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ETS-1 oncoprotein expression is decreased in aggressive papillary transitional cell carcinoma of the urinary bladder: An immunohistochemical study



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KEYWORDS

Papillary transitional cell carcinoma;
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Grade

Abstract

Introduction: ETS-1 proto-oncogene is a transcription factor that plays multiple roles in the process of oncogenesis and helps in the process of tumor invasion. ETS-1 oncoprotein correlation with high grade and invasive tumors is controversial; as it is found to be upregulated with some tumors and down regulated with others. Expression of ETS-1 in urinary bladder carcinoma (UBC) and its correlation with tumor differentiation and invasiveness are still under-investigated. So far, there is no reliable prognostic marker has been proved for detection of the tumor progression and recurrence.

Objectives: To analyze the correlation between ETS-1 oncoprotein immunohistochemical expression and the different stages and grades of the primary papillary transitional cell carcinoma of the urinary bladder.

Patients and methods: This is a retrospective cross sectional study that included archival material from 150 cancer cases and 24 control biopsies.

Results: There was a decreased ETS-1 oncoprotein expression with increasing stage and grade of the tumor with a highly significant statistical correlation ($P=0.001$). With the quantitative assessment of the immunohistochemical results and using ROC (receiver operating characteristics) curve, cut-off values were found, that were associated with high grade and muscle invasive tumors ($\leq 30\%$ and $\leq 20\%$, respectively).

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Conclusion: ETS-1 oncoprotein is down regulated with high grade and highly invasive urinary bladder papillary transitional cell carcinomas. This oncoprotein may be used as an independent prognostic marker to predict the aggressive papillary transitional carcinomas with high invasive potential. More studies are needed to confirm our results.

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Introduction

Urinary bladder carcinoma (UBC) is one of the most prevalent malignant tumors worldwide; it is the ninth in incidence order amongst others. It is the seventh most common malignancy in adult males and the 17th among adult females [1,2].

ETS-1 proto-oncogene is a transcription factor that plays multiple roles in the process of oncogenesis. It helps the process of tumor invasion by modulating the extracellular matrix via stimulating the transcription of metalloproteinases and urokinase-type plasminogen activator [3]. Furthermore, it activates the endothelial cells proliferation and activation, thus stimulating the angiogenesis and tumor progression [4]. ETS-1 oncoprotein correlation with high grade and invasive tumors is found to be controversial; as it is found to be upregulated with some tumors [5–10] and down regulated with others [11–13]. This can be explained by the different target proteins which may result in a competitive function that may be expressed differently in various organs [13].

Expression of ETS-1 in UBC and its correlation with the tumor differentiation and invasiveness are still under-investigated.

Objectives

The present study aims to evaluate the significance of ETS-1 expression in papillary transitional cell carcinoma of the urinary bladder and determine the relationship of this oncoprotein with the histopathological parameters including tumor grade and stage.

Subjects and methods

This is a retrospective cross sectional study performed in the pathology department of Ain Shams Specialized University Hospital (ASUSH). The studied material comprised of archival paraffin blocks of urinary bladder papillary transitional cell carcinoma from patients who underwent radical cystectomy or TUR. The control cases consisted of cystoscopic biopsies for chronic cystitis, in an anonymous way, so no consent was needed from the patients. The research study was approved by the Research Ethical Committee of Ain Shams University, the rules of which were in accordance with the ethical standards laid down in 1964 Declaration of Helsinki.

Specimen and data collection

We retrieved 157 cases of primary papillary transitional cell carcinoma of the urinary bladder and 24 control biopsies. Paraffin blocks were retrieved from the pathology departments of ASUSH and Alde-merdash Hospital in the period from January 2010 to December 2014. Archival files of the cases were also retrieved and all

available clinical data were registered (age, sex and stage of the tumor). Inclusion criteria included a diagnosis of primary urinary bladder papillary transitional cell carcinoma, with availability of adequate clinical data and the presence of, furthermore, adequately presented muscularis propria for proper assessment of the tumor invasion. Exclusion criteria included inadequate clinical data, tumors with much tissue necrosis and absence of muscularis propria in the examined sections.

We excluded five cases due to inadequate clinical history and another two cases due to extensive tumor tissue necrosis. Of the remaining were 150 cases, for the study; there were 81 cases had undergone radical cystectomy and the remaining 69 were transurethral biopsies.

