Clinicopathological and Immunohistochemical Study of Fascin-1 Expression as a Predictive Factor for Invasiveness in transitional cell carcinoma of Urinary bladder

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AbStRAct

background: Urothelial carcinoma of the urinary bladder represents 90% of all primary tumors of this structure. It is one of the most common ten malignancy in Iraq and worldwide. The depth of invasion, particularly for muscularis propria is the most important prognostic and therapeutic determinators. Fascin-1 is an actin bundling protein involved in cell migration and motility and up-regulated in transformed and aggressive epithelial carcinomas like urothelial carcinoma and overexpression is associated with advance stage. The aim of the present study is to evaluate the fascin -1 expression as a predictive marker for assessment of depth of invasion and as prognostic and therapeutic purposes. Methods: Fifty five patients were of age range from 16-95 years with mean age ± SD of 65.8 ± 13.7 years and with different grades and stages were taken from laboratory of histopathology of Al-Hilla Teaching Hospital. Histological sections of paraffin embedded tissue were be taken for H and E staining for assessing grades and stages according to WHO grading system and AJCC staging system, respectively. Immunohistochemical staining by using mouse monoclonal antibody for fascin-1, then correlation between clinicopathological parameters and marker expression were done. Results: The fascin-1 expression was found not related to age, gender, tumor grade and perineurial invasion($P>0.05$). The intense expression was noted predominantly in the invasive component. Tumor size, lympho-vascular invasion and necrosis were noted to be associated with aggressive tumor with increased fascin expression ($P<0.05$), Conclusion: fascin-1 can be used as a predictive marker for recurrence and invasion and as a therapeutic target.

Keywords: *Urothelial carcinoma, Fascin-1, Invasion, Tumor grade, Tumor necrosis.*

Introduction

Urothelial carcinoma represents 90% of primary urinary bladder tumors. The age of incidence is usually more than 50 years with male to female ratio about 3:1 [1]. It is one of the ten most common cancers in Iraq and worldwide. In Iraq, it represents the fifth most common tumor; it is the second most common cancer in males and the tenth most common cancer in females[2]. Females have more aggressive tumor behavior than males [3]. It is two folds higher in white people than blacks [4]. Worldwide, bladder cancer represents the ninth most frequent cancer with the highest incidence in men from Southern and Western Europe as well as North America. Bladder cancer ranks 13th in terms of deaths ranks. Bladder cancer occurs predominantly in heavy smokers and occupational exposure to chemicals such as benzidine, beta-naphthylamine, painters and ingestion of analgesics, parasitic infections, bladder calculi, and chemotherapy [5]. Pathogenesis is complex and recent mRNA sequencing studies identified two molecular subtypes: luminal and basal bladder cancers[6]. It presents grossly either exophytic or endophytic growth [7]. According to WHO/ISUP (2016), urothelial carcinoma is divided microscopically into infiltrative and non invasive urothelial neoplasms [8]. The non-invasive papillary tumors graded into low grade and high grade papillary urothelial carcinomas. About 75%

