Stromal CD10 immunohistochemical expression in urothelial carcinoma of urinary bladder and correlation with clinicopathological parameters

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Abbreviations: CD10, cluster of differentiation. IRB, International Review Board. WHO, World Health Organization. ISUP, International society of urologic pathology. TNM, Tumor, Node and metastasis. HPF, high power field**.** pT, pathologic tumor stage. Sig, significant. P value, propability value.

Abstract:

CD10 is widely expressed in various tumor types and their surrounding cancer associated fibroblasts; and correlate with poor prognosis. In present study CD10 immunohistochemical expression in stromal fibroblasts surrounding urothelial carcinoma and its relationship with clinicopathological parameters were investigated.

Patients and Methods: Formalin-fixed paraffin embedded tissue sections from 106 urothelial cancer specimens were stained with CD10 antibody using immunohistochemistry technique. Expression of CD10 in the stromal cells was then analysed to evaluate its association with different clinicopathological variables.

Results: Stromal CD10 expression was significantly associated with each of high grade (P<0.001), invasive tumor, advanced stage (P<0.001), squamous differentiation of tumor cells (P=0.02) and papillary architecture (P<0.001). There was no significant association between stromal CD10 expression and, and patient age, sex and tumor size.

Conclusion: Increased expression of CD10 in cancer associated fibroblasts was strongly correlated with tumor progression and invasion.

Keywords: CD10, stroma, urothelial, stage, grade, papillary, bilharziasis.

Introduction:

Cluster of differentiation 10 (CD10) is a member of family of membrane-bound zinc- dependent endopeptidases [1]. CD10 is an integral membrane protein, with a short N-terminal cytoplasmic domain, a transmembrane hydrophobic region and a large luminal domain containing active site [2]. CD10 also known as enkephalinase, neprilysin, neutral endopeptidase 24.11 or membrane metalloendopeptidase, and common acute lymphoblastic leukemia antigen (CALLA) [1, 3].

CD10 could directly mediate signaling events.

In addition, CD10 can degrade peptides, such as bombesin and endothelin 1, which stimulate cancer migration and invasion by bombesin-stimulated RhoA-signaling [4]. On the other hand, some tumors as urinary bladder cancer and breast cancer, an upregulation of CD10 enzymatic activity could lead to an accumulation of local CD10-cleaved peptides that inhibit epithelial cell differentiation and maintain cancer stem cell [5]. CD10 is expressed in several hematopoietic malignancies as well as several solid tumors such as Wilm's tumor and neuroblastoma [6], melanoma [7] and in other carcinomas as arise from kidney, stomach, cervix, lung, skin, liver, pancreas, prostate, breast, [5], and urinary bladder [8].
Stromal markers are valuable markers in assessing the prognosis of invasive cancers. CD10 is widely expressed in various tumor types and their surrounding stromal fibroblasts; and correlate with poor prognosis [9].
The aim of this study is to evaluate CD10 immunohistochemical expression in cancer associated fibroblasts surrounding urothelial carcinoma cells of the urinary bladder and tocorrelate this expression with various clinicopathologic parameters.

Material and Methods:

A-atient Recruitment:

This was a retrospective study, where formalin–fixed paraffin–embedded tissue blocks were collected from the archive materials of the South Egypt Cancer Institute, covering the period from January 2011 to December 2013. The protocol was approved by International Review Board (IRB).

The paraffin blocks represent 106 cases of urothelial carcinoma of the bladder removed surgically by radical cystectomy. Clinicopathological parameters such as patient age, sex, tumor size, histological grade, pathological stage, papillary architecture, squamous differentiation and bilharzial infestation were obtained from the available histopathological reports. The tumors were graded according to WHO / ISUP grading system [10] and staged according to TNM WHO pathologic staging system of urinary bladder cancer [11]

B-Methods: -

Immunohistochemistry: Three μm thick formalin fixed paraffin-embedded tissue sections were cut and mounted on coated-positive-charged glass slides. Sections were dewaxed in Xylene (for half an hour) and rehydrated through graded alcohols. Pre-treatment with heat-induced epitope retrieval (HIER) was done using Dako PT Link (code PT 100/ PT101). Slides were then washed 2-3 times with

PBS. Blocking of endogenous peroxidase activity was performed using Dako peroxidase blocking reagent and incubated 5 minutes at room temperature in humidity chamber to prevent unnecessary background staining.

Primary FLEX monoclonal mouse anti-human CD10 antibody (clone 56C6, Code IR648, Dako Denmark A/S, 117568-002) Ready–to-use was applied and incubated for 1 hour at room temperature. Then the slides were washed 2-3 times using phosphate buffer solution (PBS). After washing, immunostaining was performed using a universal staining kit "Envision Detection System Anti-Polyvalent, HRP/DAB (Ready-To-Use)" (EnVision Flex, high PH, Link, code K800021-2, Dako Denmark A/S) following the manufacturer's instructions was applied to the slides and incubated for 20 minutes at room temperature, then rinsed and washed with PBS two times. Sections were then counter stained using EnVision FLEX hematoxylin (Link) (code K8008), washed in tap water, dehydrated in ascending alcohols then cleared in Xylene and left to dry in air. DPX was applied to each slide and cover was slipped.

