Increasing expression of fascin in renal cell carcinoma associated with clinicopathological parameters of aggressiveness

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Summary. Aim: To determine whether higher expression of fascin, an actin-bundling protein associated with motility, in conventional renal cell carcinoma (RCC) is associated with more advanced stages of the disease. Methods: Immunohistochemical analysis of fascin expression was performed in tissue microarrays of 108 RCCs including 55 clear cell RCCs (CRCCs), 39 CRCCs with granular cell differentiation (GRCCs), 8 CRCCs with sarcomatoid differentiation (SRCCs) and 6 metastatic RCCs. Results: The expression of fascin was undetectable in normal renal tubules of all control cases. However, among the 108 RCC cases, fascin immunoreactivity was seen on the cell membrane and cytoplasm. The average immunostaining score for fascin was 128/400 in grade I, 170/400 in grade II, 207/400 in grade III, and 323/400 in grade IV RCC. The average immunostaining score of fascin was 187/400 for stage T1, 205/400 for stage T2, 288/400 for stage T3, and 355/400 for stage T4 cases of RCCs. Higher fascin scores in RCC were significantly correlated with higher T and N stages and nuclear grade. In addition, the fascin scores in GRCC (368±19) and SRCC (263±21) were significantly higher than in CRCC (95±18). Conclusions: Our findings demonstrate for the first time that increased expression of fascin is associated with clinicopathological parameters of aggressiveness in patients with RCC. Fascin may be a novel biomarker for diagnosis and treatment of RCC.

Key words: Fascin, Renal cell carcinoma, Clear cell type, Granular cell differentiation, Sarcomatoid differentiation

Introduction

Tumor metastasis is the main cause of treatment failure and mortality in patients with renal cell carcinoma (RCC) (Bonsib et al., 2000; Sweeney et al., 2003; Atkins et al., 2004; Flanigan, 2004). Studies have demonstrated the expression of a series of diverse genes that are switched on in invasive cancer cells (Kerbel et al., 1988; Kerbel, 1990; Su et al., 1993). These include genes encoding proteinases, adhesion molecules, angiogenesis factors, and growth factors (Kerbel et al., 1988; Kerbel, 1990; Su et al., 1993).

Tumor invasion is a complex process involving local invasion, lympho-vascular space invasion, extravasation, and growth at sites of metastasis (Aznavoorian et al., 1993). One of the mechanisms in tumor invasion is the enhancement of cell motility (Partin et al., 1989). Cell motility is driven by the activity of the actin cytoskeleton, and microfilament bundling is critical in this activity (Lauffenburger et al., 1996).

Fascin is a cytoplasmic protein that functions by fundling cytoplasmic actin filaments (Kureishy et al., 2002) and its expression in normal epithelia is absent or low (Yamashiro et al., 1998; Kureishy et al., 2002). However, since fascin is markedly upregulated in breast, lung, colon, and ovarian cancers (Grothey et al., 2000; Hu et al., 2000; Goncharuk et al., 2002; Jawhari et al., 2003; Pelosi et al., 2003a,b), it may be an important mediator of tumor cell invasion.

The expression profiles of fascin in RCC are currently unclear. In the present study, the expression of fascin in RCCs was evaluated using tissue microarrays. Our findings demonstrate for the first time that fascin is over-expressed in RCCs.

Materials and methods

Paraffin-embedded tumor tissues of Chinese patients were retrieved from the Department of Pathology, Tri-Service General Hospital, and tissue microarray slides

were constructed according to a previously published method (Hidalgo et al., 2003). The tissue microarrays consisted of 108 conventional RCCs (including 55 conventional clear cell RCCs [CRCCs, mean age 53], 39 CRCCs with granular cell differentiation [GRCCs, mean age 50] in at least 20% of the tumor area, 8 CRCCs with sarcomatoid differentiation [SRCCs, mean age 55] in at least 10% of the tumor area) and 8 metastatic RCCs. For the study on GRCCs and SRCCs, two pathologists screened histological sections and selected areas of the representative tumor cells with granular cell differentiation or sarcomatoid differentiation. One tissue core (2 mm in diameter) was taken from each of the representative tumor samples and placed in a new recipient paraffin block.

