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Is Smoothelin Useful for Detection of Muscularis Propria Invasion in Urinary Bladder Carcinoma Cases?

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A B S T R A C T

Urinary bladder carcinoma is a common urologic malignancy, particularly in males. We studied the role of smoothelin (a cytoskeleton protein) as a diagnostic marker for muscularis propria invasion, differentiating it from the muscularis mucosae for proper staging in urinary bladder carcinoma patients. Paraffin blocks from 85 cases of bladder carcinoma: 69 urothelial carcinoma cases, 10 squamous cell carcinoma cases, 5 adenocarcinoma cases and one case neuroendocrine small cell carcinoma, were stained by smoothelin immunohistochemical marker. Smoothelin proved an important diagnostic utility, allowing distinction of the muscularis propria MP (positive smoothelin expression) from the muscularis mucosa MM (negative smoothelin expression). The sensitivity and specificity of smoothelin in detecting MP invasion in the current study was 100%. It is important to use smoothelin immunohistochemistry as a routine in all cases of TURBT specimens for accurate staging and subsequent optimal patient management.

Introduction

Urinary bladder carcinoma (UBC) is an international public health problem (Malats and Real, 2015). It is the 9th frequently-diagnosed cancer worldwide, the 7th common cancer in men and the 14th leading cause of deaths due to cancer worldwide (Mahdavifar *et al.,* 2016). The highest incidence of bladder cancer is observed in Europe, the United States and Egypt, respectively, while the lowest level is in Sub- Saharan Africa, Asia and South America, respectively (Antonia *et al.,* 2017). In Egypt, bladder cancer incidence and behavior differ significantly from the developed countries, as bladder cancer has been and remains one of the most prevalent malignancies, accounting for 12.22% of male cancers and representing the main bulk of the urinary system malignancy (Salem and

Mahfouz, 2012). Smoothelin is a cytoskeletal protein that is specifically expressed in fully differentiated contractile smooth muscle (Kirna *et al.,* 2015). Smoothelin has been reported to be expressed in the muscularis propria (MP) of normal and overactive bladder (Maake *et al.,* 2006, Paner *et al.,* 2009 and Bovio *et al.,* 2010).

Pathological determination of the depth of invasion is crucial to the accurate staging and subsequent therapy of patients. Namely, tumor invasion limited to the lamina propria (LP) is pathological stage T1 (pT1), whereas tumor extending into the MP is at least pathological stage T2 (pT2) (Vakar-Lopez *et al.,* 2007).

This distinction is critical because invasion of the MP is associated with a poorer prognosis and is one indication for definitive aggressive therapy (e.g. radical cystectomy or chemo-radiation) (Humphrey, 2004).

There are several circumstances in which accurate determination of MP invasion may be difficult. The invasion of urothelial carcinoma into the LP often elicits hypertrophy of the muscularis mucosae (MM), changing the appearance from thin wisps of smooth muscle to thickened muscle bundles that can simulate the MP. Also this invasion may induce stromal desmoplastic reaction consisting of myofibroblasts giving false appearance of MP.

This may be particularly problematic in transurethral resection of bladder tumor (TURBT) specimens, in which proper orientation of the material is not possible (Jimenez *et al.,* 2000; Vakar-Lopez *et al.,* 2007).

In addition, carcinomas invading into the MP can often alter the arrangement of the MP muscle by splitting the usually thick muscle bundles, making its recognition difficult (Epstein *et al.,* 2004).

Materials and Methods

This study was carried out on 85 cases of urinary bladder carcinomas. These cases were selected from the archives of the Pathology department, Faculty of Medicine during the period from January 2017 to December 2018. Approval from the research ethics committee (REC), was taken before conducting the study. The specimens obtained were: 71 specimens of trans-urethral resection of bladder tumors (TURBT) (83.5%) and 14 radical cystectomy specimens (16.5%).

Histopathological study

Histological sections, 4-mm thick, were stained by hematoxylin and eosin (H&E) for evaluation of histopathological parameters, including: the histopathological grade, depth of invasion (T), vascular, perineural invasion, associated CIS and whenever possible, lymph node status (N) and prostatic involvement.

The studied urinary bladder carcinoma cases were classified microscopically according to the 2016 World Health Organization (WHO) classification system (Moch *et al.,* 2016).

Pathological Staging of the studied tumors was done according to American Joint Committee on Cancer (AJCC) TNM Pathologic Staging of Urinary Bladder Carcinomas (Edge *et al.,* 2010).

