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Original article

Semiquantitative Smoothelin Expression in Detection of Muscle Invasion in Transurethral Resection and Cystectomy Specimens in Cases of Urinary Bladder Carcinoma

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ABSTRACT

Objectives: To examine the usefulness of smoothelin - a new immunohistochemical (IHC) marker that is expressed predominantly in visceral smooth muscle - in recognizing muscularis propria (MP) in transurethral resection (TUR) and matched cystectomy specimens and to compare the pattern of its expression in muscularis mucosae (MM) and MP in radical cystectomy specimens.
Methods: IHC staining for smoothelin was performed in 49 cases of urothelial carcinoma removed by radical cystectomy (16 had undergone TUR before the cystectomy).
Results: In cystectomy specimens, smoothelin staining in the MP was strong (+3), moderate (+2) and weak (+1) in 49%, 44.9% and 6.1% of cases, respectively, whereas smoothelin positivity in the MM was absent and weak in 77.6% and 22.4% of cases, respectively. In TUR specimens, smoothelin immunoreactivity was moderate to strong in 68.8% and weak in 6.3% of cases and all of them proved to have MP invasion in cystectomy specimens.
Conclusion: Smoothelin is a useful marker for the detection of MP in TUR specimens. Moderate to strong smoothelin staining of the muscles included in TUR specimens and split by the tumor is a sign of MP invasion. It may be useful in cancer staging and treatment decision making.

Key Words: Cystectomy, Muscle invasive urinary bladder carcinoma, Smoothelin, Transurethral resection, Urinary bladder carcinoma

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INTRODUCTION

Bladder cancer is among the top 10 causes of cancer-related deaths in the USA[1]. In Egypt, it is the most commonly diagnosed malignancy[2]. Tumor stage is the most important prognostic factor in bladder carcinoma. Accurate staging remains a critical task that allows for appropriate prognostic and therapeutic stratification of patients. Recognition of the depth of invasion by carcinoma is a vital determinant of subsequent therapy and prognosis. In the urinary bladder, urothelial carcinoma limited to the lamina propria is staged as pT1 and involvement of muscularis propria (MP) is staged as at least pT2[3]. It is now widely known that an inconsistent layer of muscularis mucosae (MM) muscle exists in the lamina propria, which can mimic the MP, particularly when hyperplastic. This makes staging extremely challenging in small, un-oriented or highly cauterized specimens. Also the presence of a desmoplastic stromal response rich in fibroblasts may mimic muscle bundles[4–6].

Smoothelin is a smooth muscle-specific cytoskeletal protein exclusively found in differentiated smooth muscle cells. This contrasts with other smooth muscle

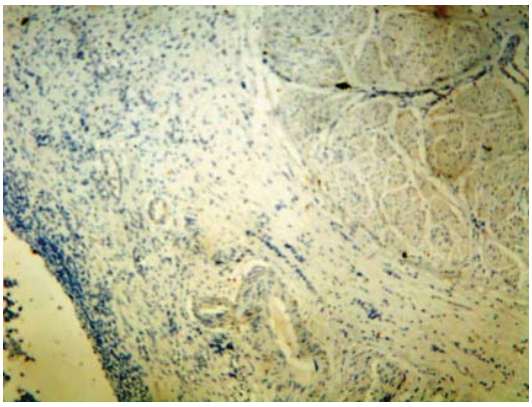

Fig. 1: Immunostaining of smoothelin in MM of cystectomy specimen showing weak positivity (IHC, original magnification x100).

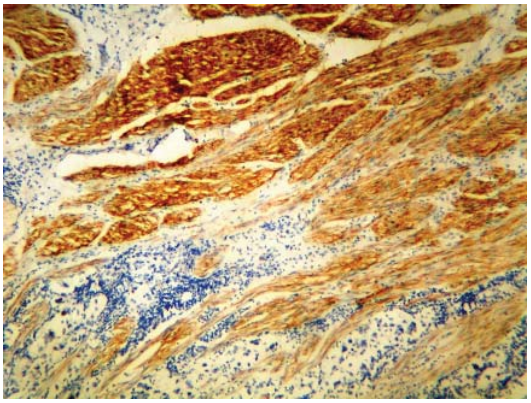

Fig. 2: Smoothelin expression in MP invaded by nests of tumor cells showing strong smoothelin expression (IHC, original magnification x200).

proteins (eg, h-caldesmon, [alpha]-smooth muscle actin, desmin, smooth muscle myosin) which are expressed in proliferative (early) stages of smooth muscle development and occasionally in other cell types (striated muscle, myofibroblasts, myoepithelial cells, pericytes). Smoothelin has been shown to be expressed predominantly in visceral smooth muscle and to a lesser extent in vascular smooth muscle[7]. Few previous reports estimate the difference in smoothelin expression in transurethral resection (TUR) or cystectomy specimens[8, 9].

