The Korean Journal of Pathology 2009; 43: 139-44
DOI: 10.4132/KoreanJPathol.2009.43.2.139

The Relationship between Prognostic Factors and the Expression Pattern of Fascin and E-cadherin in Renal Cell Carcinoma

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Received : July 8, 2008
Accepted : January 12, 2009

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Background : Fascin is associated with motility in various transformed cells. Overexpression of fascin is known to aid in the progression of some cancers and is associated with a poor prognosis. E-cadherin is a major protein of epithelial cells and its expression is involved in the regulation of cell proliferation and differentiation. The aim of this study was to determine the expression pattern for fascin and E-cadherin and how it is related to the prognostic factors for renal cell carcinoma (RCC). **Methods :** The expression of fascin and E-cadherin was evaluated in 208 RCCs including 175 clear cell, 20 papillary, and 9 chromophobe types using tissue array analysis. **Results :** The expression of fascin increased as the tumor stage (p=0.00) and Fuhrman grade (p=0.00) increased. A high positive rate of expression for fascin was observed in cases with sarcomatoid changes (p=0.27). E-cadherin expression was seen in the distal tubules and collecting ducts of normal kidneys with a membranous pattern. The positive rate of expression for E-cadherin increased as the Fuhrman grade increased (1, 0%; 2, 23.2%; 3, 34.9%; and 4, 53.8%, p=0.00). An inverse correlation in RCCs was observed in the expression of fascin and E-cadherin (p=0.026, r=-0.158). **Conclusions :** In patients with RCC, the increased expression of fascin and E-cadherin was positively correlated to poor prognostic factors such as a higher Fuhrman nuclear grade and advanced pTNM stage.

Key Words : Fascin; E-cadherin; Renal cell carcinoma

The incidence of renal cell carcinoma (RCC) has increased over the past decade due to environmental factors and increased use of imaging modalities including ultrasonography, computed tomography, magnetic resonance imaging, and other techniques.[1] RCCs account for approximately 1.9% of all malignancies worldwide and are still the most lethal of all urologic cancers.[1,2] Extensive research has been performed to predict the prognosis of RCC including tumor necrosis, tumor stage, nuclear grade, and molecular markers.[3] Fascin protein is an actin-bundling protein that has a major function in cell protrusions which are important in cell migration. Fascin is widely expressed in mesenchymal tissues and the nervous system, and has low levels of expression in adult epithelia.[4-8] However, fascin expression is elevated in a variety of cancers including breast, ovary, colon, and esophageal cancer and correlated to the clinical aggressiveness of tumors and poor patient survival.[4-9] E-cadherin is the key functional component of adherent junctions between epithelial cells. Downregulation of E-cadherin in several types of human neoplasms usually correlates with poor tumor differentiation, more advanced disease stage, lymph node metastasis, and poor survival rates.[10] A relationship between fascin and E-cadherin has been documented where cytoplasmic accumulation of fascin leads to a loss of cell-to-cell adhesion by disruption of the E-cadherin adhesion system.[11] However, the relationship between fascin and E-cadherin in RCCs has not yet been determined. The aim of this current study was to determine fascin and E-cadherin expression and its correlation to the patient's clinicopathologic prognostic factors for RCC. In addition, the relationship between fascin and E-cadherin in 208 RCCs was demonstrated by immunostaining. Here, we present results for the first time on fascin and E-cadherin expression in RCCs.

MATERIALS AND METHODS

Case selection

Tissue microarray specimens of 208 RCCs (175 clear cell, 20

papillary, 9 chromophobe, and 4 unclassified types) between 1995 and 1997 were chosen for analysis (PETA™ Array, The Genitourinary Pathology Study Group of the Korean Society of Pathologists, 2004.07.07). At least three cylindrical core biopsies, 1.0 mm in diameter, from different sites in each tumor specimen were arrayed in a recipient microarray block.