Immunohistochemical staining

Immunohistochemical staining was performed using the streptavidin-biotin method as described by (Buchwalow and Böcker, 2010), for evaluation of smoothelin expression. From each paraffin block, 4-mm-thick sections mounted onto positively charged slides, deparaffinized and rehydrated. Endogenous peroxidase was blocked by immersion in 3% hydrogen peroxide. Antigen retrieval using

microwave oven was performed. The primary antibodies used were:

Smoothelin antibody was a mouse monoclonal antibody raised against cytoskeletal extract from gizzard of chicken origin, (R4A): sc-23883 (Santa Cruz Biotechnology, Inc). Two to three drops of smoothelin were placed on each slide at a 1:50 dilution.

Interpretation of smoothelin positivity

Positive smoothelin staining was indicated by the presence of brownish cytoplasmic staining in the smooth muscle cells of the studied cases

The intensity of smoothelin expression was graded semiquantitatively as follows: Negative (0), Weak (1+), Moderate (2+), or Strong (3+) (Bovio *et al.*, 2010).

Statistical analysis

The collected data was statistically analyzed using SPSS software statistical computer package version 20. Data were expressed in terms of frequencies (number of cases) and percentages for categorical variables and range, median, mean±standard deviation (SD) for continuous variables. For comparing categorical data, Chi-square (X2) test was used as a test of significance. Fisher's exact test or Monte Carlo was used when one or more of cells have an expected frequency of five or less. P values of <0.05 were considered statistically significant (Petrie and Sabin, 2005).

Results and Discussion

Histopathological examination of the studied cases

The 85 studied cases (78 cases were males, 91.8% and 7 cases were females, 8.2% with age ranged between 38 and 86 years, mean age was 61.08), were categorized into 4 groups:

Group I: 69 cases of urothelial carcinoma (UC) (81.2%): including 10 cases of non-invasive low grade papillary UC and 59 cases of infiltrating UC. Infiltrating urothelial carcinomas included: 29 case pure UC, 13 cases UC with squamous differentiation, 4 cases UC with glandular differentiation, 3 cases sarcomatoid UC, 2 cases plasmacytoid UC, 2 cases microcystic UC, 2 cases micropapillary UC, 2 cases clear cell UC, one case UC with both squamous and glandular differentiation, and one case poorly differentiated urothelial carcinoma.

UC cases were graded into: 18 cases low grade urothelial carcinoma (26%) and 51 cases high grade urothelial carcinoma (74%). Regarding muscularis propria (MP) invasion in urothelial carcinoma group: 23 cases were NMI "Ta & T1" (33.3%) and 46 cases were MI "T2, T3 & T4" (66.7%).

Group II: 10 cases of squamous cell carcinoma (SCC) (11.8%).

Eight cases were moderately differentiated SCC and two cases were poorly differentiated SCC.

Group III: 5 cases of moderately differentiated adenocarcinoma (5.9%).

Group IV: One case of neuroendocrine small cell carcinoma (SmCC) (1.1%), confirmed by strong membranous immunoreactivity to CD56 neuroendocrine marker.

Smoothelin immunohistochemical results

Out of our 85 cases, muscularis propria (MP) invasion was detected in 62 cases (72.9%), while no muscle invasion was found in 23 cases (27.1%).

Table.1 Smoothelin expression in the muscularis propria among the four studied groups

Group		Smoothelin Scoring			Total positive	Total
		No Muscle Invasion	Moderate +2	Strong +3		
Urothelial carcinoma	N	23	6	40	46	69
	%	33.3%	8.7%	58%	66.7%	100%
Squamous cell carcinoma	N	0	0	10	10	10
	%	0%	0%	100%	100%	100%
Adeno carcinoma	N	0	0	5	5	5
	%	0%	0%	100%	100%	100%
Neuroendocrine carcinoma	N	0	0	1	1	1
	%	0%	0%	100.0%	100%	100%
Total	N	23	6	56	62	85
	%	27.1%	7.1%	65.9%	72.9%	100%

Fig.1 Muscularis propria (MP) in case of high grade infiltrating urothelial showing strong cytoplasmic smoothelin expression (+3) (Streptavidin biotin x400)

