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FASCIN 1 IN UROTHELIAL CARCINOMA OF THE URINARY BLADDER: EVALUATION AND ITS CORRELATION WITH PATHOLOGICAL STAGE OF THE TUMOR

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ABSTRACT: Introduction: In urothelial carcinomas, the degree of invasion plays a major role in prognostication and treatment assessment. In certain instances, evaluating invasion on standard histopathological sections may be challenging. Actin-bundling protein fascin is implicated in the migration of tumour cells and is expressed more intensely in invasive tumours. **Objectives:** To evaluate the expression of fascin-1 in urothelial carcinoma of urinary bladder and correlate it with different grades of tumor and level of invasion and other clinicopathological parameters. **Material and Methods:** Observation study was conducted after approval from the institutional ethical committee. Fascin-1 immunoreactivity was studied in 45 confirmed cases of urothelial neoplasms using monoclonal antibody against fascin by immunohistochemistry. The extent, intensity, and combined immunoreactivity score of fascin-1 cytoplasmic immunoreactivity were evaluated semi quantitatively. **Results:** 29 cases (64.4%) had high grade urothelial carcinoma, 14 cases (31.1%) had low grade urothelial carcinoma, and two cases (4.4%) had papillary urothelial neoplasm of low malignant potential (PUNLMP) based on histological classification. In stage pTa were two cases (4.4%), of stage pT1 were 16 cases (35.6%), and of stage pT2 were 27 cases (60%). Invasion of the muscularis propria was seen in 27 cases (60%) and invasion of the lamina propria in 43 cases (95.6%). High-grade carcinomas had considerably greater intensity, extent, and combined immunoreactivity ratings, all of which were statistically significant. Furthermore, invasive carcinomas with advanced tumour stages showed strong staining. **Conclusion:** Overexpression of fascin-1 in urothelial carcinomas may serve as a marker of aggressiveness.

INTRODUCTION: Urothelial carcinoma of bladder (UCB) is an epithelial malignancy developing in the urothelial lining of urinary bladder. Bladder cancer is the ninth most common cancer worldwide and causes a considerable amount of morbidity and mortality [1].

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At seven percent of all new cases, it ranks as the fourth most common cancer among American men. Bladder tumours are mostly epithelial in origin (95%) with urothelial neoplasms being the most common type, followed by squamous and glandular neoplasms (5%) [2].

In India, the incidence of bladder cancer has been increasing over the past few decades, and is a common cancer in the Indian population at present. The World Health Organization's 2016 classification of non-invasive urothelial (transitional cell) tumours classifies flat lesions as

urothelial dysplasia, urothelial carcinoma in situ, and urothelial proliferation of uncertain malignant potential (flat hyperplasia). Exophytic papillary lesions are further classified as papilloma, papillary hyperplasia, papillary urothelial neoplasms of low malignant potential, and papillary urothelial carcinoma low grade and high grade [2].

The urinary bladder histology gives important clues and information about the prognosis of the disease [3]. The degree of tumour differentiation and the depth of invasion in the bladder wall are the primary determinants of the biological behaviour of urothelial carcinoma [4].

The pathological staging and grading of bladder cancer based on histopathological examination of tissues serve as a foundation for defining the treatment strategy and evaluating the eventual clinical result [5]. There are several techniques for identifying and differentiating the smooth muscle in the bladder, such as hematoxylin and eosin stains, special stains like Mason's trichome, Van Gieson, and Immunohistochemistry [6]. Routine sections with H&E remain the most common method for urothelial cancer classification; however, H&E alone frequently finds it difficult to distinguish between muscularis propria and muscularis mucosa. As a result, immune-histochemistry is an effective supplementary method for precise urothelial carcinoma staging [7].