The aim of this study was to examine the capability of smoothelin in recognizing muscularis propria in TUR specimens and matched cystectomy specimen and to compare the pattern of its expression in MM and MP in radical cystectomy specimens.

PATIENTS AND METHODS

The Specimens

This study consisted of Group 1: 6 cases of TUR biopsy with matched radical cystectomy specimens of the same patients and Group 2: 33 cystectomy specimens with prominent MM in hematoxylin and eosin (H&E) stained sections. All specimens were obtained from the Urology Department, Assiut University Hospital, Faculty of Medicine, Assiut University and examined in the Departments of Pathology, Faculties of Medicine, Assiut and Sohag Universities from January 2009 to June 2009.

Immunohistochemistry (IHC)

IHC was performed according to the manufacturer's protocol. Tissue sections (4-µm thick) of formalin-fixed, paraffin-embedded specimens were deparaffinized, rehydrated in graded alcohol and transferred to phosphate buffered saline (PBS; pH 7.6). The slides were rinsed twice with PBS, then endogenous peroxidase was blocked by the use of 3% hydrogen peroxide in methanol for 5 minutes and antigen retrieved using microwave at 700W for 20 minutes in citrate buffer.

After cooling, the slides were washed three times with PBS. The slides were then incubated overnight at room temperature with primary antibody for smoothelin (Biocare Medical CA, USA) at a dilution of 1:100. The slides were then rinsed three times with PBS and incubated for 15 minutes at room temperature with the primary antibody enhancer (Thermo Scientific, Fremont, USA). The slides were rinsed three times with PBS and incubated for 15 minutes with HRP polymer (Thermo Scientific, Fremont, USA). The sections were then washed three times with PBS and diamino benzidine (DAB) was applied for 5 minutes at room temperature. The slides were then rinsed in distilled water, counterstained with Mayer's hematoxylin, dehydrated and mounted.

The interpretation of immunoreactivity was performed in a semiquantitative manner by analyzing the extent of the staining posi-

Table 1: Smoothelin expression in bladder MM and MP in cystectomy specimens.

Smoothelin immunoreactivity	MM (%)	MP (%)	Vessels
0	38/49 (77.6%)	0/49 (0%)	22/49 (44.9%)
+1	11/49 (22.4%)	3/49 (6.1%)	26/49 (53.1%)
+2	0/49 (0%)	22/49 (44.9%)	1/49 (2%)
+3	0/49 (0%)	24/49 (49%)	0/49 (0%)

tivity of the muscle cells. For MP, the inner muscle layer or muscle bundles bordering the lamina propria were selected to evaluate staining of MP bundles of the inner half of the bladder wall, because these muscle bundles were most likely to be included in TUR specimens and could be confidently evaluated with respect to the overlying urothelium in all available specimens. Staining intensity was recorded as negative, weak, moderate or strong. The staining score was evaluated as follows: 0 or negative, +1 or weak, +2 or moderate and +3 or strong positivity. Representative sections were used for evaluation by H&E and IHC. MM and MP were evaluated in H&E and corresponding smoothelin stained slides.

RESULTS

In cystectomy specimens, 38 of 49 cases had definite MM bundles and in 11 cases it was not well identified and only showed a few spindle cells in the lamina propria.

Smoothelin expression in cystectomy specimens

IHC staining for smoothelin in bladder MM, MP and blood vessels in cystectomy specimens is summarized in Table 1.

MM muscles predominantly had absent or weak smoothelin staining in 77.6% and 22.4% of cases, respectively (Fig. 1) and none had moderate or strong staining. On the other hand, MP muscles predominantly had moderate or strong smoothelin staining in 49% and 44.9% of cases, respectively (Fig. 2).

Only 3 (6.1%) cases showed a weak smoothelin staining pattern in MP. Desmoplastic reaction to invasive carcinoma always stained negatively. Considering moderate and strong smoothelin immunostaining as the cut-off value for the presence of MP, smoothelin expression was 93.9% sensitive and 100% specific for MP (vs. MM) in cystectomy specimens.

The smooth muscles of vessels showed either negative or weakly positive smoothelin reactivity especially in large caliber arteries of MP in 44.9% and 53.1% of cases, respectively and only one case (2%) showed moderate smoothelin staining.

