

## Short Communication

# Podoplanin (D2-40) is a Reliable Marker of Urinary Bladder Myofibroblasts (Telocytes)

(urinary bladder myofibroblasts / telocytes / immunohistochemistry / podoplanin (D2-40) positivity / interstitial cystitis)

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**Abstract.** Podoplanin, D2-40, has been described in a variety of normal and neoplastic tissues. It is often used for highlighting lymphatics. We evaluated the expression of podoplanin in  $\alpha$ -smooth muscle actin-positive myofibroblasts producing the suburothelial layer in tunica propria of the urinary bladder that have some similar features with telocytes. Our results showed that these cells demonstrate distinct D2-40 immunoreactivity from telocytes occurring in the renal pelvis and ureter. We observed positive reaction not only in bioptic specimens from women with interstitial cystitis, but also in a control group of women and men treated for pathological bladder lesion different from interstitial cystitis. It is interesting that identical staining reaction was observed in the ureters only exceptionally. In addition, we examined samples from myofibroblastic tumoriform lesions of soft tissue such as nodular fasciitis and fibromatosis (desmoid) and we obtained negative results. It means that the so-called myofibroblasts of urinary bladder tunica propria have a unique immunophenotype that has probably not been described until now. Our findings suggest that D2-40 can be used as a complementary immunostainer to  $\alpha$ -smooth muscle actin on urinary bladder biopsies from patients with interstitial cystitis. The role of D2-40 as an immunohistochemical marker is still being investigated.

## Introduction

Interstitial cystitis/painful bladder syndrome (IC) is a poorly understood condition that predominantly affects women (Graham and Chai, 2006; Butrick et al., 2010). It is characterized by severe urinary urgency, frequency, suprapubic pain related to bladder filling, and dyspareunia in patients with negative urine cultures. Women report exacerbation of symptoms during the premenstrual week. Cystoscopic criteria include findings of ulcers or petechial mucosal haemorrhages (glomerulations) upon bladder distention. Hunner's ulcers are present in fewer than 15 % of IC patients. The pathological features of IC include oedema and vasodilation of the submucosal vessels and suburothelial haemorrhages. There are also small inflammatory infiltrates except for the patients with Hunner's ulcers. Diagnosis of this disease is mainly a diagnosis of exclusion because there are no characteristic symptoms or pathognomonic cystoscopic or histological findings. The aetiology and pathogenesis are still undetermined and it is supposed that the pathophysiology of IC may be multifactorial (Graham and Chai, 2006; Butrick et al., 2010). Some symptoms may arise from defective urothelial lining along with mast cell activation and neurogenic inflammation and neurotransmitter receptors in the smooth muscle cells (Graham and Chai, 2006; Butrick et al., 2010). The role of other structural components of the urinary bladder wall such as suburothelial myofibroblasts is not clear.

The suburothelial myofibroblasts in the lamina propria of the bladder wall were recently identified as a novel cell type (Wiseman et al., 2003; Drake et al., 2006; Gevaert et al., 2011; Zheng et al., 2012). It has been supposed that these cells could act as modulators of the bladder behaviour and could also play a role in the pathogenesis of the overactive bladder syndrome (Fry et al., 2007; Roosen et al., 2009). Immunohistologically they express vimentin,  $\alpha$ -smooth muscle actin, desmin, but in contrast to smooth muscle cells, they do not show positivity of h-caldesmon. These cells have close contacts with nerves containing small vesicles, and so it is supposed that they function as a bladder stretch receptor

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Abbreviations: ABC – avidin-biotin complex, IC – interstitial cystitis, ICC – interstitial cells of Cajal, HE – haematoxylin and eosin.

(Wiseman et al., 2003). The most striking immunohistochemical finding is the variable expression of oestrogen and progesterone receptors (Gevaert et al., 2011). Some authors, based on the ultrastructural and immunohistochemical phenotype, suppose that these cells should be classified as so-called telocytes (Gevaert et al., 2011), as was proposed for the first time by Popescu and Fausone-Pellegrini (2010). The functional relevance of upper lamina propria in the urinary tracts remains to be elucidated, and further immunohistochemical studies are therefore needed.