Fascin belongs to the actin-bundling family and is a 55-KDa globular protein. Three forms of fascin are known [8]. Fascin-1 is the most common type, present in specialized cells with extensive surfaces or migratory potential such as neurons, glia, dendritic cells, macrophages, skeletal and smooth muscle, endothelial cells and not normal epithelial cells. Fascin-2 is present in the retina while fascin-3 is present in testis [9]. Fascin plays an important role in cell motility and adhesion. It is proven to cause membrane protrusions, which in turn causes cell motility [10]. An aggressive course of disease has been associated with overexpression of fascin in tumours. Nonetheless, in normal epithelium, it is typically missing or downregulated [11].

Fascin-1 expression in urothelial neoplasms and its association with invasiveness, however, have not been thoroughly studied. Through immune-histochemistry analysis, we aimed to assess fascin-1 expression in urothelial neoplasms and establish a relationship between its expression and tumour aggressiveness and invasiveness.

MATERIAL AND METHODS: The study was conducted in Department of Pathology in association with Department of Urology Pt. B D Sharma PGIMS, Rohtak. 45 biopsy-proven cases of urothelial neoplasms over one year were selected. Representative sections from each case diagnosed as urothelial carcinoma on routine histopathology were subjected to immunohistochemical staining for Fascin-1. Their expressions were assessed and correlated with other clinicopathological parameters.

Cases with inadequate biopsies and malignancies other than urothelial carcinoma were excluded from the study. The cases were graded and staged according to WHO histological classification of tumours of urinary tract 2016 and American Joint Committee on Cancer System 8th Edition 2018.

Immunohistochemical staining with Fascin-1 was carried out using a standard technique. The Dako PT Link, a completely automated system, was used for antigen retrieval. With minor adjustments, cytoplasmic staining was used to evaluate the extent, intensity, and cumulative immunoreactivity score of fascin-1 immunoexpression in tumour cells, following the methodology of Karasavvidou F *et al* [12].

Based on the percentage of positive immunostained neoplastic cells, the extent of immunostaining was divided into four groups.

- Score 0: Absent
- Score 1: <25%
- Score 2: 25%-50%
- Score 3: 50%-75%
- Score 4: 75%

Based on the cytoplasmic staining of endothelial cells employed as internal controls, the intensity of positive immunostaining of tumor cells was classified into:

- Score 0: Not present

- ❖ Score 1: Weak (less than endothelial cells)
- ❖ Score 2: Moderate (equivalent to endothelial cells)
- ❖ Score 3: Intense (more than endothelial cells)

The extent and intensity scores for each case were multiplied to get a combined immunoreactivity score (CIS). It was categorized further as:

- Absent (0): 0
- Mild staining (1): 1-4
- Moderate (2): 5-8
- Intense (3): 9-12

Section from colorectal carcinoma was used as positive control and negative control was obtained by substituting the primary antibody with antibody of non-specific relevance. The whole data was subjected to statistical analysis Using SPSS 24 software. Cases were compared to the control. All the data enlisted in the investigation proforma (name, age, sex, CR no, clinical diagnosis and history) was collected. Chi-square test (χ^2) and nonparametric Spearman rho (r) correlation coefficient were used to compare the expression of fascin with the tumour characteristics and stage. P value < 0.05 was considered statistically significant.

RESULTS: The patients were between the ages of 26 to 82 years. Most of the patients were in the 51–60 age group. The mean age was 58.29$\pm$13.19 years. Majority of the patients were male 91.1% and female 8.9%. The majority of cases showing 1+, 2+ and 3+ positivity all fall in the age group of 61-70 years. No statistically significant association is found between fascin expression and age and gender **Table 1.**

In the present study, 4.4% of cases were classified as PUNLMP, 31.1% low grade and 64.4% were high-grade urothelial carcinoma. Fascin showed higher i.e., 3+ immunostaining in maximum number of high-grade tumours (78.5%) while maximum number of low-grade tumour cases showed lower i.e. 1+ immunostaining (71.4%) **Fig. 1-2** and **Table 2.**

