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Original Article

Evaluation of fascin-1 expression as a marker of invasion in urothelial carcinomas

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ABSTRACT

Background: Prognostication and therapeutic evaluation of urothelial carcinomas significantly depends on the depth of invasion. The assessment of invasion on routine histopathological sections may be difficult in some cases. Fascin is an actin-bundling protein involved in tumor cell migration with enhanced expression associated with invasive tumors. The data available on fascin-1 expression in urothelial carcinoma however is limited. To characterize fascin-1 expression in urothelial neoplasms and its correlation with invasiveness in urothelial carcinomas.

Methods: A descriptive study design wherein fascin-1 immunoreactivity was studied in 126 urothelial neoplasms using monoclonal antibody against fascin by immunohistochemistry. 52/126 (41.26%) were low grade carcinomas (48/52 stage pTa and 4/52 stage pT1), 46/126 (36.5%) high grade carcinomas (13/46 stage pTa, 8/46 stage pT1 and 25/46 stage pT2), 02/126 carcinoma-in-situ, 03/126 papilloma, 12/126 papillary urothelial neoplasm of uncertain malignant potential and 11/126 were other variants of urothelial carcinomas. Fascin-1 cytoplasmic immunoreactivity was assessed semiquantitatively in terms of extent, intensity and a combined immunoreactivity score. Correlation between immunoreactivity scores and invasiveness was evaluated using Pearson's chi-square (χ^2) and Nonparametric Spearman rho (ρ) correlation coefficient two tailed.

Results: The scores for intensity, extent and combined immunoreactivity were significantly higher in invasive carcinomas. In addition, strong staining was observed exclusively in invasive carcinomas. None of the pTa tumors demonstrated intense staining, including those categorized as high grade carcinomas.

Conclusion: Fascin-1 overexpression may be used as a marker in urothelial carcinomas where it is morphologically difficult to determine the status of invasion.

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Introduction

Tumor stage is currently the most powerful effector of prognosis in urothelial neoplasms dependent profoundly on the assessment of depth of invasion in biopsy specimen. Fascin-1 is an evolutionarily conserved actin-binding protein which is an integral part of cell surface protrusions and is required for cellular motility.¹ Studies have reported correlation of invasiveness of tumor with fascin-1 overexpression in various carcinomas. However, there are limited studies on fascin-1 expression in urothelial neoplasms and its correlation with invasiveness. We undertook this study with an aim to evaluate the fascin-1 expression in urothelial neoplasms using immunohistochemical studies and correlate its expression with invasiveness of the tumor.

Material and methods

126 biopsy proven cases of urothelial neoplasms diagnosed in the Department of Pathology, at a tertiary care institution over a period of five years (Jun 2006 to Jun 2011) were selected for the present study. Slides showing quantitatively inadequate material and extensive cautery/crush artifacts were excluded.

Hematoxylin and eosin (H&E) stained slides and the paraffin blocks were retrieved from the archives. The slides were then examined by two independent observers. All the cases were graded and staged according to the 2004 World Health Organization histological classification of tumors of the urinary tract and American Joint Committee on Cancer System of 2002 respectively.^{2,3}

Immunohistochemical staining was carried out with mouse monoclonal antibody against human fascin-1 protein (FCN01 – BIOGENEX-AM488-5M) using HRP based secondary detection system from Dako (Envision, Denmark). Reed–Sternberg (RS) cells from case of Hodgkin's lymphoma were taken as positive control and transitional lining epithelial cells from normal bladder mucosa were taken as negative control. Fascin-1 immunoexpression in tumor cells was evaluated as cytoplasmic staining in terms of extent, intensity and combined immunoreactivity score as carried out by Foteini et al with few modifications.⁴

1. The extent of immunostaining was categorized into 4 groups according to the percentage of positive immunostained neoplastic cells
 - Score 0: Absent
 - Score 1: $\leq 25\%$
 - Score 2: 25%–50%
 - Score 3: 50%–75%
 - Score 4: $\geq 75\%$
2. The intensity of positive immunostaining of tumor cells was categorized according to the cytoplasmic staining of endothelial cells used as internal controls into:
 - Score 0: Absent
 - Score 1: Weak (less than that of endothelial cells)
 - Score 2: Moderate (equal to that of endothelial cells)
 - Score 3: Intense (more than that of endothelial cells)

3. A combined immunoreactivity score (CIS) was calculated by multiplying the score for extent by the score for intensity for each case.⁴ It was further grouped as:

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

SPSS software version 13.0 was used to analyze the data. Pearson's chi-square (χ^2) and nonparametric Spearman rho (ρ) correlation coefficient two tailed was used to compare the expression of fascin with the tumor characteristics and stage. In all the tests, p value of <0.05 was taken as significant.

Results

Mean age of study population was 62 ± 12.82 years ranging from 25 to 90 years with male predominance (male to female ratio - 7:1). 60.7% cases presented with painless hematuria and rest 39.3% presented with other symptoms as dysuria, increased urinary frequency and hematuria. 48% cases presented with solitary growth and 49.2% cases had multiple lesions. No statistically significant correlation of fascin immunostaining was observed with age, sex, clinical presentation or number of lesions.