Smoothelin expression in TUR specimens is shown in Table 2

In TUR specimens 11/16 (68.8%) of the muscles showed moderate to strong smoothelin expression while 1/16 (6.3%) showed weak expression as shown in table 2. Cystectomy specimens of these patients proved muscle invasion and also showed smoothelin positivity. The blood vessels of vascular plexuses in the lamina propria were consistently negative or weakly stained.

Table 2: Smoothelin expression in bladder MM and MP in TUR biopsies.

Smoothelin immunoreactivity	Muscles (%)	Vessels
0	(25%) 4/16	(81.3%) 13/16
+1	(6.3%) 1/16	(18.7%) 3/16
+2	(31.3%) 5/16	(0%) 0/16
+3	(37.5%) 6/16	(0%) 0/16

DISCUSSION

Tumor stage is the most important prognostic factor in bladder carcinoma and the cornerstone of the pathologic tumor (pT) categories is evaluating the depth of carcinoma invasion in the bladder wall. The urinary bladder MP serves as an important anatomic landmark in the assessment of the depth of invasive carcinoma and is most often the critical dividing line between conservative and aggressive management in patients with bladder cancer. Involvement of the MP in a TUR specimen would stage the urothelial carcinoma as "deeply" invasive (at least pT2) indicating the need for cystectomy[5]. Thus, accurate identification of the MP by the pathologist is important in every TUR biopsy. Determination of the type of muscles (MM versus MP) invaded by carcinoma can occasionally be difficult, due to small sample size, tissue distortion, cautery artefact, poor orientation, fibrosis, inflammation or hypertrophy of the normally thin, wispy MM[1,5].

A few studies have reported that smoothelin immunoreactivity was useful in the discrimination between MM and MP in TUR and cystectomy specimens with unequivocal morphological findings[8,9].In this study, we provide further demonstration that smoothelin positivity can serve as an adjuvant tool in the evaluation of MP invasion by bladder carcinoma in diagnostic bladder biopsy.

Paner et al found that MM typically showed absent or weak positivity for smoothelin in 88% of cases and MP showed strong positivity in 86% of cases[8]. In the current study we found that 93.9% of MP showed moderate or strong positivity for smoothelin; whereas 100% of MM showed no or weak positivity.

MP showed moderate or strong smoothelin immunoreactivity in 46/49 (93.9%) and weak positivity in 3/49 (6.1%) of cystectomy specimens. This means that moderate to strong smoothelin expression was 93.9% sensitive and 100% specific for MP detection in cystectomy specimens. Also, MP showed moderate or strong smoothelin immunoreactivity in 11/16 (68.8%) and weak reactivity in 1/16 (6.3%) TUR specimens. As all these cases were shown to have MP invasion in cystectomy specimens, smoothelin could be used as a marker of MP invasion in TUR biopsies.

In TUR specimens included in the present study, smoothelin immunoreactivity confirmed the presence of MP in 68.8% of cases. This highlights the importance of smoothelin immunostaining, since the recognition of MP in small TUR specimens may obviate the need to perform a restaging TUR, especially in the case of high-grade invasive urothelial carcinomas. These finding were consistent with a recent report by Bovio et al 2010[10].

Although smoothelin reactivity is useful as adjunct to light microscopic evaluation, it should not be used as the sole determinant of muscle invasion, as we have three cases in which MP showed weak smoothelin reactivity in cystectomy specimens and one case of weak positivity in TUR biopsies. However, in these cases MM and blood vessels showed smoothelin negativity.

Myofibroblasts are distinct cells with characteristics of both smooth muscle cells and fibroblasts. They are thought to play a critical role in inflammation, repair and neoplasia[11]. Especially in cases of schistosoma-associated bladder cancer, differentiation between the characteristic prominent desmoplastic reaction and muscles split by tumor cells is very important. In the current study, desmoplastic reaction to invasive carcinoma and reaction around bilharzial ova were always negative

for smoothelin reactivity, whereas smoothelin was expressed at a similar level in MM and blood vessels of the lamina propria.

The reactivity of the medium-sized vessels is related to the expression of different isoforms of smoothelin: smoothelin A in contractile visceral smooth muscle and smoothelin B in vascular smooth muscle. The smoothelin antibody clone R4A used in this study binds to both visceral and vascular isoforms of smoothelin, accounting for the reactivity observed at these sites. This finding is consistent with a recent report by Bovio and colleagues who suggest using this finding as internal control for IHC, since vessels are present in nearly all specimens of non-superficial bladder cancer[10].

CONCLUSIONS

Our data confirm the value of moderate to strong smoothelin immunostaining to distinguish between MP, MM and desmoplastic reactions, thereby facilitating appropriate pathologic stage designation in often challenging TUR specimens.

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