Out of 45 cases, two cases (4.4%) were of stage pTa, 16 cases (35.6%) were of stage pT1 and 27 cases (60.0%) cases were of stage pT2. The muscularis propria showed invasion in 27 cases (60.0%). The lamina propria showed invasion in 43 (95.6%) cases, while it was absent in two (4.4%) cases. So, the tumor showed invasion beyond basement membrane into lamina propria and beyond in 43 out of 45 (95.6%) cases **Table 3.** Fascin showed higher immunostaining 3+ in pT2 (68.2%) than in pT1 (21.8%) and none of the tumours with pTa stage. Similarly, immunostaining 2+ was observed in majority of pT1(54.5%) followed by pT2 (45.5%) tumours while none of the pTatumours. Immunostaining 1+ was observed in 16.7% of pTa, 25.0% of pT1 and 58.3% of pT2 **Table 4.**

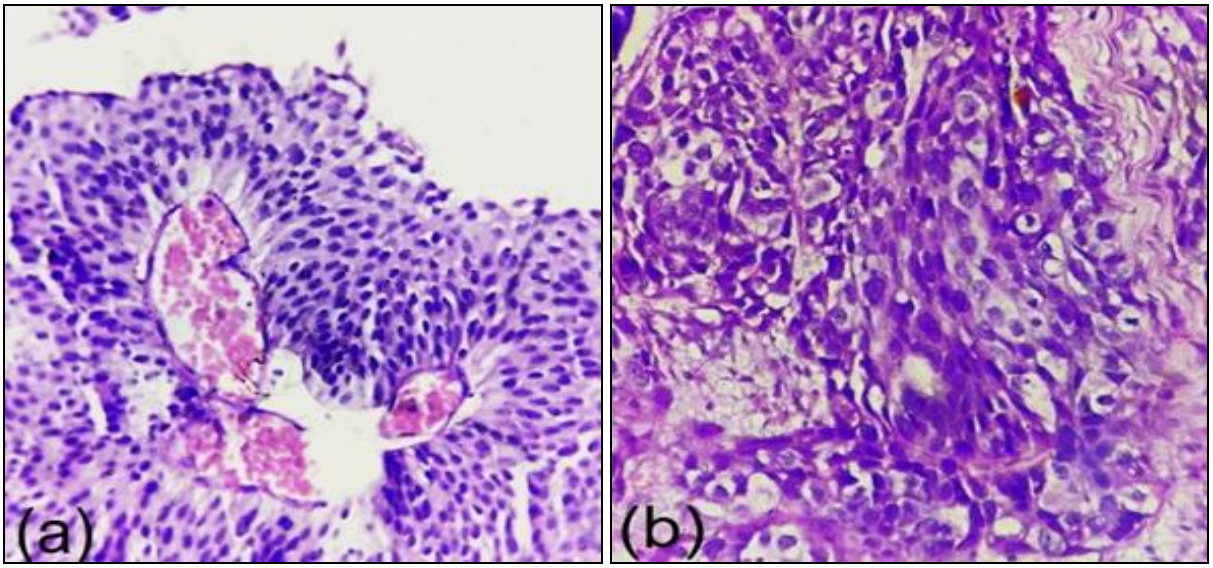

FIG. 1: (A) LOW GRADE UROTHELIAL CARCINOMA 400X; (B): HIGH GRADE UROTHELIAL CARCINOMA 400X

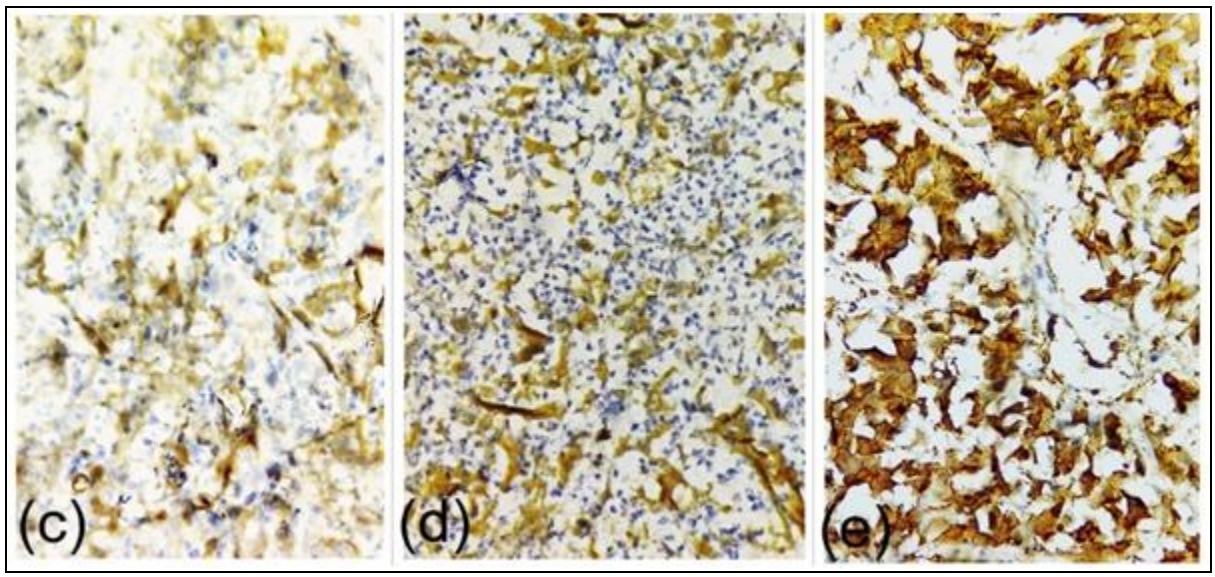

FIG. 2: (C): UROTHELIAL CARCINOMA WITH FASCIN 1+ STAINING EXPRESSION 400X: (D) UROTHELIAL CARCINOMA WITH FASCIN 2+ STAINING EXPRESSION 400X: (E) UROTHELIAL CARCINOMA WITH FASCIN 3+ STAINING EXPRESSION 400X