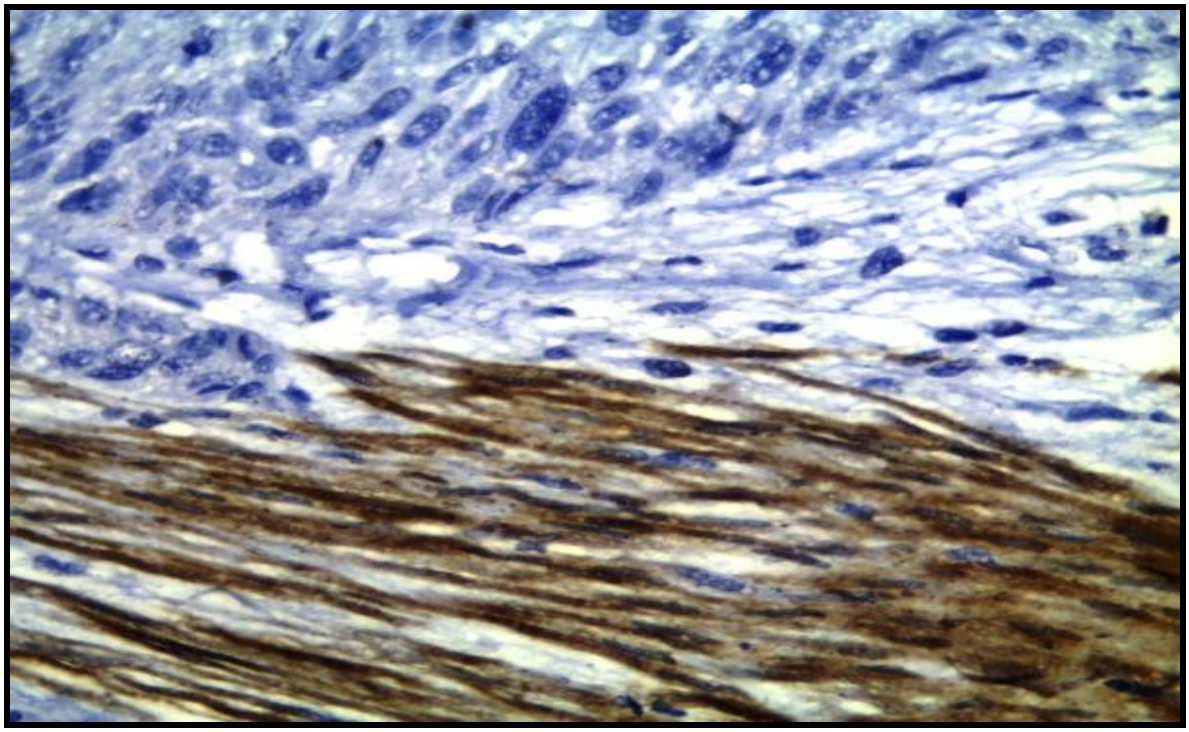


Fig.2 Muscularis propria (MP) in a case of moderately differentiated SCC showing strong cytoplasmic smoothelin expression of the MP (+3) (Streptavidin biotin x400)