The aim of this study was to analyse the podoplanin expression in human telocytes immunohistochemically. Our study demonstrates that podoplanin (D2-40) is a highly effective marker of subepithelial myofibroblasts occurring in tunica propria of the urinary bladder.

## Material and Methods

### Patients

This study included 10 women, aged 28–35 years, examined for interstitial cystitis at the General University Hospital in Prague (Czech Republic) in the period 2010–2013. Control bioptic specimens were obtained from six women and five men examined and treated for different pathological bladder lesions distinct from interstitial cystitis. In addition, samples from six ureters and five renal pelvises were studied. We also examined nine myofibroblastic lesions (four samples of nodular fasciitis and five samples of myofibromatosis and desmoid) of soft tissue immunohistochemically to compare their phenotype with bladder myofibroblasts. All patients received information about the study and signed an informed consent.

### Histological evaluation

The specimens from the bladder biopsies performed with a flexible cystoscope obtained from control subjects and from both women with IC and control ureters and renal pelvises were fixed in 10% formalin and embedded in paraffin wax. The histological evaluation was done on slides routinely stained with haematoxylin and eosin (HE).

### Immunocytochemical staining

For the purposes of immunohistochemical studies, we used the avidin-biotin complex (ABC) technique. Primary monoclonal antibodies against  $\alpha$ -smooth muscle actin (1 : 400, Sigma-Aldrich, St. Louis, MO), muscle-specific actin HHF35 (1 : 400, DAKO, Glostrup, Denmark), desmin (1 : 200, Dako) and h-caldesmon (1 : 50, DAKO), ER (1 : 200, clone ER-6F11, Novocastra, Leica Biosystems, Newcastle upon Tyne, UK), PR (1 : 100, clone PGR-312, Novocastra), c-kit (1 : 200, DAKO), CD 34 (1 : 50) and podoplanin-D2-40 (1 : 100, DAKO) were used.

All immunostainings were performed in a Benchmark automatic immunostaining device (Ventana Medical

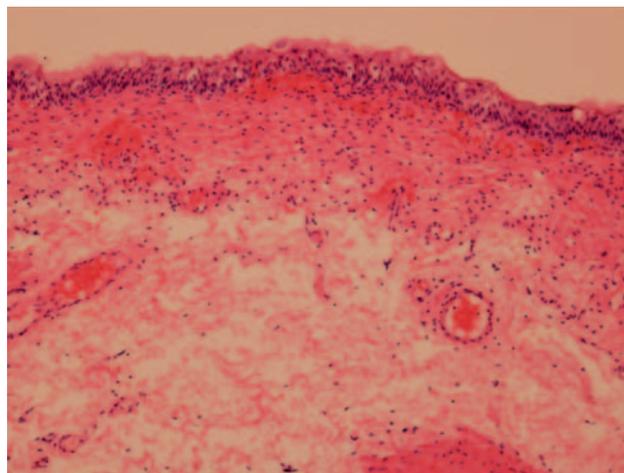


Fig. 1. Myofibroblasts in the upper lamina propria in a urinary bladder sample from a woman with interstitial cystitis. Haematoxylin-eosin,  $\times 90$

System, Tucson, AZ). Cases were considered positive for ER or PR when strong nuclear staining was observed in at least 10 % of tumour cells tested.

## Results and Discussion

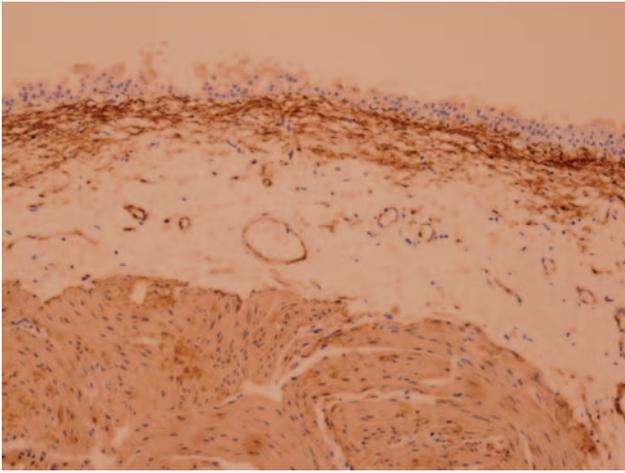
In biopsies from patients with IC, within the lamina propria of the urinary bladder we observed oedema and vasodilatation of vessels, and there were also small lymphocytic inflammatory infiltrates. The subepithelial layer was composed of small spindle cells corresponding to  $\alpha$ -smooth muscle actin and muscle-specific actin-positive myofibroblasts. These cells also expressed podoplanin, in contrast to  $\alpha$ -smooth actin-positive and h-caldesmon-positive smooth muscle cells of the detrusor. Other immunoreactions with antibodies against CD 34, desmin, S-100 protein, CD 31, and c-kit (CD 117) were completely negative.