Divergent differentiation was noted in 4.4 % of cases in the form of squamous differentiation. 8.9% of the cases showed evidence of lymphovascular invasion while 2.2% of cases showed perineural invasion. Lymphovascular and perineural invasion were evaluated and no association was found between fascin expression and lymphovascular invasion (p-value 0.25) as well as perineural invasion (p-value 1.0) **Table 5.**

TABLE 1: CLINICOPATHOLOGIC FINDINGS OF TUMOURS (N=45)

Parameter		Frequency	95% CI
Age	Age Group	26-82 years	
	Mean Age	58.29± 13.19 years	
Sex	Male	41 (91.9%)	77.9% - 97.1%
	Female	04 (8.9%)	2.9% - 22.1%
Tumor Grade	High	29 (64.4%)	48.7% - 77.7%
	Low	14 (31.1%)	18.6% - 46.8%
	Punlmp	02 (4.4%)	0.8% - 16.4%
Tumour Stage	Pta	02 (4.4%)	0.8% - 16.4%
	Pt1	16 (35.6%)	22.3% - 51.3%
	Pt2	27 (60.0%)	44.4% - 73.9%
Squamous	Present	02 (4.4%)	0.8% - 16.4%
Differentiation	Absent	43 (95.6%)	83.6% - 99.2%
Invasion in Lamina	Present	43 (95.6%)	83.6% - 99.2%
Propria	Absent	02 (4.4%)	0.8% - 16.4%
Invasion in Muscularis	Present	27 (60.0%)	44.4% - 73.9%
Propria	Absent	18 (40.0%)	26.1% - 55.6%
Necrosis	Present	02 (4.4%)	0.8% - 16.4%
	Absent	43 (95.6%)	83.6% - 99.2%
Lymphovascular	Present	04 (8.9%)	2.9% - 22.1%
Invasion	Absent	41 (91.1%)	77.9% - 97.1%
Perineural Invasion	Present	01 (2.2%)	0.1% - 13.2%
	Absent	44 (97.8%)	86.8% - 99.9%

TABLE 2: CATEGORIES OF FASCIN COMBINED IMMUNOREACTIVITY SCORE (CIS) IN UROTHELIAL CARCINOMA

Fascin Expression Score	Frequency
1+	12 (26.7%)
2+	11 (24.4%)
3+	22 (48.9%)