Briefly, tissue microarray sections were deparaffinized by xylene, rehydrated in diluted alcohols, and treated for 5 min with 3% hydrogen peroxide. Then, the sections were microwaved in Tris/EDTA buffer (pH 9.0) for 15 min for antigen retrieval. The sections were then incubated for 30 min with primary monoclonal antibodies for fascin (1:100; DAKO, Carpinteria, CA, USA) and E-cadherin (1:100; BD, Biosciences, Franklin Lakes, NJ, USA). After rinsing three successive times with washing buffer, the specimens were treated with standard streptavidin-biotin complex (DAKO REAL™ EnVision™ Detection System; Glostrup, Denmark), 3,3′-diaminobenzidine tetrahydrochloride was used as a chromogen and Mayer's hematoxylin was used for counterstaining.

Immunohistochemical (IHC) evaluation and controls

Immunoreactivity was assessed in a semiquantitative fashion by three pathologists who were unaware of the clinicopathologic data. The fascin scoring method[12] was used to evaluate the IHC staining intensity and the proportion of stained epithelial cells. The cytoplasmic staining intensity was subclassified as follows: 1, weak; 2, moderate; and 3, strong.

The number of positive cells were expressed as the percentage of the total number of epithelial cells and assigned to one of the following five categories: 0, <5%; 1, 5-25%; 2, 26-50%; 3, 51-75%; and 4, >75%.

By multiplying the two scores, the product was designated as the immunoreactive score for each tumor specimen. The score was subclassified as follows: 0, 0-1; 1, 2-7; and 2, >8. Scores 1 and 2 were considered positive for statistical analysis. The E-cadherin grade was evaluated by the proportion of positive cells and subclassified into negative (<10%) and positive (≥10%).

Statistical analysis

Statistical analysis was performed using the SPSS 13.0 software package. The association between the expression of fascin, E-cadherin, and the clinicopathologic prognostic factors were analyzed using the Pearson χ^2 test. p-values <0.05 were considered statistically significant.

RESULTS

Clinicopathologic features

The study population included 208 patients (159 males and 49 females) with TNM stage I-IV RCC as follows: I, 85 (42.7%); II, 52 (26.1%); III, 38 (19.1%); and IV, 24 (12.1%). The patients' ages ranged from 17-77 years (mean±SD, 54.8±10.8 years). The tumor size ranged from 1-19 cm (mean±SD, 6.1±3.2 cm). The distribution of histologic types was as follows: clear cell, 175 (85.8%); papillary, 20 (9.8%); chromophobe, 9 (4.4%); and unclassified, 4 and the distribution of Fuhrman grades was as follows: 1, 17 (8.4%); 2, 106 (52.5%); 3, 65 (32.3%); and 4, 14 (6.9%) (Table 1).

Fascin expression

Fascin immunoreactivity was characterized as a fine, granular weak cytoplasmic staining in normal glomerular endothelial cells (Fig. 1A), but diffuse strong immunoreactivity was noted in the cytoplasm of tumor cells (Fig. 1D). Through immunostaining of fascin, scores 1 and 2 were found in 114 cases (54.8%) which were categorized as the positive group. Negative fascin expres-

Table 1. Summary of clinicopathologic factors in renal cell carcinomas

Features		N (%)
Age (years)		54.78±10.82
Size (cm)		6.10±3.23
Gender	Male	159 (76.4)
	Female	49 (23.6)
Site	Right	90 (53.3)
	Left	77 (45.6)
	Bilateral	2 (1.2)
Histologic type	Clear cell	175 (85.8)
	Papillary	20 (9.8)
	Chromophobe	9 (4.4)
Fuhrman grade	Grade 1	17 (8.4)
	Grade 2	106 (52.5)
	Grade 3	65 (32.2)
	Grade 4	14 (6.9)
Necrosis	Absent	111 (53.4)
	Present	97 (46.6)
Multiplicity	Single	163 (78.4)
	Multiple	45 (21.6)
Sarcomatoid changes	Absent	192 (92.3)
	Present	16 (7.7)
TNM stage	I	85 (42.7)
	II	52 (26.1)
	III	38 (19.1)
	IV	24 (12.1)

sion (score 0) occurred in 94 cases (45.2%) (Table 2). The expression of fascin increased as the tumor stage (p=0.00) and Fuhrman grade (p=0.00) increased. A high positive rate of expression for fascin was observed in cases with sarcomatoid changes (p=0.27) and during necrosis (p=0.006), but there was no significant correlation between the expression of fascin and necrosis. Also, fascin expression was significantly higher in the papillary histologic type for RCCs (p=0.27) (Table 3).