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are non invasive of low grade. The infiltrating urothelial tumors include tumors with divergent differentiation toward sequamous, glandular, trophoblastic, nested, microcystic, micropappilary, lymphoepithelioma-like, sarcomatoid, plasmacytoid [9].In this study, only the papillary urothelial tumors were included. The treatment depends on age, grade, stage and presence of dysplasia or carcinoma in situ [10]. Low grade and non muscle invasive treated by transurethral resection, while high grade and muscle invasive are treated by radical cystectomy with or without preoperative radiation therapy or chemotherapy [11]. The invasion of muscularis propria is either superficial invasion (submucosa and inner half of muscle) or deep (outer half of muscle and perivesical tissue and lymphatic) [12]. Fascin-1(FSCN1) is a 55 KD monomeric actin filament bundling protein that plays a role in cell adhesion and motility. Three forms of fascin are found in vertebrates; Fascin-1(most common) present in specialized cells with extensive surfaces or migratory potential such as neurons, glial cells, dendritic cells, macrophages, skeletal and smooth muscle cells and endothelial cells [13]. The level of fascin-1 is low or undetectable in normal epithelial cells; overexpression was seen in transformed epithelial cells and associated with high grade and aggressive tumors. Fascin binds beta-catenin and colocalizes with it at the leading edges and borders of epithelial and endothelial cells so it has a role in the maintenance of cell adhesion, coordinating motility and invasion [13]. Fascin-1 localizes to actin-rich protrusions at the cell surface called filopodia. A recent study showed that fascin also localizes to invadopodia, membrane protrusions formed at the adherent cell surface that facilitates extracellular matrix (ECM) invasion, this provide a mechanism for how fascin increases the invasiveness of cancer cells [15]. Studies have shown that fascin plays a major role in immune suppression. T regulatory cell adhesion to antigen presenting dendritic cells causes sequestration of Fascin-1, which is essential for immunological synapse formation, and skews Fascin-1–dependent actin polarization in antigen presenting dendritic cells toward the T regulatory cell adhesion zone, this sequestration of cytoskeletal components causes a lethargic state of dendritic cells leading to reduced T cell priming [16, 17].

The aim of the present study is to evaluate the fascin -1 expression as a predictive marker for assessment of depth of invasion and as prognostic and therapeutic purposes.

Materials and Method

This study was carried out in the Department of Pathology and Forensic Medicine, Faculty of Medicine/ Babylon University. The samples were taken from the laboratory of histopathology at Al-Hilla Teaching Hospital. Fifty-five paraffin embedded blocks were included. The clinical data were obtained from the reports at Al-Hilla Teaching Hospital, these included age, gender, tumor size, grade, stage, vascular invasion, perineurial invasion and tumor necrosis. The age of patients ranged from 16-95 years with mean age ± SD of 65.8 ± 13.7 years. Subjects were 46 males and 9 females, cases with tumor size <1cm were (40) and those >1cm were (15) cases; low grade tumor cases were (30) and high grade cases were (25). In addition, (35) cases were stage Ta, (8) cases stage T1, (8) cases were stage T2 and 1/8 case was with squamous differentiation and of high grade, (2) cases were stage T3, (2) cases were T4 and all cases from T2-T4 were high grade except one case. The cases without vascular invasion were (48) cases and those with vascular invasion were (7) cases. Also, (53) cases were without perineurial invasion and (2) cases with perineurial invasion, (49) cases were negative for tumor necrosis, while (6) cases were positive for tumor necrosis.

Fascin-1 is a monoclonal mouse anti-human fascin-1 protein of isotype IgG2a/k, Bio SB, Santa Felida, USA. The detection system was mouse/rabbit poly detector plus HRP/DAP kit. The positive immunoreaction is evaluated by the diffuse brownish staining of the cytoplasm and two parameters were evaluated semi-quantitatively according to [18] scoring system:

1. The extent of immunostaining according to the percentage of stained neoplastic cells.
2. The intensity of immunostaining according to the staining of endothelial cells as positive internal controls.

Then for each case, a combined immunoreactivity score was evaluated by multiplying the score for extent by the score for intensity so the combined immunoreactivity score (CIS) ranged from(0-12) as the following:

Score 0: Absent (no staining).

Score 1: Mild staining 1-4 (≤25% of cells with Weak (less than that of endothelial cells)).

Score 2: Moderate staining 5-8 (25%-50% with moderate (equal to endothelial cells staining)).

Score 3: Intense staining 9-12 (50%- ≥75% of cells with intense (more than of endothelial cells staining)).

The statistical analysis was done with SPSS software version 22. P-value less than 0.05 was considered statistically significant and less than 0.01 as highly significant.