In addition, 72 of 108 RCC cases received surgical excision after tissue proved and had the information about clinical stages. All tumors were pathologically staged according to the 1997 TNM system and assigned a Fuhrman nuclear grading. Stage T1 was defined as a tumor less than 7 cm confined to the kidney. Stage T2 was defined as a tumor greater than 7 cm confined to the kidney. Stage T3 was defined as direct tumor infiltration of the perirenal fat or tumor extension into segmental renal veins or the inferior vena cava. Stage T4 was defined as tumor extension beyond Gerota's fascia.

Immunohistochemistry

Tissue microarray sections were dewaxed in xylene, rehydrated in alcohol, and immersed in 3% hydrogen peroxide for 10 min to suppress endogenous peroxidase activity. Antigen retrieval was performed by heating (100°C) each section for 30 min in 0.01 mol/L sodium citrate buffer (pH 6.0). After 3 rinses (each for 5 min) in phosphate buffered saline (PBS), sections were incubated for 1 h at room temperature with a mouse monoclonal anti-human fascin antibody (NEOMARKERS, Freemont, CA, USA, 1:100) diluted in PBS. After 3 washes (each for 5 min) in PBS, sections were incubated with horseradish peroxidase-labeled rabbit anti-mouse immunoglobulin (DAKO, Carpinteria, CA, USA) for 1 h at room temperature. After 3 additional washes, peroxidase activity was visualized with a solution of diaminobenzidine (DAB) at room temperature.

For evaluation of immunoreactivity and histological appearance, all tissue microarray slides were examined and scored by two authors concurrently. The intensity of membranous and cytoplasmic immunostaining of fascin in individual tumor cells was scored on a scale of 0 (no staining) to 4 (strongest intensity), and the percentage of cells with fascin staining at each intensity was estimated from 0 to 100. The absolute value of the proportion of cells at each intensity level was multiplied by the corresponding intensity value, and these products were added to obtain an immunostaining score ranging from 0 to 400 (Rosenthal et al., 2003; Jin et al., 2006). Previous study showed that the endothelial cells of vessels are

strong positivity with fascin and this was interpreted as positive control (Grothey et al., 2000; Hu et al., 2000; Goncharuk et al., 2002). As negative controls, the slide was treated by replacement of primary antibody with non-immune serum.

Statistical analysis

All results are expressed as mean ± standard error of the mean (SEM). The immunostaining score of fascin for different histopathological differentiation of RCC was compared with the score of normal renal tubules. Statistical analysis was performed using the Student t-test between groups. A P value less than 0.05 was considered to be statistically significant. SigmaState software (Jandel Scientific, San Rafael, CA, USA) was used to perform linear regression testing to analyze the relationship between fascin immunostaining score and clinicopathological parameters.

Results

Fascin expression in RCCs

The expression of fascin was detected in renal vessels and glomerular capillaries (Fig. 1B). In contrast, no fascin was detectable in the renal tubules of all 12 control normal renal tissue specimens (Fig. 1D). By review of hematoxylin and eosin stained slides, two pathologists confirmed the diagnoses of RCC in the 108 tumor specimens (Figs. 2,3). In all 108 tumor specimens, fascin immunoreactivity was significantly enhanced on the tumor cell surface and in the cytoplasm (Figs. 2, 3 and Table 1). Fascin immunostaining score ranged from a high of 368±19 for GRCCs to a low of 95±18 for CRCCs (Table 1). All 39 GRCCs and 8 SRCCs were strongly (intensity score >2) and diffusely (>90% tumor cells) positive for fascin. In addition, fascin expression was significantly higher in GRCCs (368±19) and SRCCs (263±21) than in CRCCs (95±18). In metastatic RCCs, the fascin immunostaining score was also significantly increased to 215±23 (Table 1, Figs. 1,3).

Correlation of fascin immunostaining scores with clinicopathological features

In grade I, grade II, grade III, and grade IV RCCs, the average fascin expression intensity was 1.6, 2.0, 2.3, and 3.4, respectively, average percentage of tumor staining was 80, 85, 90, and 95, and average immunostaining score was 128, 170, 207, and 323 (Table 2). Statistical analyses revealed significant positive correlation of fascin staining intensity, % tumor staining, and immunostaining scores with the histological grading system (Table 2, P<0.05).