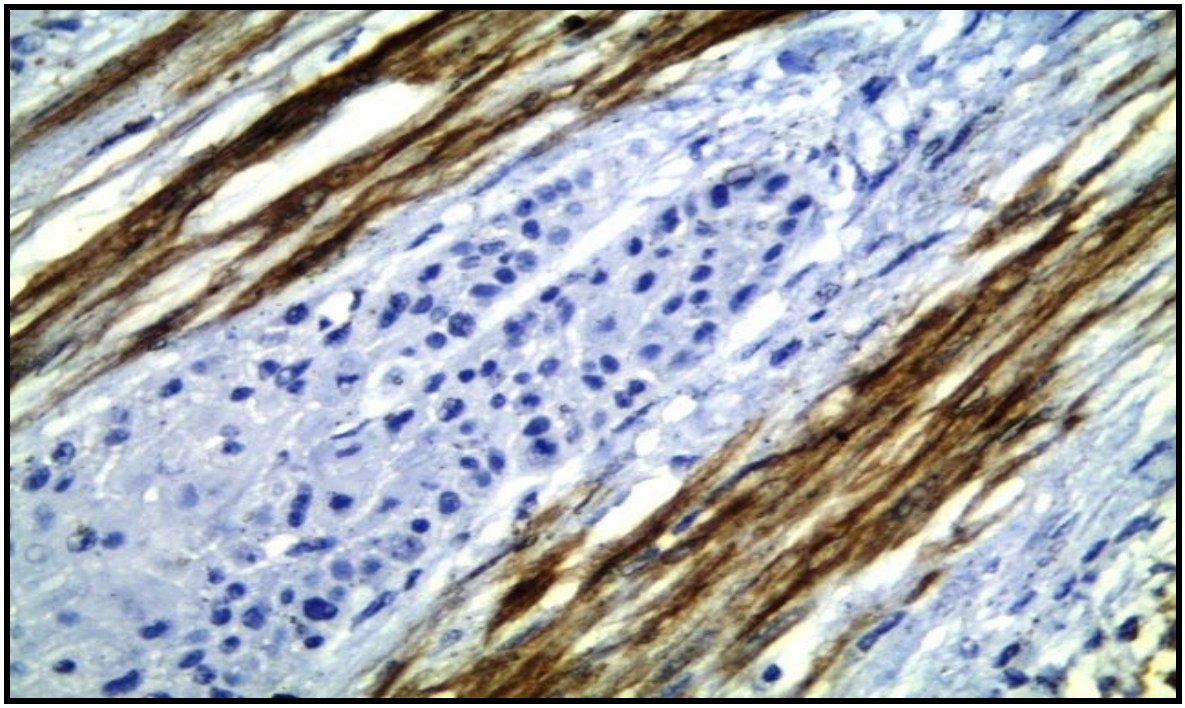
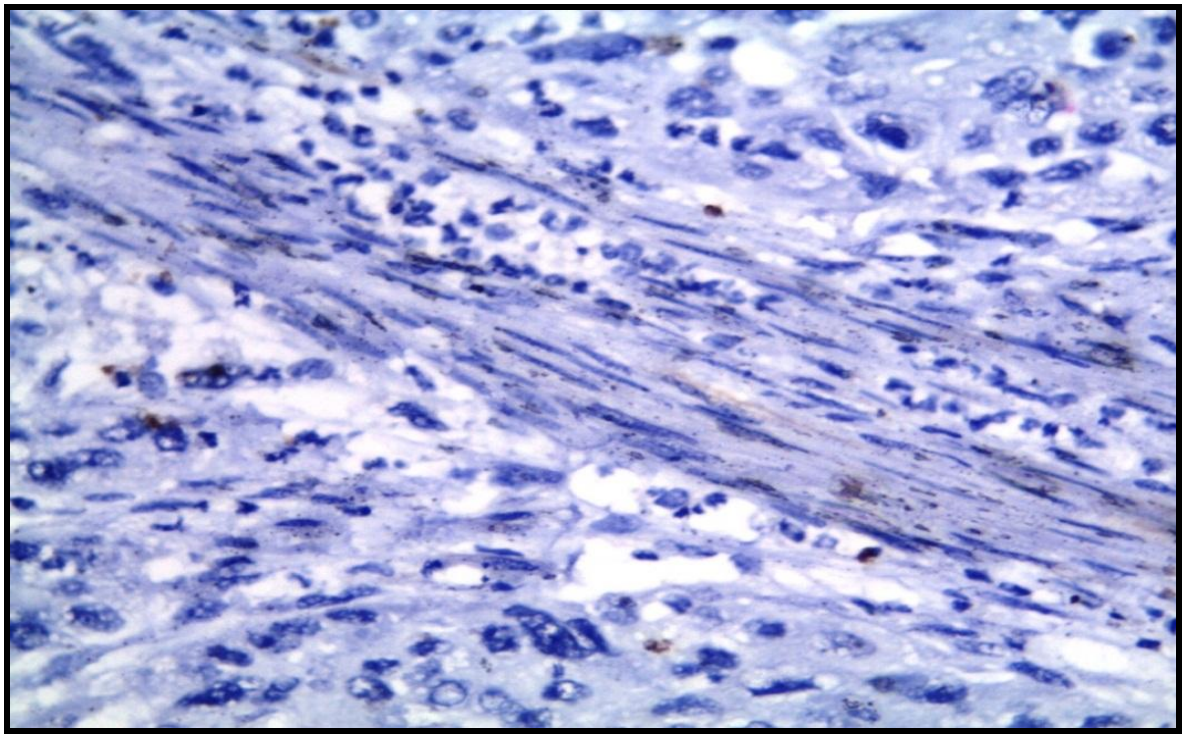


Fig.3 Muscularis mucosa (MM) in a case of high grade infiltrating urothelial carcinoma showing negative smoothelin expression (Streptavidin biotin x400)



Positive cytoplasmic smoothelin expression was present in the smooth muscle cells of the MP in all of the studied cases. All cases with MP invasion (62 cases) showed positive cytoplasmic smoothelin expression (100%) with varying intensity, as 56 cases showed strong +3 smoothelin expression, while 6 cases showed moderate +2 smoothelin intensity (Table 1, Figure 1 and 2).