52/126 (41.26%) were low grade (LG) papillary urothelial carcinomas, 46/126 (36.5%) were classified as high grade (HG) papillary urothelial carcinomas, 2/126 (1.5%) carcinoma-in-situ, 3/126 (2.3%) papillomas and 12/126 (9.5%) papillary urothelial neoplasm of uncertain malignant potential (PUNLMP). 11/126 (8.7%) were urothelial carcinomas with other differentiations as squamous differentiation 3/11, glandular differentiation 3/11, signet ring cell differentiation 2/11, rhabdoid differentiation (9%), sarcomatoid differentiation (9%) and 1/11 undifferentiated.

All papillomas and PUNLMPs were completely negative for fascin expression. Two cases of carcinoma-in-situ exhibited weak staining and that too in less than 25% of tumor cells. 23/52 (44.2%) LG papillary carcinomas were positive for fascin-1 expression while 52/57 (91.22%) HG papillary carcinomas (including 11 variants) displayed fascin-1 expression.

98 cases of papillary urothelial carcinomas were then analyzed for association of grade and stage with fascin expression. 11 variants were excluded to maintain homogeneity of papillary urothelial carcinomas. 61/98 cases (62.2%) were in stage pTa, 12/98 (12.2%) in pT1 and 25/98 (25.6%) in pT2. It was observed that all 37 invasive carcinomas (pT1 + pT2) were positive for fascin expression while only 29/61 (47%) of pTa exhibited fascin immunostaining ($\chi^2 = 28.8$, $p = 0.001$). Positive statistical association was also observed between intensity, extent and CIS of immunostaining with the invasiveness of the tumor (pTa vs pT1 + pT2) with significant P value of 0.001 respectively. (Intensity: $\chi^2 = 69.06$, extent: $\chi^2 = -69.5$, CIS: $\chi^2 = 65.09$). 31/34 tumors exhibiting moderate to intense staining and more than 50% extent were invasive. When fascin-1 immunoreactivity was compared within the three stages there was statistically significant increase in the immunoreactivity scores from pTa, to pT1 and pT2. (Intensity: $\chi^2 = 71.01$, extent: $\chi^2 = -81.02$, CIS: $\chi^2 = 65.03$).

Fascin immunostaining and grade

Fascin-1 immunoreactivity parameters were also compared within the same grade. Though the number of invasive LG carcinomas were low (4/52), significant increase in all three immunoreactivity parameters was observed in the invasive low grade group. All four pT1 LG tumors exhibited more than 50% immunostaining of moderate intensity. Low grade pTa tumors exhibited weak staining which was patchy that too in less than 50% of the extent (Fig. 1).

31 of 33 invasive HG urothelial carcinomas exhibited moderate to intense fascin-1 positivity and none was negative (Fig. 2). The intense positivity was observed in invasive component and also in the noninvasive component. Moreover, 03/10 (30%) cases of HG papillary carcinomas which belonged to pTa stage were completely negative for Fascin-1 staining. Rest 07/10 (70%) of the cases showed positive immunostaining which was less intense than the endothelial cells and in less than 50% of the tumor cells. Only two noninvasive high grade tumors exhibited intense positivity. The blocks were retrieved, tissues were flipped and sections recut which revealed invasive component. Similar correlation was observed with the extent and CIS. Hence within the same grade the immunoreactivity parameters correlated significantly with the invasiveness both for LG and HG tumors. (Table 1).

Discussion

Grade and stage of urothelial carcinomas play important role in determining the overall prognosis, treatment modality to be adopted and outcome. Many molecules are under study which can predict the behavior of the tumor but none has been completely successful to achieve the purpose so far. The molecules targeted belong to various groups involved in different pathways of tumorigenesis. The property of motility which is essential for tumor cells to migrate is currently under exploration. Many proteins and molecules related to motility of malignant cells like gelsolin, cathepsins, MMP-2, stromelysins, E-cadherin and motility related protein-1 have been studied to correlate with invasiveness and prognosis of the urothelial neoplasms.^{5,6} Fascin-1 is one such protein which helps in formation of cellular surface protrusions and mediates cell motility. This property of malignant cells to migrate in invasive tumors has been utilized in this study.

Mammalian genomes encode a small gene family consisting of fascin-1, fascin-2 and fascin-3. Fascin-1 is widely expressed in mesenchymal tissues and in the nervous system, whereas fascin-2 and fascin-3 are specifically expressed in retinal photoreceptor cells and the testis, respectively.^{7–9} Enhanced fascin-1 expression has been found to be associated with tumor progression and enhanced metastatic

Fig. 1 – Photomicrograph of (A): negative fascin-1 expression in papillary urothelial neoplasm of uncertain malignant potential. (Immunoperoxidase staining $\times 200$), (B) weak patchy fascin-1 cytoplasmic staining in low grade papillary urothelial carcinoma stage pTa (Immunoperoxidase staining $\times 100$), (C and D) intense cytoplasmic staining of fascin-1 in low grade papillary urothelial carcinoma stage pT1, both in urothelial lining and in infiltrating tumor nests. (Immunoperoxidase staining $\times 200$).