E-cadherin expression

E-cadherin expression was observed in the distal tubules and

Table 2. Positive rate of fascin and E-cadherin expression in renal cell carcinomas

	Negative	Positive
Fascin	94 (45.2%)	114 (54.8%)
E-cadherin	144 (72.7%)	54 (27.3%)

Table 3. Correlation between fascin expression and clinicopathologic factors in renal cell carcinomas

		Negative n (%)	Positive n (%)	p
Histologic type	Clear cell	82 (46.9)	93 (53.1)	0.027
	Papillary	5 (25.0)	15 (75.0)	
	Chromophobe	7 (77.8)	2 (22.2)	
Necrosis	Absent	60 (54.1)	51 (45.9)	0.006
	Present	34 (35.1)	63 (64.9)	
Fuhrman grade	Grade 1	10 (58.8)	7 (41.2)	0.000
	Grade 2	59 (55.7)	47 (44.3)	
	Grade 3	22 (33.8)	43 (66.2)	
	Grade 4	2 (14.3)	12 (85.7)	
Sarcomatoid changes	Absent	91 (47.4)	101 (52.6)	0.027
	Present	3 (18.8)	13 (81.3)	
TNM stage	I	68 (80.0)	17 (20.0)	0.000
	II	38 (73.1)	14 (26.9)	
	III	27 (71.1)	11 (28.9	
	IV	14 (58.3)	10 (41.7)	
Site	Right	50 (55.6)	40 (44.4)	0.030
	Left	27 (35.1)	50 (64.9)	
	Bilateral	1 (50.0)	1 (50.0)	