Results

Fascin-1 overexpression was reported in 35(63.6%) of 55 cases while 20(36.3%) were negative. Also, 46(83.6%) of total cases were males with 28(60.9%) positive staining and 18(39.1%) cases were negative; females totally were 9(16.3%) with 7(77.8%) positive and 2(22.2%) negative and there was no relation between gender and fascin-1 expression (P= 0.3). According to age groups (Figure 1), there were only 2 cases (3.6%) of 16-35 years group and all were positive, 8(14.5%) of 36-55 years group and 4(50.0%) positive, 32(58.1%) of 56-75 years group with 22(68.8%) cases were positive, 13(23.6%) of 76-95 years group and 7(53.9%) cases were stained with fascin and there was no correlation found between expression and age groups (P= 0.4). Tumor size <1cm was 40(72.7%) and ≥1cm 15 (27.3%) of cases (Table 1) and a strong association between increased fascin expression and tumor size (P=0.007) (Table 2). Low grade were 30(54.5%) cases and high grade were 25(45.5%) cases (Table 1) and no correlation was found between expression score and grade (P= 0.08) (Table 2). Cases of stage Ta were 35(63.6%),T1 were 8(14.5%), T2 were 8(14.5%) and one case of T2 with squamous differentiation, T3 were 2(3.6%), T4 were 2(3.6%) and all T2-T4 cases were stained and no negative cases (Table 1) and there was a strong relation between fascin expression score and stage of tumor (P= 0.001; Table 2). Vascular invasion was only present in 7(12.7%) cases and was absent in 48(87.2%) of cases (Table 1) and a relation was found between vascular invasion and score (P= 0.04). Moreover, all 7 cases stained moderately and intensely with no negative staining (Table 2). Perineurial invasion was reported in only 2(3.6%) of cases and was absent in 53(96.3%) of cases (Table 1), both 2 cases were score 3 and of high grade (stage pT2) and there was no correlation between expression and invasion (P= 0.1; Ttable 2). Tumor necrosis was reported in 6 (10.9%) of cases (Table 1),1/6 of cases were score 2; whereas 5/6 of cases were score 3 without negative staining and there was a relation between score and necrosis (P= 0.01; Table 2).

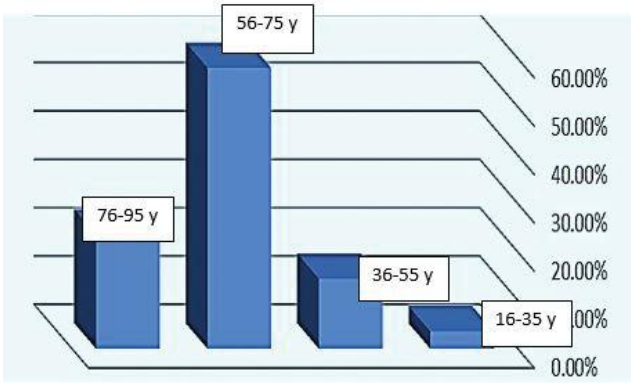

Figure 1: Frequency of the age groups among patients

table 1: Fascin-1 expression in relation to tumor size, grade, stage, vascular invasion, perineurial invasion and necrosis

Parameter	total no of cases no.(%)	negative staining no.(%)	Positive staining no.(%)
tumor size			
<1cm	40(72.7)	19(47.5)	21(52.5)
≥1cm	15(27.3)	1(6.6)	14 (93.3)
Grade			
Low	30(54.5)	14(46.6)	16(53.4)
High	25(45.4)	6(24.0)	19(76.0)
Stage			
Ta	35(63.6)	20(57.1)	15(42.9)
T1	8(14.5)	0(0.0)	8(100)
T2	8(14.5)	0(0.0)	8(100)
T3	2(3.6)	0(0.0)	2(100)
T4	2(3.6)	0(0.0)	2(100)
Vascular invasion			
Negative	48(87.2)	20(41.6)	28(58.3)
Positive	7(12.7)	0(0.0)	7(100)
Perineurial invasion			
Negative	53(96.3)	20(37.7)	33(62.2)
Positive	2(3.6)	0(0.0)	2(100)
tumor necrosis			
Negative	49(89.0)	20(40.8)	29(59.1)
Positive	6(10.9)	0(0.0)	6(100)

table 2: Relation of tumor size, grade, stage, vascular invasion, perineurial invasion and necrosis with score of fascin expression