Of all the 72 RCC cases with surgical excision, 11 cases of T1 stage were found, 42 cases of T2 stage, 18 cases of T3 stages, and 1 case of T4 stage (Table 2). In our study, the more advanced stages of RCC had higher
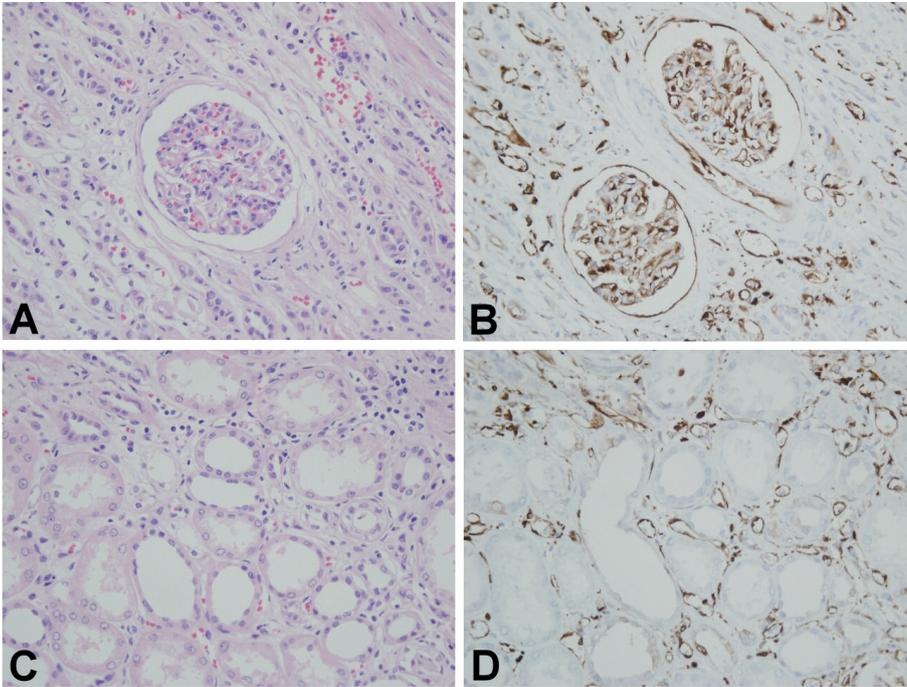
Fig. 1. Hematoxylin and eosin staining of normal renal glomerulus **(A)** and normal renal tubules **(C)** and immunohistochemical staining of fascin in normal renal glomerulus **(B)** and normal renal tubules **(D)**. x 400
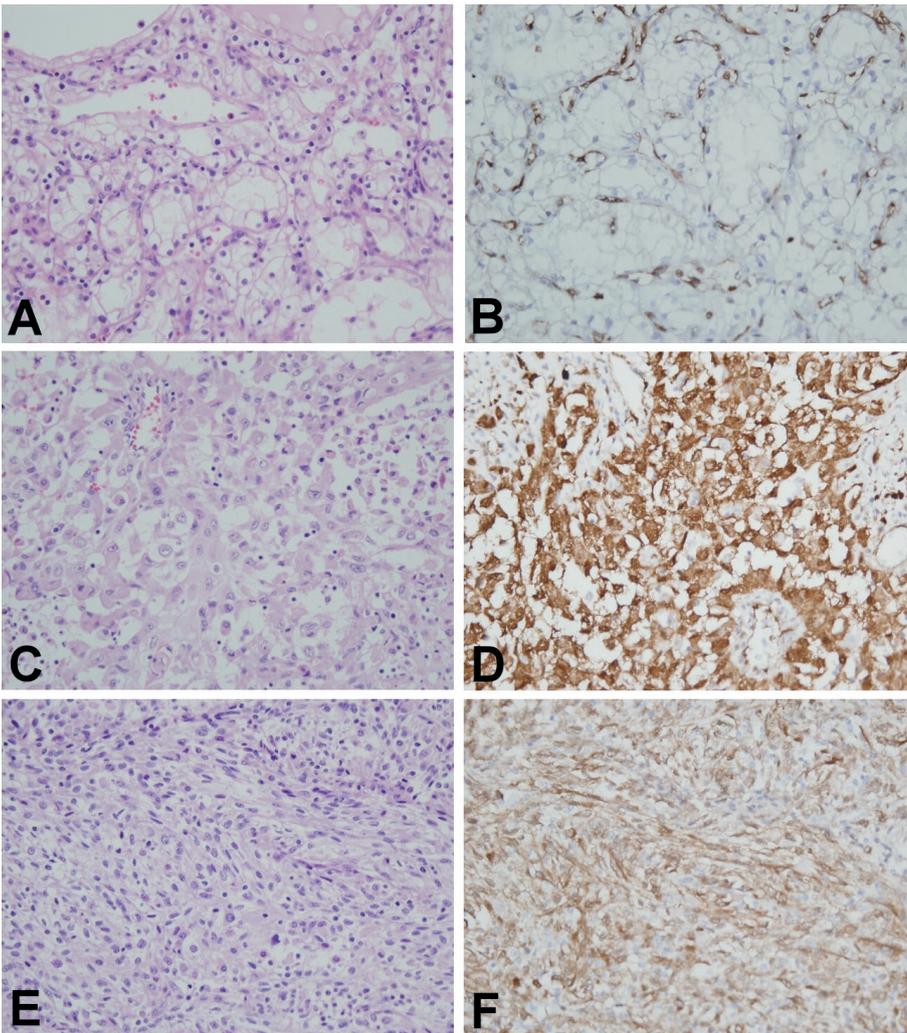
Fig. 2. Hematoxylin and eosin staining of clear cell RCC **(A)**, clear cell RCC with granular cell differentiation **(C)**, and clear cell RCC with sarcomatoid differentiation **(E)** and immunohistochemical staining of fascin in clear cell RCC **(B)**, clear cell RCC with granular cell differentiation **(D)**, and clear cell RCC with sarcomatoid differentiation **(F)**. x 400