These findings were compared to those in the control group that had no symptoms of cystitis. We obtained identical immunohistochemical results, including bioptic specimens from men.

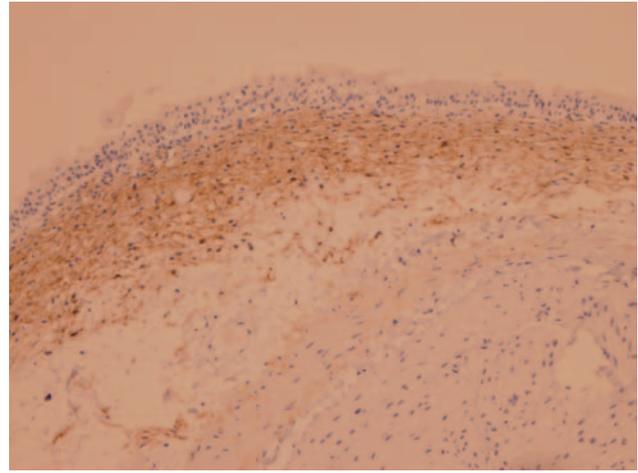
In the samples from renal pelvis, the layer of telocytes in this zone also expressed podoplanin in different short subepithelial areas. In contrast, the podoplanin positivity in ureters was observed only in one sample out of six cases.

We also determined the ER and PR receptor status of myofibroblasts and smooth muscle cells in all our patients. Smooth muscle cells were completely negative in contrast to subepithelial myofibroblasts. In all examined patients we found the nuclear expression of PR in different parts of subepithelial myofibroblasts. The ER-positive status was observed in eight patients. Co-expression of both hormonal receptors was observed in seven patients.

Immunohistochemical examination did not reveal podoplanin expression in any patients with myofibroblastic lesion of the soft tissue, i.e. nodular fasciitis and



*Fig. 2.* Immunophenotype of upper lamina propria myofibroblasts (telocytes) in a urinary bladder biopsy sample obtained from a woman with interstitial cystitis: immunoreactivity of  $\alpha$ -smooth muscle actin.  $\times 90$



*Fig. 3.* Immunophenotype of upper lamina propria myofibroblasts (telocytes) in a urinary bladder biopsy sample obtained from a woman with interstitial cystitis: immunoreactivity of podoplanin (D2-40).  $\times 90$

fibromatosis. However, they were typically  $\alpha$ -smooth muscle actin positive and h-caldesmon negative, as it is their characteristic immunophenotype.

Human bladder suburothelial myofibroblasts consist of long spindle-shaped cells that form a distinctive layer below the urothelium. In their cytoplasm, they contain bundles of fine smooth muscle myofilaments with focal densities and are surrounded by an interrupted basal lamina and produce fibronexuses (Drake et al., 2006). It is supposed that they act as an amplification stage in the sensory response to bladder-wall stretch, as it occurs during the bladder filling (Fry et al., 2007). They have close contacts with nerves (Wiseman et al., 2003). Immunohistochemical characterization of spindle cells with  $\alpha$ -smooth muscle actin has been used to define myofibroblasts without electron microscopy in some studies (Roosen et al., 2009). However, we should add that these cells are h-caldesmon negative, in contrast to smooth muscle cells also occurring in tunica propria of the human bladder.

