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Prognostic significance of tenascin-C expression in clear cell renal cell carcinoma

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Abstract. Tenascin-C is an extracellular matrix protein that plays an important role in cell proliferation, migration and tumor invasion in various types of cancer. However, few reports exist on tenascin-C expression in renal cell carcinoma (RCC). This study aimed to assess the prognostic significance of tenascin-C in clear cell RCC. Using immunohistochemistry, 137 formalin-fixed, paraffin-embedded tissue sections obtained from patients with clear cell RCC were examined for tenascin-C expression. Tenascin-C expression was observed in 55 (40.1%) of the 137 clear cell RCC sections. Tumor cells displayed membranous and/or cytoplasmic staining for tenascin-C. Tenascin-C expression was more prominent near the pseudocapsule of the tumor and around the tumor vessels. Tenascin-C expression was significantly associated with a higher stage (P=0.0065) and higher nuclear grade (P=0.0001). However, there was no correlation between the tenascin-C expression and venous involvement. The cancer-specific survival rate in patients with a tenascin-C-positive primary tumor was significantly lower than that in those with a tenascin-C-negative primary tumor in univariate analysis (P=0.0017). However, tenascin-C expression did not exhibit a significant value for cancer-related death in the Cox regression analysis. In patients with stage 1-3 disease, the 5-year metastasis-free rate in patients with the tenascin-C-positive primary tumor was significantly lower than that in those with the tenascin-C-negative primary tumor (67.8 vs. 88.5%, respectively; P=0.0038). The Cox regression analysis showed that tenascin-C expression is a significant predictor of metastasis (P=0.0345). The tenascin-C expression was strongly related to the stage, nuclear grade and 5-year metastasis-free rate. Therefore, tenascin-C expression may be a possible marker for the metastatic potential of clear cell RCC.

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Introduction

Renal cell carcinoma (RCC) accounts for ~3% of adult malignancy (1). At the time of diagnosis, approximately one-third of the patients with RCC had metastatic disease (2) and 20-30% of patients with localized disease subsequently developed metastasis after nephrectomy (3). RCC is inherently resistant to chemotherapy and radiotherapy. Immunotherapy with interferon-α and/or interleukin-2 has been effective against metastatic RCC, however, its efficacy is not satisfactory (4). Recent progress in understanding the biology of RCC has led to the development of molecular-targeted therapy. Several anti-vascular endothelial growth factor agents have demonstrated clinical activity in patients with metastatic RCC (5). It is therefore important to establish an individual follow-up protocol for the early detection and treatment of metastasis and to identify novel prognostic markers of metastasis.

We succeeded in establishing 2 RCC cell lines, which were derived from a matched pair of the primary tumor and adrenal metastasis. A DNA microarray analysis of these cell lines identified tenascin-C as one of the important genes. Tenascin-C is an extracellular matrix protein that is transiently expressed during fetal development and is absent or greatly reduced in most adult tissues. Furthermore, tenascin-C is highly expressed in various types of cancers, and it plays an important role in cell proliferation, migration and tumor invasion (6). However, few reports exist on tenascin-C expression in RCC. Therefore, we examined the prognostic significance of tenascin-C in RCC, particularly in clear cell RCC.

Materials and methods

Tissue samples. A total of 137 formalin-fixed, paraffin-embedded tissues were obtained from patients with clear cell RCC who were treated at The Tokyo Medical University Hospital between September 1986 and March 2003. The mean age of the patients (100 men and 37 women) at the time of diagnosis was 59 years (range 24-81). The patients had undergone radical nephrectomy at our hospital where, lymphadenectomy is not included in routine nephrectomy. No patient had received irradiation or chemotherapy prior to surgery. The tumors were staged according to the 1997 Tumor-Node-Metastasis staging system: 76 tumors belonged to TNM stage I; 11, stage II; 25, stage III; and 25, stage IV.