TABLE 3: FASCIN- 1 EXPRESSION IN DIFFERENT TUMOR GRADE

Parameter		Fascin Expression (n=45)				Fisher's Exact Test	
		1+	2+	3+	Total	$\chi 2$	P value
Tumour Grade	High	00	08	21	29	32.195	<0.001
	Low	10	03	01	14		
	Punlmp	02	00	00	02		

TABLE 4: CORRELATION OF FASCIN-1 WITH TUMOR STAGE

Parameter		Fascin Expression (N=45)				Fisher's Exact Test	
		1+	2+	3+	Total	X2	P Value
Tumour Stage	pTa	02	00	00	02	7.717	0.156
	pT1	03	06	07	16		
	pT2	07	05	15	27		

TABLE 5: ASSOCIATION OF FASCIN-1 WITH INVASIVENESS

Parameter		Fascin Expression (n=45)				Fisher's Exact Test	
		1+	2+	3+	Total	X^2	P Value
Invasion in Lamina Propria	Present	10	11	22	43	5.756	0.122
	Absent	02	00	00	02		
Invasion in Muscularis Propria	Present	07	05	15	27	1.597	0.455
	Absent	05	06	07	18		
Lymphovascular Invasion	Present	00	02	02	04	2.345	0.251
	Absent	12	09	20	41		
Perineural Invasion	Present	00	00	01	01	1.069	1.000
	Absent	12	11	21	44		

DISCUSSION: Urothelial carcinoma's biological behaviour is mostly determined by the tumour's level of differentiation and the depth of its invasion into the bladder wall. However, the creation of reliable molecular markers might offer helpful data to improve the diagnosis method, defining the course of treatment, and forecasting the eventual clinical result of bladder cancer [13]. The overall prognosis, the chosen treatment approach, and final outcome are all influenced by the grade and stage of urothelial carcinomas [14].

The molecules of interest are associated with diverse groups that play roles in various tumorigenesis pathways. Fascin is known to induce membrane protrusions and cell motility [10]. According to in vitro studies, fascin plays a role in cellular functions such cell proliferation, adhesion molecule-mediated cell-cell contact loss, and motility. Loss of anchorage reliance, loss of cell-cell adhesion and junctional contacts, and changes in cell shape with the emergence of membrane protrusions are common characteristics of invasive tumour cells. Numerous alterations are a result of cytoskeletal microfilament rearrangements involving several actin cross-linking protein types, including fascin, which is a crucial component of filopodia, spikes, lamellipodial ribs, dendrites, and microvilli. Overexpression of fascin led to an increase in the motility of epithelial cells and a decrease in cell-to-cell adhesion. This property might suggest the potential mechanism linking Fascin-1 overexpression to increased tumoraggrasiveness and invasiveness. Fascin overexpression was correlated with high-grade tumours, risk of metastasis, and poor prognosis in many human neoplasms, including lung, stomach, pancreas, colon, gallbladder, thyroid, and kidney [15]. This property of malignant cells to migrate in invasive tumours has been utilized in this study. Fascin, recognized as a novel prognostic marker, is currently under investigation across a range of tumours, including colorectal, esophageal, ovarian, and lung carcinomas.

Various biomarkers are currently used in clinical practice for urothelial carcinoma like Ki-67, P53, her2-neu, FGFR3 and others. Each of these biomarkers has its own strengths and limitations, and their expression can provide valuable information about the tumor's characteristics and potential behavior. Fascin-1 stands out for its association with tumor invasiveness and aggressiveness, making it a valuable marker for identifying high-risk urothelial carcinomas. Tong *et al* studied the expression of Fascin in urothelial neoplasms for the first time in 2005 [16]. In concordance with the study of Tong *et al*, fascin-1