Sections from healthy renal tissue were used as positive control for evaluation of the CD10 specificity. The positivity was identified as brown membranous and cytoplasmic staining of glomerular and tubular cells. Sections from urothelial carcinoma tissue with the same protocol while omitting primary antibody using as negative control.Assessment of CD10 positivity:

Brown staining of the cell membrane and/or cytoplasm by CD10 was considered positive, with a 5% cut-off point in stromal fibroblasts [12].

Statistical Analysis: -

Data are presented as numbers and percentage in qualitative data; and median value (as abnormal distribution) in quantitative data. A 2x2 table and ***χ2* test** was used to assess the association between CD10 protein expression in stromal fibroblast surrounding urothelial carcinoma cells and other clinicpathologic features. **Fissure value** was used when there cells have expected count less than 5. **MannWhitney U test** was used in association

between CD10 protein expression in stromal fibroblast surrounding urothelial carcinoma cells and tumor size after application of normality test. Results were statistically analyzed using statistical package for Social Sciences (SPSS version 20).

Results:

Demographic data:

Analysis of clinicopathologic data were summarized in (table 1).

CD10 expression by IHC

Using 5% stained cells as a cutoff for CD10 expression in stromal fibroblasts. 86 (81.1%) were positive for CD10 while 20 (18.9%) were negative.

Correlation between CD10 expression in tumor associated stromal fibroblasts and clinicopatologic data

Potential association between CD10 and clinicopathologic characteristics was in (table 2). A significant association was present between CD10 expression in stromal fibroblasts and each of high grade ($P < 0.001$), invasive tumor, advanced stage ($P<0.001$) and squamous differentiation of tumor cells ($P < 0.001$).

The papillary architecture was negatively correlate with CD10 expression ($P<0.001$). No significant correlation between CD10 expression in stromal fibroblasts, and patient age ($P = 0.76$), gender ($P = 0.76$) and tumor size ($P =0.3$).

Discussion

several research studies have been conducted trying to elucidate the role of CD10 in pathogenesis of different tumors and its prognostic significance; however, the results of such studies were conclusive in many tumor types and inconclusive and conflicting in another group of tumors [13].

This study was conducted to investigate the role of CD10 expression in cancer associated fibroblasts invested urothelial carcinoma of bladder. To achieve our goal, we investigated a small population group treated from urothelial carcinoma in South Egypt Cancer Institute (SECI) for their clinicopathologic characteristics and CD10 protein expression.

Stromal markers are now emerging as novel markers in assessing the prognosis of invasive cancers [9]. CD10 is widely expressed in various tumor types and their surrounding stromal fibroblasts; and correlate with poor prognosis. One study revealed stromal expression of CD10 in cases of invasive duct carcinoma of breast was found to be significantly associated with high tumor grade, brisk mitotic activity, poorer prognosis, and aggressive molecular subtypes [9]. Another study done by Zhu Y et al (2016) found that high CD10 expression in stromal fibroblasts and neighboring colonic carcinoma cells associated with progression of colon cancer [14].

In the current series, we studied the expression of CD10 protein in cancer associated fibroblasts invested the urothelial carcinoma cells. We found a positive significant correlation between CD10 expression in cancer associated fibroblasts and tumor grade. This was in keeping with the study done by Omran [15], and in a study done by Zhu et al (2016) which revealed that CD10 expression in cancer associated fibroblast surrounding colon cancer was significantly different in differentiation degree of tumor. However, Abdou revealed no significant correlation between CD10 expression in stromal fibroblasts and tumor grade [12]. This difference may contribute to smaller number of cases of Abdo study.

Our study showed significant association between CD10 protein expression cancer associated fibroblasts and tumor stage. This was in agreement with study done by Omran [15]. Abdou revealed no significant correlation between CD10 expression in stromal fibroblasts and tumor stage [12]. This may contribute to smaller number of cases of Abdo study.

In this study, no significance correlation was detected between CD10 expression in stromal fibroblasts with patient age, sex, tumor size and bilharzial infestation. These results were agreed with the study done by Abdou [12]. In contrast, Omran showed that the frequency of stromal expression of CD10 was higher in bilharzial-associated bladder carcinomas than in non-bilharzial and was statistically significant [15]. This result was not matched with ours which may contributed to different primary antibody used and immunohistochemical steps.

Conclusion

Increased expression of CD10 in cancer associated fibroblasts are significantly correlated with high tumor grade and advanced stage. This finding suggesting the role of CD10 protein in contributing urothelial carcinoma pathogenesis, progression and invasion.

Limitations

They include that this cohort of urothelial carcinoma of urinary bladder wasrelatively small, derived from consecutive cases from one hospital over a period of many years and the systemic treatment were not standardized, making

outcomes stratified by treatment difficult to interpret.

Most cases involved in this study were mainly invasive urothelial carcinoma. Absence of other histological variants in our series restrict the analysis of data.