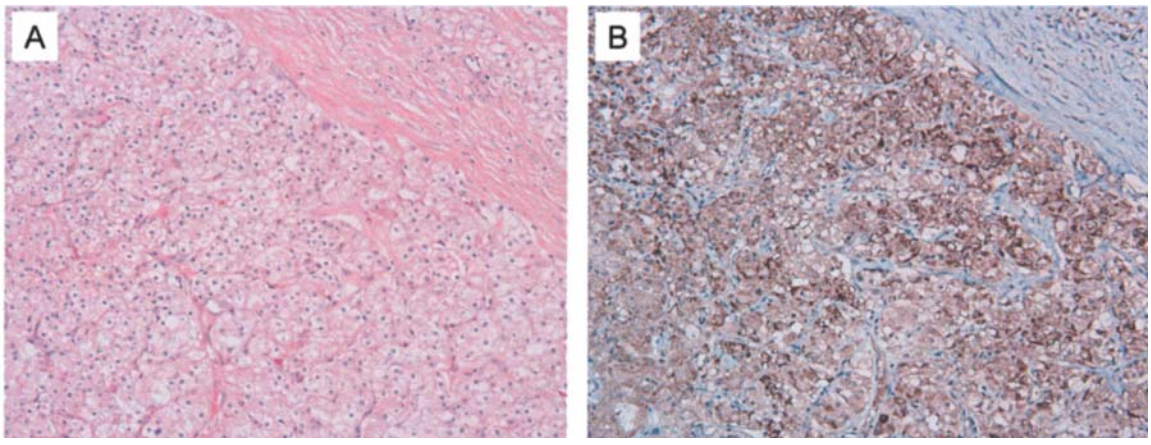

Figure 1. Tenascin-C expression in clear cell RCC. (A) The tumor was diagnosed as a grade 2 clear cell RCC. HE staining, x200. (B) The tumor cells show membranous and/or cytoplasmic staining for tenascin-C.

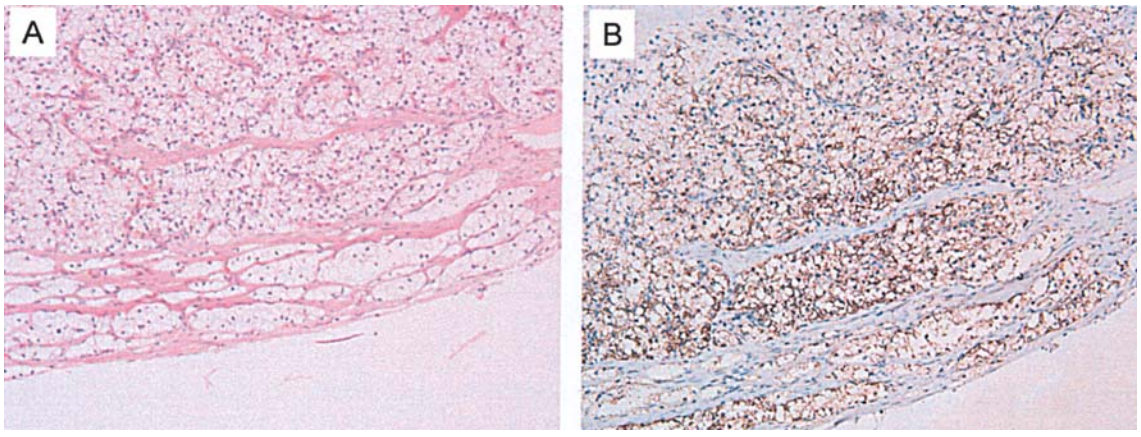

Figure 2. Tenascin-C staining of clear cell RCC. (A) The tumor was diagnosed as a grade 1 clear cell RCC. HE staining, x200. (B) The tenascin-C expression was more prominent near the pseudocapsule of the tumor.

The histological types were determined according to the World Health Organization classification. Nuclear grading was performed according to Fuhrman's nuclear grading system: 46 tumors were grade 1; 64, grade 2; and 27, grade 3 or 4. The patients were followed up with clinical and radiological examinations at regular intervals. Patients with metastatic disease received interferon-α or interleukin-2 therapy. At the last follow-up, 77 patients showed no evidence of disease, 22 were alive with metastases, 28 had succumbed due to cancer and 10 patients succumbed to non-cancer-related events. The mean follow-up period was 78.8 months (range 2.3-214).

Immunohistochemistry. From the archival formalin-fixed, paraffin-embedded tissues of the representative area of each surgical specimen, which included the highest nuclear grade cancer, 4-μm sections were obtained and mounted on poly-L-lysine coated slides. They were deparaffinized with xylene and rehydrated in graded alcohols. Endogenous peroxidase was blocked using 3% hydrogen peroxide for 20 min. The slides were treated with the antigen retrieval technique (121˚C for 10 min in 10 mM citrate buffer, pH 6.0). Endogenous biotin was blocked using 0.01% biotin for 20 min at room temperature. After blocking non-specific conjugation with 1% casein, the slides were incubated for 60 min at room temperature with anti-tenascin-C mouse monoclonal antibody (clone 49; 1:100, Novocastra, UK). The bound antibodies were detected by using the avidin-biotin complex peroxidase method (Vectastain ABC kit, Vector Laboratories, CA, USA) and visualized with diaminobenzidine. The slides were counterstained in Harris' hematoxylin, dehydrated and then mounted.

Tenascin-C expression was considered positive when >10% of the cancer cells showed clear staining.

