,15] signaling.

The deep LP, which is the area between the upper LP and the detrusor, houses a looser network of ICs that has received less attention in the literature than the upper LP. The organizational difference between the densely packed upper LP ICs and the looser network of deep LP ICs led us to hypothesize that the 2 IC populations might have some differences in phenotype. In the current study we looked for phenotypic differences between human bladder upper and deep LP ICs using morphological, immunohistochemical and ultrastructural techniques.

METHODS

Tissue Sampling and Processing

The study protocol was in accordance with European Union guidelines and approved by the institutional ethical committee. Bladder tissue samples were obtained from cystectomies performed for invasive bladder cancer in patients without previous intravesical or systemic neoadjuvant treatment. Samples from a total of 9 cystectomies done for bladder cancer in 6 females and 3 males with a mean age of 67 years were used and fully processed.

All cystectomy specimens were received fresh from the operating room on dry ice and processed within 15 minutes. The bladder was opened from the anterior side and full-thickness bladder wall biopsies were taken from the bladder dome as far as possible from macroscopically visible tumor. One part of each biopsy was immediately fixed in 6% formalin and subsequently embedded in paraffin. A second part was placed on a cork, snap frozen in isopentane cooled with liquid nitrogen and stored at −80C. The third part was prepared for electron microscopy and fixed in glutaraldehyde. The latter procedure consisted of microscopic separation at the urothelium, and upper and deep LP ICs from the detrusor, which was easily done at the transition between the deep LP and the detrusor. This was followed by microscopic dissection of this last part in the upper LP with urothelium and the deep LP. All paraffin embedded and frozen biopsies were assessed histologically for normal bladder and samples with carcinoma in situ were excluded from study. Electron microscopy samples were evaluated histologically on semifine sections for the presence (upper LP) and absence (deep LP) of urothelium before including them in analysis.

Immunohistochemistry and Immunofluorescence

The panel of immunohistochemical markers used to characterize IC included the broad mesenchymal marker Vim, the markers of myogenic differentiation desmin and α-SMA, the ICC markers CD34 and c-kit (CD117), the mast cell marker Tryp (Dako, Glostrup, Denmark), the caveolar markers Cav-1 (Santa Cruz Biotechnology, Santa Cruz, California) and Cav-2 (BD™), the ICC markers PDGFRα (R&D Systems®) and ANO-1 (Leica Biosystems, Diagem, Belgium), and Cx43, the main element of gap junctions and hemichannels reported to be present on upper LP ICs.[3] For Cx43 we used Cx43P (Sigma®), an antibody against a phosphorylated form,[16] and Cx43NP (Invitrogen™) against an unphosphorylated form.[17]

Immunofluorescence was done on paraffin embedded and frozen tissue on 5 μm slides. The rationale for this double approach was the observation that some antibodies are more effective on frozen tissue and some are more effective on paraffin embedded tissue with the advantage of increased validity of immunofluorescence results when antibodies are effective for each.

For immunofluorescence on paraffin embedded tissue 5 μm sections were deparaffinized in xylene followed by immersion in alcohol and rehydration. Before staining heat induced epitope retrieval was performed for 30 minutes at 120C in Bond Epitope Retrieval Solution 2 (Leica Bioystems). For immunofluorescence on frozen tissue 5 μm sections were immersed in acetone. All staining consisted of a sequential approach. Sections were incubated with the first primary antibody for 30 minutes at room temperature followed by the first secondary antibody for 30 minutes. These steps were followed by the same cascade for the second set of primary and secondary antibodies. Each step was followed by a 3 × 5-minute wash in Bond Wash Buffer (Leica Biosystems). Before each incubation with primary antibody slides were incubated with normal goat serum (diluted 1:5 in phosphate buffered saline) for 30 minutes to block nonspecific epitopes. Nuclear counterstaining was done with DAPI (300 nM in phosphate buffered saline). Secondary antibodies were Alexa Fluor® 568 goat anti-mouse and goat anti-rabbit, and Alexa Fluor 488 goat anti-mouse and goat anti-rabbit.

Images were collected with the laser scanning TCS SP5 Confocal Microscope using a HCX PL APO 40× (numerical aperture 1.25) oil immersion lens (Leica Microsystems, Mannheim, Germany). Different fluorochromes were detected sequentially using the excitation lines 405 nm (DAPI, blue signal), 488 nm (Alexa Fluor 488, green signal) or 561 nm (Alexa Fluor 568, red signal). Overlap