Histopathological examination

The slides of the selected cases were reviewed under light microscopy. The tumors were graded according to the World Health Organization 2004 system [14] and staged into: stages 0 and I (early bladder cancer), stages II and III (invasive bladder cancer) and stage IV (advanced bladder cancer) [15].

Immunohistochemical staining and scoring

Four micron-thick sections were cut from the selected paraffin blocks and stained immunohistochemically by using ETS-1 antibody (mouse monoclonal antibody) (Clone 1G11: Cat. #MS-1762-R7) (7.0 ml) (Thermo Fisher Scientific Inc, MA, USA). It was a ready-to-use reagent; the manufacturer's staining protocol was followed. For positive control we used human tonsillar tissue and omitting the use of primary antibody was done for negative controls to ensure correct staining procedure [16].

ETS-1 expression was assessed semi-quantitatively into 3 categories according to the percentage of cells with positive nuclear staining; the results were expressed as follows: score 0 = 0–10% positive cells; score 1 = 10–50% positive cells; score 2 = >50% positive cells [17].

Statistical analysis

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc., Chicago, IL, 2001). Data was presented and suitable analysis was done; Mean, Standard deviation (\pm SD) and range for parametric numerical data, Student's *t*-test to assess the statistical significance of the difference between two study group means, ANOVA test was used to assess the statistical significance of the difference between more than two study group means. Chi-square test was used to examine the relationship between two qualitative variables. Fisher's exact test was done to examine the

Table 1 Correlation between the patients' demographic data and their clinicopathologic features.

Parameter	Age Mean \pm SD	Sex		Total (100%)
		Male	Female	
Tumor stage				
0 (9)	50.7 \pm 11.8	9 (7.7%)	0 (0%)	6%
I (54)	56.9 \pm 13.9	39 (33.3%)	15 (45.5%)	36%
II (38)	58.6 \pm 13	28 (73.7%)	10 (26.3%)	25.3%
III (42)	57.5 \pm 8.8	30 (71.4%)	12 (28.6%)	28%
IV (7)	60 \pm 6.3	5 (71.4%)	2 (28.6%)	4.7%
Total		117 (78%)	33 (22%)	150
<i>P</i> *	0.6		0.8	
Tumor grade				
Low 84 (56%)	55.6 \pm 14.8	63 (53.8%)	21 (63.6%)	84 (56%)
High 66 (44%)	60 \pm 10.6	54 (46.2%)	12 (36.4%)	66 (44%)
Total		117 (78%)	33 (22%)	150
<i>P</i> **	0.3		0.7	

*P** Anova test results revealed no significant correlation.

*P*** Student's *t*-test results revealed no significant correlation.

relationship between two qualitative variables in a table with a cell containing value less than five. The ROC curve (receiver operating characteristic) was used to evaluate the sensitivity and specificity of the quantitative diagnostic measures. The cut-off value of significance was $P < 0.05$.

Results

Patients and tumor characteristics

The age of study cases ranged from 48–75 years with a mean value of 57.5 ± 13.2 years. The majority of cases were males (78%) with a male/female ratio of 3.5:1. Bilharzias infection was present in 39

cases (26%). More than half of cases had early bladder cancer (stage I, 36%) and of low grade type (56%). There were no significant statistical correlation between both age and sex on one hand and tumor grade and stage on the other hand (Table 1).

Statistical relationships

The microscopic examination of the immunohistochemically stained slides revealed cytoplasmic and nuclear staining of the tumor cells.

The entire normal (non-neoplastic) urothelium in the control group showed positive ETS-1 staining (Fig. 1A).

