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Original Article

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

Maj Arun Sharma [a], *Col Sonia Badwal* [b,*], *Brig Vibha Dutta, SM* [c], *Col Atoshi Basu* [d]

[a] *Graded Specialist (Pathology), Military Hospital Yol, C/o 56 APO, India*
[b] *Associate Professor, Dept of Pathology, Armed Forces Medical College, Pune 411040, India*
[c] *Consultant (Pathology & Oncopath), Army Hospital (R&R), Delhi 10, India*
[d] *Senior Advisor (Pathology), Command Hospital (Eastern Command), Kolkata, India*

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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 ($\chi 2$) 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.

* *Corresponding author*. Tel.: +91 9823967924 (mobile).
E-mail address: deepsbadwal@hotmail.com (S. Badwal).

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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: ≤25%
   - Score 2: 25%–50%
   - Score 3: 50%–75%
   - Score 4: ≥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 ($x^2 = 28.8$, $p = 0.001$). 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: $x^2 = 69.06$, extent: $x^2 = -69.5$, CIS: $x^2 = 65.09$). 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: $x^2 = 71.01$, extent: $x^2 = -81.02$, CIS: $x^2 = 65.03$).