fascin intensity and immunostaining scores. The average immunostaining score of fascin was 187 in stage T1, 205 in stage T2, 288 in stage T3, and 355 in stage T4 RCCs.

The positive correlation of fascin staining scores with T stage was validated based on P value less than 0.05 (Table 2 and Fig. 4). In addition, we also demonstrated
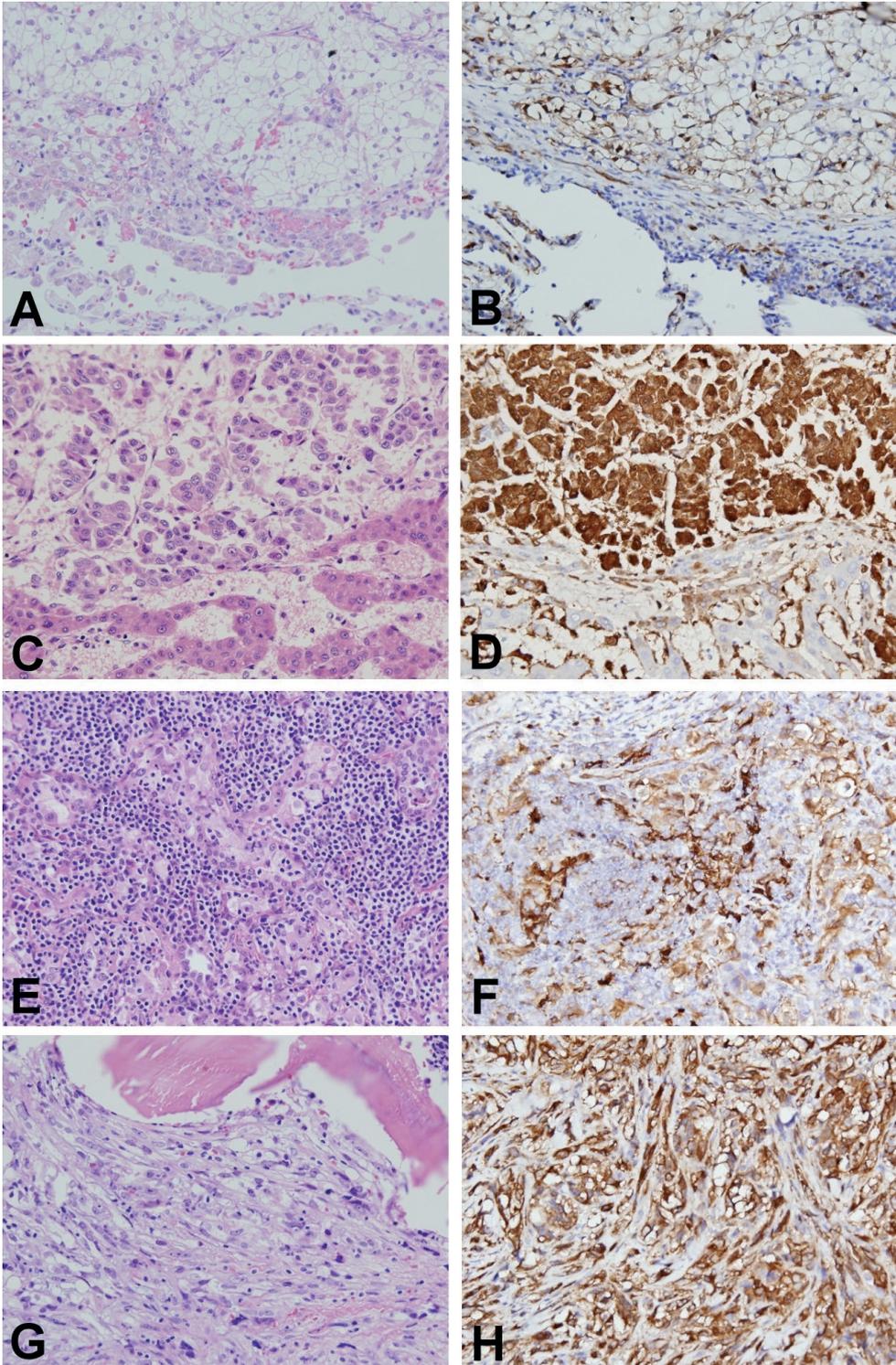