Recommendations:

1. CD10 advised to be routinely done as it has an effect on overall and recurrence free survival.
2. All studied parameters require more depth studying to understanding the prognostic role of CD10 protein and its underlying mechanisms, such as performing large number of UCB cases and its variants for more accurate statistical analysis.
3. Analysis of other molecules interacting with CD10 protein.
4. Data base for all patient should be done according to strict institutional protocol to facilitate accurate detection of relations between the molecular profile with overall survival, recurrence free interval and metastasis free intervals.

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Table 1: Clinicopathological features of patient'ssamples:

Parameters	NO. (%)
Age	
< 50 Years	23 (21.7%)
≥ 50 Years	83 (78.3%)
Sex	
Male	84 (79.2%)
Female	22 (20.8%)
Grade	
Low	14 (13.2%)
High	92 (86.8%)
Stage	
pTa	8 (7.5%)
pT1	12 (11.3%)
pT2a	5 (4.7%)
pT2b	15 (14.2%)
pT3	48 (45%)
pT4	18 (17%)
Papillary architecture	
Present	32 (30.2%)
Absent	74 (69.8%)
Bilharziasis	
Present	43 (40.6%)
Absent	63 (59.4%)
Squamous differentiation	
Present	51 (48.1%)

Absent	55 (51.9%)
Tumor Size in cm	
Range	1 to 10 cm
Median	4 *

NO.; Number, *; used median as this variable is abnormally distributed.

Table 2: Correlation between clinicopathologic characteristics and CD10 expression:

Parameters	CD10 expression in stromal fibroblast		Test of sig.
	Positive	Negative	P
	No. (%)	No. (%)	
Age			
< 50 Years	18 (20.9%)	5 (25%)	P = 0.77 #
≥ 50 Years	68 (69.1%)	15 (75%)	
Sex			
Male	67 (77.9%)	17 (85%)	P = 0.76 #
Female	19 (22.1%)	3 (15%)	
Grade			
Low	2 (2.3%)	12 (60%)	**P < 0.001#**
High	84 (97.7%)	8 (40%)	
Stage			
pTa	0 (0%)	8 (40%)	
pT1	5 (5.8%)	7 (35%)	**P < 0.001**
pT2a	5 (5.8%)	0 (0%)	

pT2b	14 (16.3%)	1 (5%)	
pT3	47 (54.7%)	1 (5%)	
pT4	15 (17.3%)	3 (15%)	
Papillary architecture			
Present	17 (19.8%)	15 (75%)	**P < 0.001**
Absent	69 (80.2%)	5 (25%)	
Bilharziasis			
Present	35 (40.7%)	8 (40%)	P = 0.95
Absent	51 (59.3%)	12 (60%)	
Squamous differentiation			
Present	46 (53.5%)	5 (25%)	**P =0.02**
Absent	40 (46.5%)	15 (75%)	
Tumor Size in cm			
median	4	4.5	P = 0.3*

Sig, significant; #, Fischer's exact test; **Bolded vales**, Significant; *, Mann-Whitney.

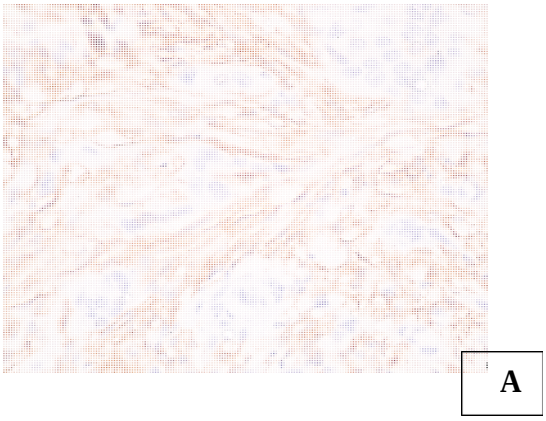
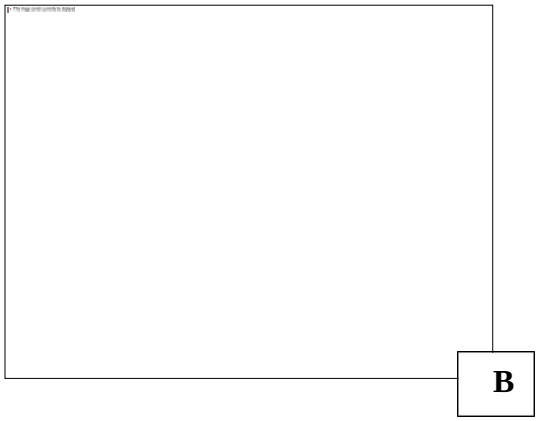


Figure 1: **Microscopic assessment of CD10 positivity in stromal fibroblasts as ≥ 5% stained cells cutoff point:** (A) Stromal fibroblasts showed diffuse positive staining for CD10 (examination at power of 10x HPFrevealed).



(B) Stromal fibroblasts were negative for CD10 (examinationn at power of 10x HPF).