immunoexpression correlates positively with the aggressiveness of the tumor. There were 2 cases of papillary urothelial neoplasm of low malignant potential (PUNLMP) which showed Fascin 1+ immunostaining. Majority of the low-grade carcinoma showed Fascin immunostaining as 1+ (ten out of 14 cases); followed by 2+ (three cases) and only one case showed 3+ immunostaining. On the other hand, majority of the high-grade tumours showed 3+ immunostaining (21 out of 29 cases) followed by 2+ immunostaining (8 out of 29 cases). None of the high-grade tumour showed 1+ immunostaining. Thus, a statistically significant association of Fascin immunostaining was seen with the histological grade of tumour (p-value <0.001). Our study is in concordance with the study by Jian-bin Bi et al and El-Rahim et al where fascin expression was positively correlated with histological grade (p= 0.024 and 0.003 respectively), indicating that Fascin can be used as a marker for the aggressiveness of the tumor [17].

Two pTa instances were identified, and both had 1+ Fascin immunostaining. The majority of pT1 cases (54.5%) had 2+ fascin immunostaining, whereas 68.2% of pT2 cases had 3+ fascin immunostaining. In the majority of cases, there is an increase in immunostaining grade with a higher tumour stage, even though there was no statistically significant correlation between fascin expression and tumour stage (p-value = 0.156). Therefore, in both low-grade and high-grade tumours, immunoreactivity scores i.e., the degree and intensity of staining correlated favourably with invasive carcinomas. Our study is in concordance with study by Gomma *et al*, showing no statistically significant relation between tumor stage and fascin immunoreactivity (p value 0.55) [15]. While studies conducted by Obaid *et al* and Ibrahim et al show statistically significant relation between tumor stage and fascin immunoreactivity (p value 0.0001 and 0.01 respectively).

Out of 45 cases, 43 had tumour invasion in the lamina propria and 27 had tumour invasion in the muscularis propria. Both the invasive tumour cells and the tumour cell nests that had infiltrated the muscularis propria or the lamina propria showed strong fascin-1 immunostaining. The vast majority of cases had increased fascin expression during invasion. However, no statistically significant association could be demonstrated between Fascin expression and invasion in muscularis propria. (p-value>0.05). According to our study, there is no statistically significant correlation between the clinical profile and patient demographics with fascin expression. Age, sex, tumour stage, level of invasion, lymphovascular and perineural invasion of urothelial carcinoma of the bladder did not correlate with fascin immunostaining. Similar results are obtained in studies by Obaid *et al* and Jianbin *et al.* Given that the incidence of urothelial carcinoma rises sharply with age and is higher in men, our data was consistent with that of other studies. In modern society, men are more likely than women to smoke and be exposed to industrial risks, which may explain why men are more likely than women to get urothelial carcinoma. Further explanations for the higher disease stages that our study's participants presented with include general ignorance and delayed investigation because of low socioeconomic position.

Research has indicated that fascin-1 overexpression is linked to clinicopathological parameters such as pathological stage, metastasis, and reduced disease-free survival. Several fascin-1 inhibitors, such as G2 and NP-G2-044, have been evaluated in vitro and in preclinical models, showing promising potential as therapeutic agents [10]. However, further investigation is needed to fully understand the therapeutic potential of fascin-1 targeting and to develop effective treatment strategies.

CONCLUSION: We conclude that Fascin overexpression in urothelial carcinoma of urinary bladder can be used as a marker of aggressiveness of the tumour. The study confirms a strong association between Fascin-1 expression and tumor grade. However, the correlation with tumor stage is weak, suggesting that Fascin-1 is not ideal marker for staging urothelial carcinoma. Also, the role of Fascin as a surrogate marker for invasion cannot be stressed upon. fascin-1 could potentially be used as a prognostic marker in routine pathology for urothelial carcinoma. Studies have shown that fascin-1 expression is associated with tumor invasiveness and aggressiveness. Various research has indicated that fascin-1 immunoreactivity is significantly higher in invasive urothelial carcinomas compared to non-invasive ones. Limitation of this study however are relatively

small sample size and lack of long term follow-up. Nonetheless, a larger studies with a tighter and longer follow-up design is required to validate the therapeutic use and prognostic/predictive significance of fascin as a marker in urothelial carcinoma.

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