The upper lamina propria area of interstitial cells has been studied extensively in the bladder and in the rest of the urinary tract during the last several years (Gevaert et al., 2011; Zheng et al., 2012). The interstitial cells of the telocyte type in renal urethra, ureter and pelvis had similar ultrastructural features, but they were not totally identical with bladder myofibroblasts. They had thinner and longer cytoplasmic processes, no peripheral actin filaments and contained dense core granules and microtubules (Gevaert et al., 2012). Together with their immunohistochemical profile, these features are most compatible with the phenotype of telocytes, a recently discovered group of stromal cells (Popescu and Fausone-Pellegrini, 2010). Gevaert et al. (2012) suggested that myofibroblasts in the human bladder should also be classified as telocytes, in spite of some differences in the ultrastructural features. Our new immunohistochemical findings demonstrating podoplanin positivity in urinary

bladder myofibroblasts and in renal pelvis support such conclusion.

The term telocytes was proposed by Popescu and Fausone-Pellegrini (2010) using the Greek affix *telos*. Telocytes, playing a role in intercellular signalling, were detected in a number of tissues and organs in mammals, e.g. skin, urinary tract, heart, blood vessels, exocrine pancreas, intestine tract, lungs, pleura, skeletal muscle, uterus and fallopian tube, endometrium, parotid glands or meninges and choroid plexus, as summarized by Zheng et al. (2012). Two different telocyte subpopulations express different markers in the human gastrointestinal tract. Interstitial cells of Cajal (ICC) are c-kit positive and CD34 and PDGER- $\alpha$  (platelet-derived growth factor receptor) negative, in contrast to c-kit-negative and PDGFR-positive fibroblast-like cells (Vanucchi et al., 2013).

Immunohistochemically, we can confirm the known phenotype of myofibroblasts occurring in the human bladder. These cells express vimentin, smooth muscle actin, muscle specific-actin and progesterone and oestrogen receptors. No expression of c-kit and CD34, which is rather common for interstitial cells of the gastrointestinal tract, was observed. We could confirm positivity of D2-40 antibody against podoplanin in human subepithelial myofibroblasts as was described by Gevaert et al. (2011). Podoplanin positivity occurred not only in interstitial cells of the myofibroblast type in the urinary bladder, but also in true telocytes occurring in other locations in the urinary tract, i.e. in renal pelvis and exceptionally in the ureter. To determine whether podoplanin represents an effective telocyte marker, we compared the results of immunohistochemical studies for podoplanin with those of the traditional myofibroblast marker  $\alpha$ -smooth muscle actin. The staining pattern of D2-40 was comparable with that of  $\alpha$ -smooth muscle actin. Both antibodies intensively stained elongated cells in the area between the urothelium and detru-

sor called lamina propria. These positive cells were localized directly underneath the urothelium and no other cells in the surrounding tissues expressed this immunohistochemical marker, with the exception of rarely occurring lymphatic vessels.

Urinary bladder actin-positive suburothelial cells have some ultrastructural features similar (identical) to myofibroblasts. That was the reason why we also examined several  $\alpha$ -smooth muscle actin myofibroblastic lesions such as nodular fasciitis and fibromatosis with antibody against podoplanin. In all these lesions the results were negative. These findings support the supposition that subepithelial cells in the urinary bladder have a partially different immunophenotype from the true myofibroblasts in spite of some identical immunohistochemical and ultrastructural features. It is possible that they may represent a new variant of myofibroblasts, as yet undescribed, with unusual function. To the best knowledge of the authors, this comparative study is the first to examine podoplanin expression in different organs and tissues of the urinary tract.

The monoclonal antibody D2-40 recognizes the membrane 40 kD sialoglycoprotein podoplanin. Podoplanin is an established marker for germ cell tumours, mesotheliomas, Kaposi sarcoma, lymphovascular tumours, hemangioendothelioma, schwannoma, some skin tumours (Kalof and Cooper, 2009), dermatofibroma (Kaddu and Leinweber, 2009), epithelioid sarcoma (Karagkounis et al., 2013) and ependymoma (Ishizawa et al., 2009). It is also expressed in a variety of normal cells including lymphatic endothelial cells, follicular dendritic cells (Marsee et al., 2009) and myoepithelial cells of the breast (Kanner et al., 2010), chondrocytes and osteocytes (Ariizumi et al., 2010). Our findings suggest that D2-40 can be used as a complementary immunostainer to  $\alpha$ -smooth muscle actin in urinary bladder biopsies from patients with interstitial urocystitis.

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