Parameter	Fascin expression score no.(%)				P value
	Score 0	Score 1	Score 2	Score 3	
tumor size					
<1cm	7(17.5)	14(35.0)	7(17.5)	40(72.7)	0.007 (Significant)
≥1cm	0(0.0)	1(6.6)	5(33.4)	9(60.0)	
Grade					
Low	6(20.0)	8(26.6)	11(36.7)	5(16.7)	0.08 (Not significant)
High	1(4.0)	5(20.0)	8932.0)	11(44.0)	
Stage					
Ta	7(20.0)	13(37.1)	12(34.3)	3(8.6)	0.001 (Significant)
T1	0(0.0)	0(0.0)	5(62.5)	3(37.5)	
T2	0(0.0)	0(0.0)	2(25.0)	6(75.0)	
T3	0(0.0)	0(0.0)	0(0.0)	2(100)	
T4	0(0.0)	0(0.0)	0(0.0)	2(100)	
Vascular invasion					
Negative	7(14.6)	13(27.08)	17(35.4)	11(22.9)	0.04 (Significant)
Positive	0(0.0)	0(0.0)	2(28.6)	5(71.4)	
Perineurial invasion					
Negative	7(13.2)	13(24.5)	19(35.8)	14(26.4)	0.1 (Not significant)
Positive	0(0.0)	0(0.0)	0(0.0)	2(100)	
tumor necrosis					
Negative	7(14.3)	13(26.5)	18(36.8)	11(22.4)	0.01 (Significant)
Positive	0(0.0)	0(0.0)	1(16.7)	5(83.3)	

Discussion

In the present study there was no detectable fascin immuno-reactivity in normal urothelium which was agreed with [18-20].

It was found that there was no correlation between age and gender with fascin expression (P>0.05). A similar correlation was observed by [21-23].

Tumor size was also evaluated and a strong expression of fascin-1 was found with large tumor size (P= 0.007) which may be related to the aggressive behavior of the tumor, this also observed by [23]. 3].

Tumor grade was found not related to fascin expression (P= 0.08). In variance, the only study of [24] found positive correlation between fascin expression and histological grade (P= 0.02).

Regarding the tumor stage, there was strong correlation between fascin expression intensity and tumor stage (P=0.001) with the depth of invasion and only 3/35 (8.6%) of non-invasive tumors (pTa) showed intense staining and 1/8(12.5%) of pT2 was urothelial with squamous differentiation (Table 2).There is no difference within the same stage between low and high grade tumors. This result was also reported by [19] who found that 42% of superficial papillary urothelial carcinomas (pTa) and 95% of invasive urothelial carcinomas (pT2 and higher) demonstrated strong staining for fascin-1. Also, they found the micro-invasive foci in the lamina propria were positive for fascin-1 but not strongly as the deeply invasive tumors. In agreement with findings is the study of [21] who found that the expression of fascin correlated with invasive carcinomas in low and high grade tumors, and staining was intense in the invading tumor cells lamina propria or the muscularis propria and absent or very low expression in tumors pTa stage.

However, in contrast to these findings are those reported by [18,25] who found that none of pTa tumors showed intense staining.

Vascular invasion was associated with fascin-1 overexpression which may be related to advanced tumors, this was similar to [23].

The perineurial invasion was evaluated and no association was found between fascin expression and perineurial invasion (P= 0.1) which may be due to very small sample size and no previous study to compare with it.

On the other hand, tumor necrosis shown a correlation with fascin expression although there was no similar study to compare with it.

conclusions

The fascin-1 overexpression by tumor cells is associated with advanced stage and can be used as predictive marker for recurrence and invasion. Fascin level reduction can be used as therapeutic target in the future.

Ethical clearance: The study was approved by the Research Ethics Committee at College of Medicine/ University of Babylon, Iraq.

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Conflict of Interest: None to declare.

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