Fig. 3. Representative hematoxylin and eosin staining of metastatic clear cell RCC **(A)**, metastatic RCC with granular cell differentiation **(C, E)**, and metastatic RCC with sarcomatoid differentiation **(G)** and immunohistochemical staining of fascin in metastatic clear cell RCC **(B)**, metastatic RCC with granular cell differentiation **(D and F)**, and metastatic RCC with sarcomatoid differentiation **(H)**. x 400

that more advanced N stage of RCCs is associated with higher intensity, and greater percentage of tumor staining and immunostaining scores of fascin expression (Table 2 and Fig. 4). However, fascin immunostaining score did not significantly correlate with M stage of RCCs.

Discussion

When localized, RCC can be treated effectively but not after the disease has spread outside the renal capsule (Bonsib et al., 2000; Sweeney et al., 2003). Improvement in survival is likely to depend on an increase in the ability of treatments to arrest local invasion and tumor spread. Understanding the mechanisms that involve motility-associated cell structures may lead to therapies directed at limiting distant spread of disease. Our results demonstrated that

the actin bundling protein, fascin, is overexpressed in RCCs and that overexpression is significantly correlated with clinicopathological parameters of aggressiveness.

Table 1. Fascin immunostaining scores for different differentiations of renal cell carcinoma.

Differentiation	% staining	Intensity	Total score
CRCC (n = 55)	53±5*	1.7±0.3*	95±18 *
GRCC (n = 39)	96±6*	3.5±0.5*	368±19 *+
SRCC (n = 8)	98±5*	2.8±0.4*	263±21 *+
Metastatic RCC (n = 6)	86±10*	2.5±0.7*	215±23*
Renal tubules (n = 12)	0	0	0

Data are means ± standard error of the mean (SEM) of the fascin immunostaining score in renal cell carcinomas. *p<0.05 vs. normal renal tubules. + indicates significant difference (p<0.05) compared to CRCC. Clear cell RCC, CRCC; clear cell RCC with granular cell differentiation, GRCC; and clear cell RCC with sarcomatoid differentiation, SRCC.
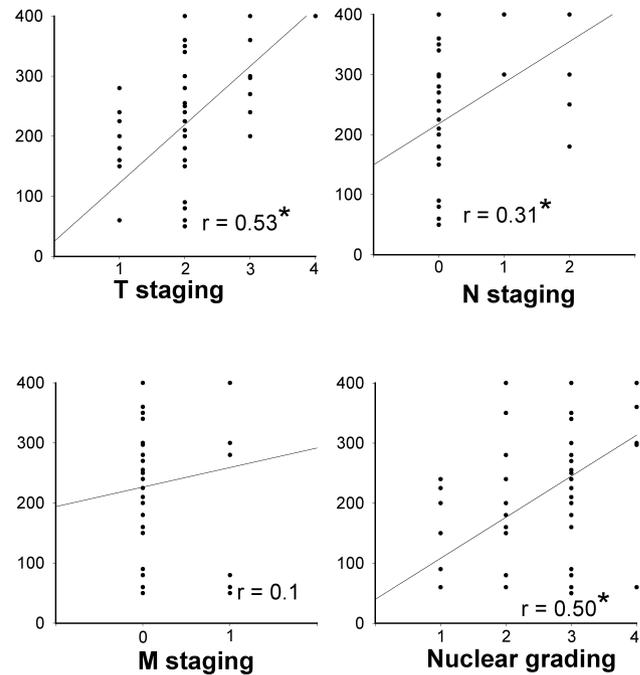
Fig. 4. Clinicopathological features (TNM stage and nuclear grade) correlate with fascin immunostaining score in renal cell carcinoma. * indicates statistical significance of linear regression testing (p<0.05).