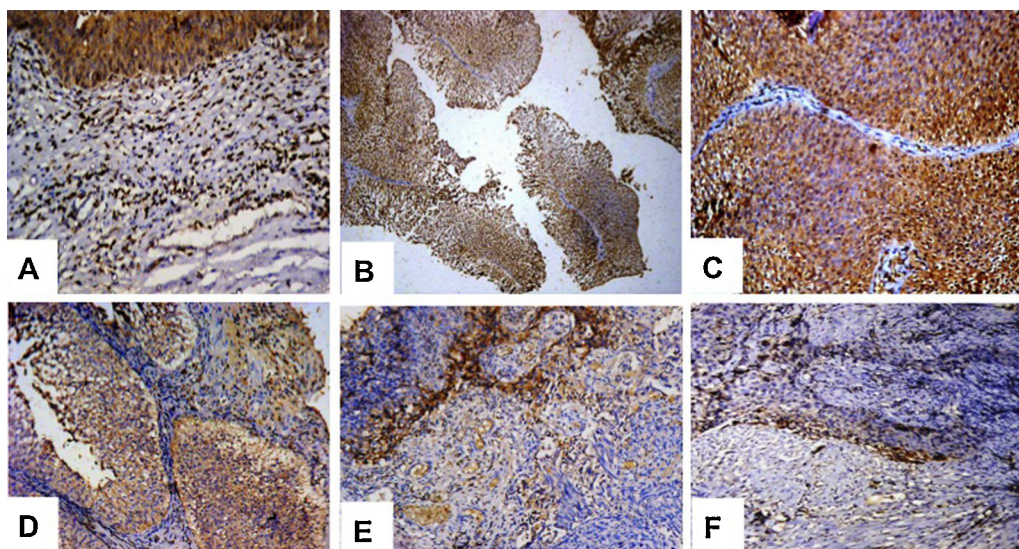


Figure 1 (A) Entire normal urothelium showing positive ETS-1 staining. The lymphocytes showed positive ETS-1 staining and served as internal positive control ($\times 200$). (B) ETS-1 expression in low grade, non-invasive urothelial carcinoma (pTa). ETS1 expression percentage is 80%, score 2 ($\times 100$). (c) ETS-1 expression in low grade, non-invasive urothelial carcinoma (pTa). ETS1 expression percentage is 80%, score 2 ($\times 200$). (D) ETS-1 Expression in low grade urothelial carcinoma with lamina propria invasion (pT1) ($\times 200$). (E) ETS-1 Expression in high grade urothelial carcinoma with lamina propria invasion (pT1). ETS1 expression percentage is 30%, score 1 ($\times 200$). (F) ETS-1 expression in high grade, muscle invasive urothelial carcinoma. ETS1 expression percentage is $< 10\%$, score 0 (Original magnification $\times 100$).

Table 2 Correlation between ETS-1 expression and tumor stage and grade.

Parameter	ETS-1			P
	0	1+	2+	
Control (24)	0	0	24	
Tumor stage (N)				0.001*
0 (9)	0	0	9 (100%)	
I (54)	9 (16.7%)	20 (37%)	25 (46.3%)	
II (38)	15 (39.5%)	15 (39.5%)	8 (21%)	
III (42)	10 (23.8%)	32 (76.2%)	0	
IV (7)	5 (71.4%)	2 (28.6%)	0	
Total 150	39	69	42	
Tumor grade				0.001**
Low	21 (25%)	27 (32.1%)	36 (42.9%)	
84 (56%)				
High	18 (27.3%)	42 (63.6%)	6 (9.1%)	
66 (44%)				
Total	39	69	42	

* Highly significant correlation between control and tumor stage regarding ETS-1 expression.

** Highly significant correlation between control and tumor grade regarding ETS-1 expression.

There was a significant statistical correlation between ETS-1 expression and tumor stage in comparison with the control group ($P=0.001$). The ETS-1 expression revealed down regulation with increased tumor stage (Stages II, III and IV in comparison to stages 0 and I). (Table 2, Fig. 1B–F). On the other hand, there was also a significant correlation between ETS-1 expression and the tumor grade with an observed down regulation with the high grade tumors ($P=0.001$) (Table 2, Fig. 1B–F).

With quantitative assessment of the extent of immunohistochemical staining and using ROC curve, it was found that ETS-1 expression <30% was associated with high grade tumors with 90.9% sensitivity and 50% specificity (Table 3, Fig. 2). Furthermore, it was found that ETS-1 expression <20% was associated with tumors that showed high invasive potential (muscle invasive, stage II or higher) with 75.8% sensitivity and 80.9% specificity (Table 3, Fig. 3).

Discussion

The current study showed down regulation of ETS-1 immunohistochemical expression with increased tumor stage with a high significant statistical correlation ($P=0.001$). On the other hand, there was also a significant correlation between ETS-1 expression and the tumor grade with an observed down regulation with the high grade tumors ($P=0.001$). It means that decreased ETS-1 expression occurred with more aggressive tumors.

Table 3 Performance of ETS-1 immunohistochemical expression in association with papillary transitional cell carcinoma of high grade and stage.

Parameter	High grade tumors	Stages (II, III and IV)
ETS-1 cut-off value	≤30	≤20
Sensitivity	90.9%	75.9%
Specificity	50%	81%
Positive predictive value (PPV)	58.8%	84.6%
P	0.001	0.001

Using ROC curve, it was found that ETS-1 expression <30% could be used as a cut-off value for prediction of high grade tumors with 90.9% sensitivity and 50% specificity. Furthermore, it was found that ETS-1 expression <20% could be used as another cut-off value in prediction of tumor with high invasive potential (muscle invasive) with 75.8% sensitivity and 80.9% specificity.