No case of detected MP showed negative or weak staining for smoothelin.

Regarding smoothelin expression in the muscularis mucosa (MM) which was detected as thin wispy muscle bundles, all detected cases showed negative smoothelin expression (Figure 3).

The smooth muscle of the blood vessel walls in the LP showed moderate smoothelin expression (+2) which served as internal control for smoothelin immunohistochemical staining.

Sensitivity and specificity of smoothelin as diagnostic marker for MP invasion

Considering strong and moderate intensity as positive smoothelin staining, the sensitivity and specificity for smoothelin in MP detection (vs. MM) were 100%. In the current study, all cases with MP invasion showed positive cytoplasmic smoothelin expression (as 90.3% showed strong +3 smoothelin expression**,** while 9.7% showed moderate +2 smoothelin intensity). No case of detected MP showed negative or weak staining for smoothelin.

Regarding smoothelin expression in the muscularis mucosa (MM) which was detected as thin wispy muscle bundles, all detected cases showed negative smoothelin expression. No case of the detected MM showed any reactivity for smoothelin.

The results of this study were in agreement with Kamel *et al.,* (2015) who found that smoothelin showed strikingly different immunoreactivity between MM and MP. In their study, the MM showed absent (73%) or weak staining +1 (25%), only one case showed moderate positive staining +2 (1.6%), in this case the MP had strong +3 reactivity and none showed strong staining (0%). In contrast to the MM, the MP predominantly showed strong staining (60%) and moderate staining (40%), with none showing weak or negative staining (0%). Council and Hameed (2009), Paner *et al.,* (2009), Bovio *et al.,* (2010), Gladell *et al.,* (2010) & El-Osaily *et al.,* (2015) also found that the MP of all evaluated cases showed moderate or strong smoothelin expression. Absent or focal staining with smoothelin was not observed in the MP in any of their studied cases, with all MM of their cases showing negative or weak expression.

Kirna *et al.,* (2015) also reported that smoothelin Immuno-histochemistry corrected their diagnosis in 3/70 cases that were wrongly diagnosed as invasive (florid desmoplastic response was mistaken for MP). Hence these 3 cases were down-staged after smoothelin IHC.

On the contrary among non-invasive tumors in their study, there were 5 cases which were proven invasive based on smoothelin IHC. These 5 cases were up-staged after IHC. They concluded that it was important to use smoothelin IHC as a routine in all cases of TURBT specimens for proper staging.

The sensitivity and specificity of smoothelin in detecting MP invasion in the current study was 100%. This is in agreement with Council and Hameed, (2009), Kamel *et al.,* (2015) who reported 100% sensitivity and 100% specificity for smoothelin in detecting MP invasion.

Our results were slightly higher than that of Bovio *et al.,* (2010) who stated that smoothelin had a sensitivity of 92% for detecting MP and a specificity of 97% for distinguishing between MP and MM.

Elkady *et al.,* (2017) also reported that the intensity of smoothelin expression showed significant difference between MM and MP with 97.5% sensitivity and 95% specificity.

They conclude that combined moderate to strong smoothelin and negative vimentin offered 100% sensitivity and 100% specificity towards the identification of MP.

Roberts *et al.,* (2014) used smoothelin immunohistochemistry in colorectal carcinoma and reported that all sections of the colonic MP (100%) had diffuse, strong +3 immunoreactivity for smoothelin while the desmoplastic areas of these tumors, composed of spindled fibroblasts and myofibroblasts, showed negative immune-staining for smoothelin. So smoothelin can help in the accurate staging of cancer and subsequent optimal patient management.

In contrast to the current study and all the previous studies, Poletajew *et al.,* (2017) highlighted a 32% incidence of moderate smoothelin expression in cases of hyperplastic MM, and as many as 25% of cases, the reaction intensity within MM and MP was similar, concluding that the use of smoothelin immunohistochemistry as a diagnostic tool for MP invasion requires caution and that no single marker is reliable for differentiating between MM and MP, so they suggested to use a combination of anti-smoothelin, and anti-vimentin antibodies for diagnostic purposes.

This discrepancy in the results may be due to different staining methodology and interobserver variability.

The relatively distinct immunohistochemical staining pattern of smoothelin between MP and MM with 100% sensitivity and specificity, makes it a useful marker to be incorporated in the routine TURBT assessment, could potentially reduce the number of cases that are re-staged, helps in the accurate staging of bladder carcinoma and subsequent optimal patient management.

We haven't submitted this work elsewhere before and the manuscript had been read and approved by all the authors.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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