Table 2. The pattern of fascin immunostaining and clinicopathological parameters of renal cell carcinoma.

	No. of cases	Average intensity	Average % tumor	Average score	Correlation
Nuclear grading					
Grade I	6	1.6	80	128	Positive correlation (P<0.05)
Grade II	18	2.0	85	170	
Grade III	32	2.3	90	207	
Grade IV	16	3.4	95	323	
TNM stage					
T stage					
T1	11	2.2	85	187	Positive correlation (P<0.05)
T2	42	2.2	93	205	
T3	18	3.1	93	288	
T4	1	3.7	96	355	
N stage					
N0	62	2.5	58	145	Positive correlation (P<0.05)
N1	6	3.6	96	346	
N2	4	3.3	92	304	
M stage					
M0	61	2.1	89	187	(P=0.21)
M1	11	2.2	87	191	

Fascin is typically expressed in endothelial cells and at very low levels in normal epithelia (Grothey et al., 2000; Hu et al., 2000; Goncharuk et al., 2002; Jawhari et al., 2003; Pelosi et al., 2003a,b). Recent studies show high expression of fascin in carcinomas of the skin (Goncharuk et al., 2002), lung (Pelosi et al. 2003a,b), breast (Grothey et al., 2000), ovary (Hu et al., 2000), and colon (Jawhari et al., 2003). The expression of fascin in lung and breast cancers correlated with a shorter survival and was an independent prognostic predictor of unfavorable clinical course of the disease (Pelosi et al., 2003a,b; Yoder et al., 2005). Our current results also demonstrated that the endothelial cells of normal renal vessels and glomerular capillaries are strongly fascin positive, whereas the normal renal tubules are fascin negative. In addition, the marked overexpression of fascin shown in RCCs was associated with aggressive clinicopathological features.

Fascin immunostaining score was significantly higher in SRCC and GRCC than in CRCC. This higher expression of fascin in SRCC and GRCC suggests that fascin expression is associated with more invasive phenotypes such as GRCC and SRCC. Our results led us to consider fascin as a potentially useful marker of more aggressive histopathological differentiations of RCC such as SRCC and GRCC.

The expression of fascin was significantly correlated with nuclear grading and the T and N stages. However, the expression of fascin was not significantly correlated with the M stage of RCCs in our current study. This may be due to the limited number (11 cases) of cases in M1 stage. Future efforts will be directed at confirming fascin's role in tumor metastasis and assessing the mechanism by which it contributes to the invasive phenotype of RCC cells.

Our current study was designed using tissue microarray. One potential limitation of tissue microarray is the correct representation of each tumor with the level of heterogeneity. However, a study has demonstrated that when the number of cases is increase to more than 54 cases in tissue microarray preparation, the probability that results from one core would correctly represent the whole section was more than 91% (Rosen et al., 2004). Tissue microarray technique enables simultaneous histological and immunohistochemical analysis of a collection of tumor samples (Lam et al., 2004). Previous studies using immunohistochemistry for quantitation in individual samples were of limited value because environmental conditions and therefore signal intensity often varied from sample to sample (Lam et al., 2004). However, the advantage of the tissue microarray technique is that it is carried out under the same conditions and all samples are evaluated simultaneously on a single tissue microarray slide (Lam et al., 2004). Thus, the higher fascin immunostaining score of SRCC and GRCC samples may indicate higher expression of fascin in the SRCC and GRCC subtypes.

In conclusion, we demonstrated that fascin is over-expressed in RCCs. Increased expression of fascin in RCC is significantly related to the aggressive behavior of this tumor. Fascin may be a novel biomarker for diagnosis and treatment of RCC.

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