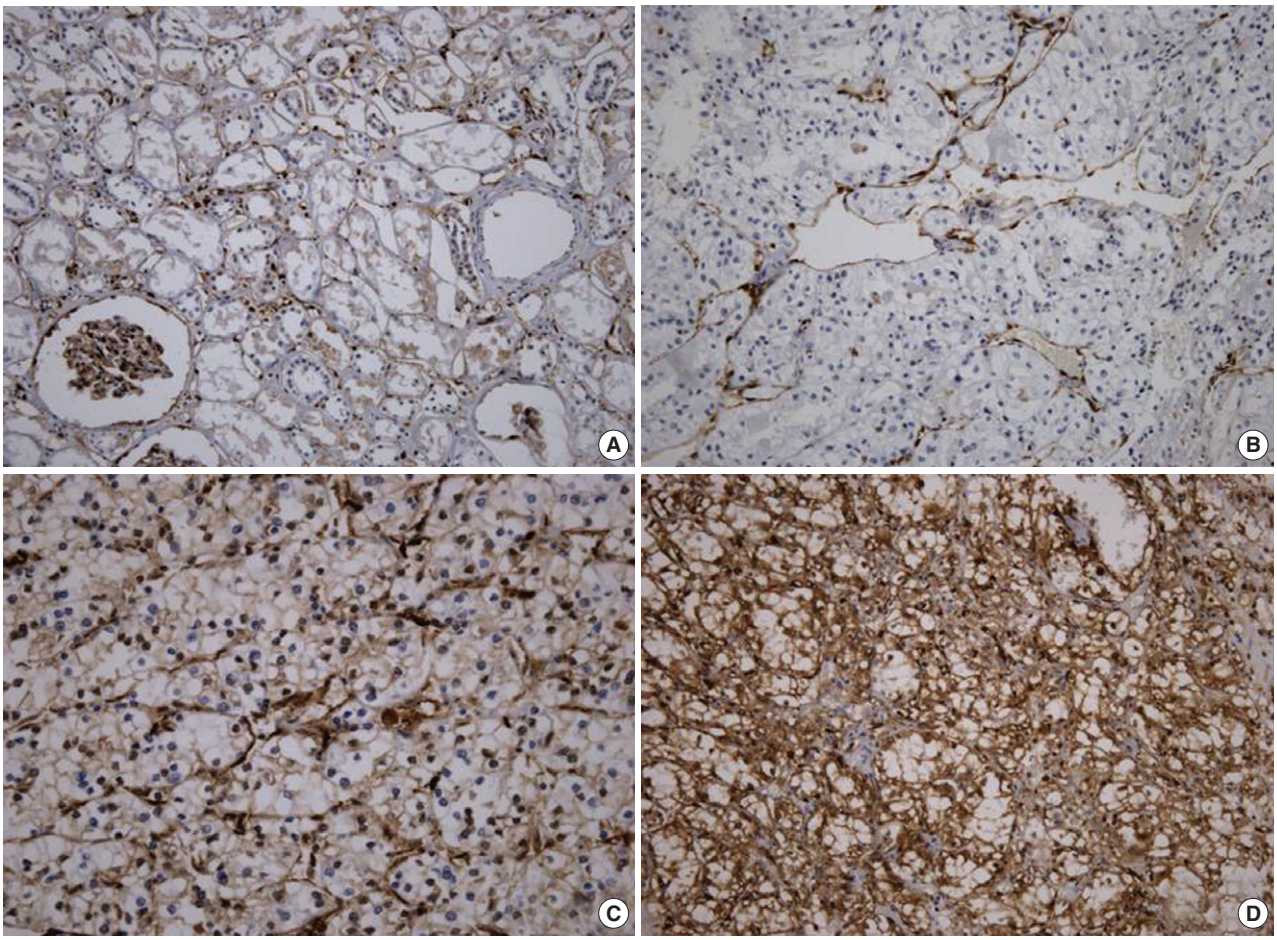

Fig. 1. Immunohistochemical stain for fascin in normal kidney and renal cell carcinomas. Fascin is expressed in the cytoplasmic staining in normal kidney (A). Representative fascin stains in renal cell carcinoma samples show negatively (B), positively patch cytoplasmic expression (C), and strong and diffuse cytoplasmic expression (D).

collecting ducts of a normal kidney with a membranous pattern (Fig. 2A). The expression of E-cadherin was seen in 54 cases (27.3%) (Table 2) and was higher in the chromophobe type of RCCs (75.0%) compared with the clear cell and papillary types (23.5 and 40.0%, respectively, p=0.001). The positive rate of expression for E-cadherin increased as the Fuhrman grade increased (1, 0%; 2, 23.2%; 3, 34.9%; and 4, 53.8%; p-value=0.00). The positive rate of expression for E-cadherin was also identified in 34 cases with necrosis (63.0%; p=0.04) and 8 cases with sarcomatoid changes (50.0%; p=0.033) (Table 4).

Fascin and E-cadherin expression in clear cell type RCCs

Because significant differences were noted in the different histologic types, we analyzed separately the clinicopathologic features in the clear cell type. In the clear cell type, fascin expression was correlated to necrosis, a higher TNM stage, and Fuhrman grade (p=0.027, 0.025, and 0.001, respectively). The positive rate of expression for E-cadherin increased as the Fuhrman grade increased

Table 4. Correlation between E-cadherin expression and clinicopathologic factors in renal cell carcinomas

		Negative n (%)	Positive n (%)	p
Histologic type	Clear cell	122 (76.5)	39 (23.5)	0.001
	Papillary	12 (60.0)	8 (40.0)	
	Chromophobe	2 (25.0)	6 (75.0)	
Necrosis	Absent	86 (81.1)	20 (8.9)	0.004
	Present	20 (37.0)	34 (63.0)	
Fuhrman grade	Grade 1	17 (100.0)	0 (0.0)	0.000
	Grade 2	76 (76.8)	23 (23.2)	
	Grade 3	41 (65.1)	22 (34.9)	
	Grade 4	6 (46.2)	7 (53.8)	
Sarcomatoid changes	Absent	136 (74.7)	46 (25.3)	0.033
	Present	8 (50.0)	8 (50.0)	