Fig. 2 – Photomicrograph of (A and B) high grade papillary urothelial carcinomas exhibiting intense and diffuse cytoplasmic positivity for fascin-1 in tumor nests infiltrating lamina propria and also in the noninvasive component. (Immunoperoxidase staining in A $\times 400$ and B $\times 200$), (C and D) intense and diffuse cytoplasmic fascin-1 staining in tumor nests infiltrating the muscularis propria. (Immunoperoxidase staining in $\times 400$).

potential. Fascin as a newer prognostic marker is presently being evaluated in various tumors e.g. colonic, esophageal, ovarian, lung carcinomas etc.^{10–15}

Tong et al studied the expression of Fascin in urothelial neoplasms for the first time in 2005.¹⁶ In concordance with Tong et al, we found absent fascin-1 expression in normal urothelium, papillomas and PUNLMPs. Their study indicated that fascin-1 immunoexpression correlates positively with the aggressiveness of the tumor. No statistically significant association, however, was found with patient demographics and

clinical profile with fascin expression by us, which is in agreement with Bi et al.¹⁷

The method adopted by us for evaluation of fascin-1 expression using objective numerical scores was similar to Foteini K et al.⁴ They however applied different statistical method using mean scores for the calculation of test of significance. We used the numerical scores directly for calculation of P value. Since the data was ordinal and nonparametric Spearman rho correlation co-efficient was also used. The numerical scores were employed since the fascin-1 staining exhibited wide variations from weak patchy to diffuse intense in majority of urothelial carcinomas.

Immunoreactivity scores i.e the intensity and extent of staining correlated positively with invasion carcinomas in both low and high grade tumors. Further, the fascin-1 immunostain was intense in the invading tumor cells as well as tumor cell nests infiltrating the lamina propria or the muscularis propria. The outer transitional cell layers in the urothelial carcinoma component also showed fascin-1 immunostaining. Positive statistical correlation was also observed between the depth of invasion and the immunoreactivity scores in concordance with other similar studies. Tong et al in their study, found increased expression of Fascin in the invasive component of the tumor which progressively increased from superficial to deeply invasive tumors.¹⁷ They also found that the tumor cells at the tips of microinvasive foci exhibited similar intense immunostaining for fascin-1 indicating that increased cell motility and decreased cell to cell

Table 1 – Correlation of fascin-1 expression with invasion within same grade. Low grade urothelial carcinomas = 52, high grade urothelial carcinomas n = 46.

	pTa vs pT1 + pT2 n = 52	pTa vs pT1 + pT2 n = 46	P value (low grade)	P value (high grade)
Pearson's chi square (χ^2)				
Intensity	52.00	16.32	0.000	0.001
Extent	41.4	19.08	0.000	0.001
CIS	14.6	22.1	0.001	0.000
Spearman's rho coefficient (ρ)				
Intensity	0.645	0.544	0.000	0.000
Extent	0.505	0.598	0.000	0.000
CIS	0.338	0.624	0.015	0.000

CIS: combined immunoreactivity score.

adhesion plays a role in stromal invasion. Similar results have also been reported by Foteini K et al and Bi et al^{4,17} In variance, Soukup et al displayed no correlation of fascin expression with the depth of tumor invasion.¹⁸ Also, no correlation between Fascin-1 expression and tumor recurrence or progression was seen by them. Cellular motility however can be used for targeted therapy as a management modality for urothelial carcinomas.¹⁷

Absent to very low fascin-1 expression was seen in majority of HG papillary carcinomas which belonged to pTa stage very similar to that in LG papillary carcinomas with similar stage. Also within same grade the fascin-1 expression correlated with the invasiveness of the tumor. The limitation however in the present study is less number of invasive low grade carcinomas which needs to be further evaluated. Two of our cases which were initially labeled as pTa were upstaged to pT1 after re-evaluation by fascin-1 stain indicating that intense score is seen exclusively in the invasive carcinomas. Hence intensity and extent of fascin-1 staining can act as surrogate marker to overcome certain morphological problems in determining the status of invasion.¹⁹ Moreover fascin also correlates positively with metastatic disease, a property which would enhance diagnostic accuracy and correctly identify primary site for appropriate patient management.²⁰

Conclusion

There are various pathways by which tumor cells can increase their malignant potential. Fascin-1, an actin bundling protein, is expressed by the malignant cells and can be detected by immunohistochemistry. It can be used as a useful marker of invasion in urothelial neoplasms where determining the extent of invasion is paramount for deciding therapeutic modalities. Limitations of this study however are less number of low grade carcinoma cases with invasive component and lack of long term follow-up. We recommend further studies with inclusion of low grade carcinomas exhibiting invasive component and evaluation, preferably with long term follow up of the cases.

Conflicts of interest

All authors have none to declare.

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