However, the retrospective cross sectional nature of this study with the lack of follow up information hinders the confirmation of its findings.

Strengths of the study include the fair number of both study and control groups, with sample stratification into low and high tumor grades as well as all the tumor stages. Furthermore, the semi-quantitative assessment of the immunohistochemical stain results adds more

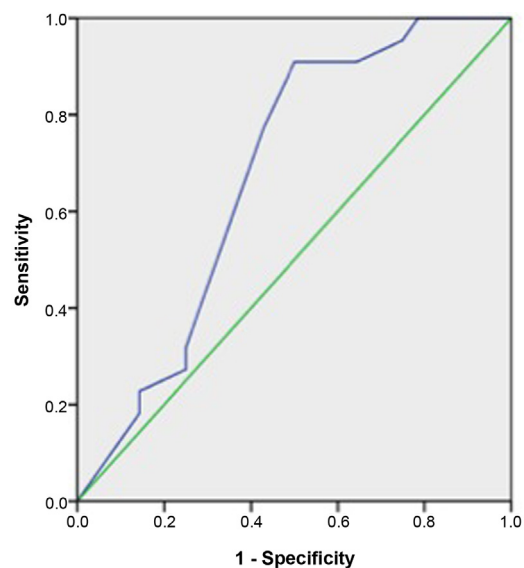


Figure 2 ROC curve illustrating the performance of ETS-1 immunohistochemical expression with different tumor grades.

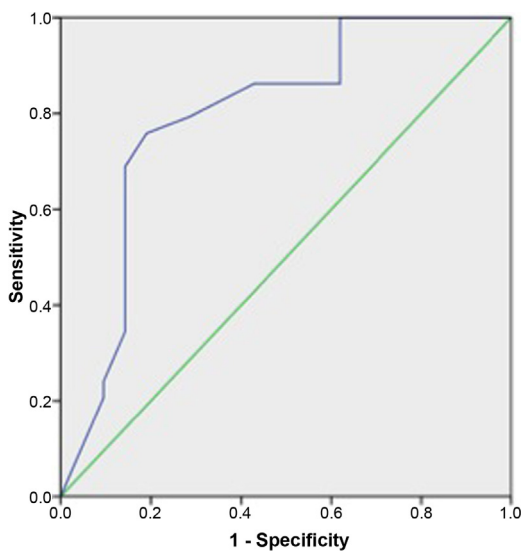


Figure 3 ROC curve illustrating the performance of ETS-1 immunohistochemical expression with different tumor stages.

accurate evaluation. To the best of our knowledge this is the first study to suggest cut-off values for prediction of tumors with high grade and invasive potentials.

Here in our study, we observed that the majority of low-grade and noninvasive urothelial carcinomas intensely expressed ETS-1, whereas high-grade and invasive carcinomas showed a frequent decrease of ETS-1 expression. This was in accordance with Sari et al. [17] who found that ETS-1 expression showed a strong negative correlation with the tumor grade ($P < 0.001$) and also found that ETS-1 expression showed a strong negative correlation with the tumor stage ($P < 0.001$). The topographic distribution of the oncoprotein stain in both nucleus and cytoplasm is in agreement with Nakayama et al. [11] who found similar results in colorectal carcinoma, also the study of Ebel et al. [14] and Yamaguchi et al. [18] where the stain revealed the same distribution in pulmonary and endometrial adenocarcinoma respectively. This can be explained by the oncoprotein overproduction which gives positive cytoplasmic staining [10,18].

ETS-1 immunohistochemical expression can be used as an adjunct prognostic tool with Hematoxylin and Eosin for better evaluation of the papillary transitional cell carcinoma of the urinary bladder. The evaluation of extent of immunohistochemical staining with use of the suggested cut-off values may help in predicting the tumors with higher invasive potentials. This may be valuable in making the decision of either conservative or radical operations. However, further researches with follow up data are needed to prove our results. Furthermore, more studies using the same marker for non papillary transitional cell carcinoma and those arising from the renal pelvis or ureter are needed for comparison with our findings.

Conclusion

ETS-1 immunohistochemical expression showed down regulation with increased grade and stage of the primary urinary bladder papillary transitional cell carcinoma. This may give a clue for more understanding of this tumor carcinogenesis. In addition, it may be

used as a prognostic marker especially for early urinary bladder cancer.