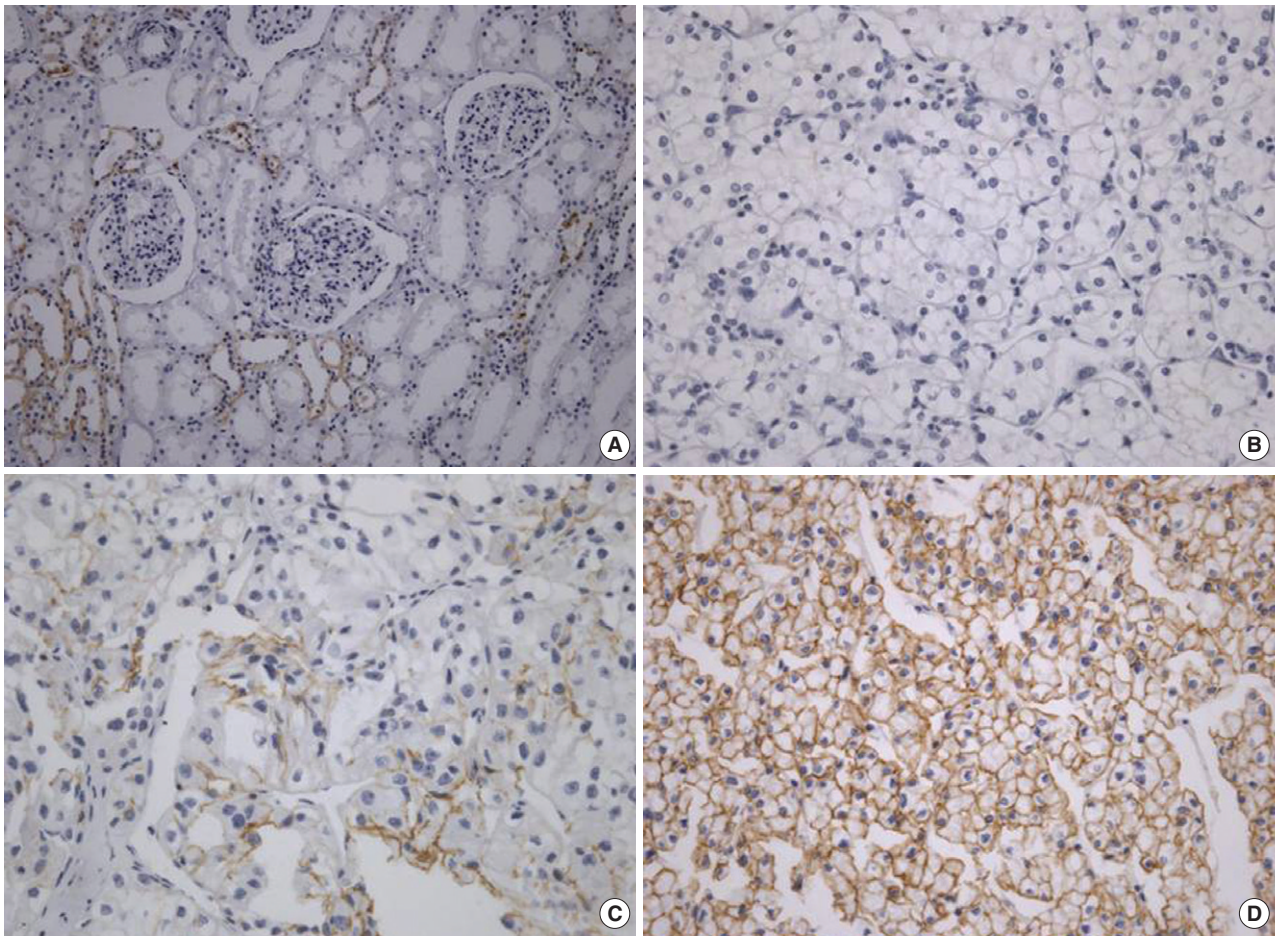

Fig. 2. Immunohistochemical stain for E-cadherin in normal kidney and renal cell carcinomas. E-cadherin is expressed in distal tubules and collecting ducts in normal kidney (A). Representative E-cadherin stains in renal cell carcinoma samples show negatively (B), weak membranous expression (C), and intense membranous expression (D).

Table 5. Inverse correlation between fascin and E-cadherin expression

		E-cadherin		p
		Negative	Positive	
Fascin	Negative	122 (84.7%)	22 (15.3%)	0.026
	Positive	52 (96.3%)	2 (3.7%)	

(p=0.002) and during necrosis (p=0.032).

Correlation between fascin and E-cadherin

In RCCs cases which were fascin negative (144 cases), E-cadherin expression was seen in 22 cases (15.3%). On contrary, in RCCs cases which were fascin positive (54 cases), E-cadherin expression was observed only in 2 cases (3.7%). These results exhibit an inverse correlation with respect to fascin and E-cadherin expression in RCCs (p=0.026, r=-0.158) (Table 5).

DISCUSSION

In Korea, renal cell carcinoma is the 10th most common cancer and the incidence of RCC appears to be on the rise.[1] Fascin is a globular actin cross-linking protein with a major function in forming parallel actin bundles in cell protrusions. Fascin is involved in cell adhesion, motility, and signaling.[13] Overexpression of fascin is correlated to a variety of aggressive human neoplasms such as ovarian, breast, pancreatic, colon, lung, and skin tumors compared to normal tissue epithelium which exhibits no expression or low expression of fascin.[14-22] The current study has demonstrated that the expression of fascin is significantly elevated in the presence of sarcomatoid changes, higher nuclear grade, and higher TNM stage.[23] Our data indicates that fascin expression may be closely linked with the aggressiveness of tumors which is supported by previous reports suggesting that fascin expression is associated with high tumor stage, high tumor grade, and large tumor size.[2,23,24] In addition, sarcomatoid morphology showed increased fascin expression. Therefore, fascin has been shown to be a novel prognostic marker in RCC. In the present study, fascin expression was increased in papillary type RCCs compared to the other types. This result may indicate that fascin is highly expressed in the more proliferative histologic types of RCCs which is similar to the association of fascin immunoreactivity with the increased proliferative activity in carcinoid tumors.[25]

The cadherin family of transmembrane glycoproteins is important for cellular adhesion in epithelial cells. It is known that E-cadherins mediate homotypic adhesions in epithelial tissues and serve to keep the epithelial cells together. However, in this study, E-cadherin expression was significantly correlated to poor prognostic factors including high nuclear grade, necrosis, and sarcomatoid changes. This can explain why E-cadherin is expressed aberrantly in transformed cells in RCCs. Markovic *et al.*[21] showed that tumor aggressiveness was determined by the impaired function and downregulation of E-cadherin which was confirmed by our study. These results indicate that E-cadherin overexpression might result in some genetic or epigenetic alteration in cancer progression. One possible explanation for our results is that fascin expression is inversely correlated to E-cadherin expression (p=0.026, r=-0.158).[11,20,22] Ying *et al.*[22] suggested that fascin and cadherin binding sites within β-catenin overlap and that fascin and cadherins *in vitro* compete for binding to β-catenin . In transformed epithelial cell systems, Yamashiro *et al.*[20] observed transfection of the fascin gene leads to cell-to-cell contact disorganization and increased cell motility by inducing the emission of microspikes on apical surfaces and on the extended lamelipodia on basolateral surfaces. Some of these changes are due to the downregulation and altered cytoplasmic distribution of the E-cadherin-based adhesion complex induced by fascin overexpression. This agrees with of Okada *et al.*[11] who showed that increased immunoreactivity for fascin had a tendency to disrupt membranous immunoreactivity for E-cadherin. Therefore, it may be postulated that the altered expression of E-cadherin is involved in fascin-mediated cell motility. Although many controversial theories have been advanced about the correlation between fascin and E-cadherin in other cancers, we demonstrate for the first time that fascin overexpression was inversely correlated to E-cadherin expression in RCCs. Increased fascin expression in RCCs was correlated to poor prognostic factors including high Fuhrman nuclear grade and advanced tumor stage. In addition, sarcomatoid morphology was associated with increased fascin overexpression in RCCs. In conclusion, fascin expression can be used as a novel prognostic marker for RCCs. Our findings not only provide a biomarker for the prediction of tumors, but also would be useful in studies pertaining to tumor progression on a genetic level.

Further studies involving the molecular mechanisms underlying the relationship between fascin and E-cadherin in RCCs are required.

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