Authors' contribution

Eman Abdel-Salam Ibrahim: Histopathological examination, statistical analysis, writing and revision of the manuscript.

Mona Refaat Hassan: Data collection, histopathological examination and statistical analysis.

Sanaa A Sammour: Writing and revision of the manuscript.

Ethical committee approval

The research study was approved by the Research Ethical Committee of Ain Shams University. The rules of which were in accordance with the ethical standards laid down in 1964 Declaration of Helsinki.

Conflict of interest

The authors declare that there are no conflicts of interest.

Source of funding

None.

References

- [1] Parkin DM. The global burden of urinary bladder cancer. *Scand J Urol Nephrol Suppl* 2008;218:12–20.
- [2] Ploeg M, Aben KK, Kiemeny LA. The present and future burden of urinary bladder cancer in the world. *World J Urol* 2009;27(3):289–93.
- [3] Dittmer J. The biology of the Ets1 proto-oncogene. *Mol Cancer* 2003;2:29.
- [4] Keehn CA, Smoller BR, Morgan MB. Expression of the ets-1 proto-oncogene in melanocytic lesions. *Mod Pathol* 2003;16(8):772–7.
- [5] Nakayama T, Ito M, Ohtsuru A, Naito S, Nakashima M, Fagin JA, et al. Expression of the Ets-1 proto-oncogene in human gastric carcinoma: correlation with tumor invasion. *Am J Pathol* 1996;149(6):1931–9.
- [6] Nakayama T, Ito M, Ohtsuru A, Naito S, Sekine I. Expression of the ets-1 proto-oncogene in human colorectal carcinoma. *Mod Pathol* 2001;14(5):415–22.
- [7] Takanami I, Takeuchi K, Karuke M. Expression of ETS-1 is correlated with urokinase-type plasminogen activator and poor prognosis in pulmonary adenocarcinoma. *Tumour Biol* 2001;22(4):205–10.
- [8] Pande P, Mathur M, Shukla NK, Ralhan R. Ets-1: a plausible marker of invasive potential and lymph node metastasis in human oral squamous cell carcinomas. *J Pathol* 1999;189(1):40–5.
- [9] Buggy Y, Maguire TM, McGreal G, McDermott E, Hill ADK, O'Higgins N, et al. Overexpression of the Ets-1 transcription factor in human breast cancer. *Br J Cancer* 2004;91(7):1308–15.
- [10] Taki N, Mizaki T, Fujisawa K, Nasu K, Miyakawa I. Expression of c-Ets 1 is associated with malignant potential in endometrial carcinoma. *Cancer* 2000;89(10):2059–67.
- [11] Nakayama T, Ito M, Ohtsuru A, Naito S, Nakashima M, Sekine I. Expression of the ets-1 proto-oncogene in human thyroid tumor. *Mod Pathol* 1999;12(1):61–8.
- [12] Ito Y, Miyoshi E, Takeda T, Sakon M, Noda K, Tsujimoto M, et al. Expression and possible role of ets-1 in hepatocellular carcinoma. *Am J Clin Pathol* 2000;114(5):719–25.
- [13] Watson DK, Turner DP, Scheiber MN, Findlay VJ, Watson PM. ETS transcription factor expression and conversion during prostate and breast cancer progression. *Open Cancer J* 2010;3:24–39.

- [14] Eble JN, Sauter G, Epstein JL, Sesterhenn IA. Pathology and genetics of tumours of the urinary system and male genital organs. Lyon: IARC Press; 2004. p. 90–102.
- [15] American Joint Committee on Cancer. Urinary bladder. In: AJCC cancer staging manual. 7th ed. New York, NY: Springer; 2010. p. 497–505.
- [16] Hsu S, Raine L. Protein, avidin and biotin in immunohistochemistry. *J Histochem Cytochem* 1981;29:1349–53.
- [17] Sari A, Calli A, Gorgel SN, Altinboga AA, Kara C, Dincel C, et al. Immunohistochemical determination of ETS-1 oncoprotein expression in urothelial carcinomas of the urinary bladder. *Appl Immunohistochem Mol Morphol* 2012;20(2):153–8.
- [18] Yamaguchi E, Nakayama T, Nanashima A, Matsumoto K, Yasutake T, Sekine I, et al. Ets-1 protooncogene as a potential predictor for poor prognosis of lung adenocarcinoma. *Tohoku J Exp Med* 2007;213:41–50.