Statistical analysis. All statistical analyses were performed using JMP IN 5.1 (SAS Institute Inc., NC, USA). Tenascin-C expression was considered a dichotomous variable (i.e., positive or negative) in all statistical analyses. The correlations between tenascin-C expression and other clinicopathological parameters were assessed using the Chi-square test, Fisher's exact test and Student's t-test. Cancer-specific survival and metastasis-free rates were estimated using the Kaplan-Meier method, and the differences between the curves were tested using the log-rank test. Metastasis-free time was calculated

Correlation between tenascin-C expression and clinicopathological factors.

	Negative	(%)	Positive	(%)	P-value
Primary tumor	82.00	(59.9)	55.00	(40.1)	
Age (years)	57.60		60.90		0.05
Size (cm)	5.43		6.53		0.05
Gender					
Male	56.00	(56.0)	44.00	(44.0)	n.s.
Female	26.00	(70.3)	11.00	(29.7)	
TNM stage					
I	55.00	(72.4)	21.00	(27.6)	0.0065
II	4.00	(36.4)	7.00	(63.6)	
III	13.00	(52.0)	12.00	(48.0)	
IV	10.00	(40.0)	15.00	(60.0)	
T stage					
T1	57.00	(69.5)	25.00	(30.5)	0.0467
T2	7.00	(41.2)	10.00	(58.8)	
T3	17.00	(47.2)	19.00	(52.8)	
T4	1.00	(50.0)	1.00	(50.0)	
M stage					
0	73.00	(63.5)	42.00	(36.5)	0.047
1	9.00	(40.9	13.00	(69.1)	
Grade					
1	38.00	(82.6)	8.00	(13.4)	0.0001
2	34.00	(53.1)	30.00	(46.9)	
3 + 4	10.00	(37.0)	17.00	(63.0)	
Microscopic venous invasion					
Negative	53.00	(60.2)	35.00	(39.8)	0.98
Positive	24.00	(60.0)	16.00	(40.0)	

n.s., not statistically significant.

from the day of radical nephrectomy to that of the radiological detection of metastases. A multivariate analysis was performed using the Cox proportional hazard regression model to test for independent prognostic values. P<0.05 was considered statistically significant.

Results

Tenascin-C expression was observed in 55 (40.1%) of the 137 clear cell RCCs. The tumor cells displayed membranous and/or cytoplasmic staining for tenascin-C (Fig. 1). The tenascin-C expression was more prominent near the pseudocapsule of the tumor and around the tumor vessels (Fig. 2). The mean size of tenascin-C-positive tumors was significantly greater than that of tenascin-C-negative tumors (6.53 vs. 5.43 cm, respectively; P=0.05). Tenascin-C expression was significantly associated with a higher stage (P=0.0065) and higher nuclear grade (P=0.0001). However, there was no correlation between tenascin-C expression and the microscopic venous involvement. The correlations between the tenascin-C immunoreactivity and clinicopathological factors are summarized in Table I. A total of 28 (20.4%) patients succumbed due to the disease. The cancer-specific 5- and 10-year survival rates were 85.7 and 82%, respectively, in patients with tenascin-C-negative tumors. These rates were significantly higher than those in patients with tenascin-C-positive tumors (74.7 and 56.2%, respectively; P=0.032) (Fig. 3). Although the univariate analysis showed that tumor stage, nuclear grade, microscopic invasion and tenascin-C immuno-reactivity are significant prognostic factors, the Cox regression analysis revealed that the T stage and nuclear grade were independent predictors of cancer-related death (Table II).

Among the 112 patients with stage 1-3 disease, 30 (26.8%) eventually developed local recurrence and/or metastases. The median time to recurrence or metastasis was 76 months (range 1.5-212). Local recurrence was observed in 2 patients. The sites of metastasis were lung (19 cases), brain (6 cases), bone (2 cases), liver (1 case), skin (1 case), adrenal gland

Table II. Cancer-specific survival rates.

	Survival (%)		Likelihood ratio		
	5-year	10-year	Log-rank test	Chi-square test	Cox
T stage					
1	94.0	88.3	<0.0001	19.24998	0.0002
2	100.0	66.7			
3	75.7	64.1			
4	26.3	0.0			
Grade					
1	97.5	97.5	<0.0001	13.38033	0.0012
2	79.1	64.3			
3 + 4	56.0	42.0			
Microscopic venous invasion					
Negative	88.1	80.6	0.0182	0.34892	n.s.
Positive	71.9	59.1			
Tenascin-C expression					
Negative	85.7	82.0	0.032	1.60821	n.s.
Positive	74.7	56.2			

n.s., not statistically significant.

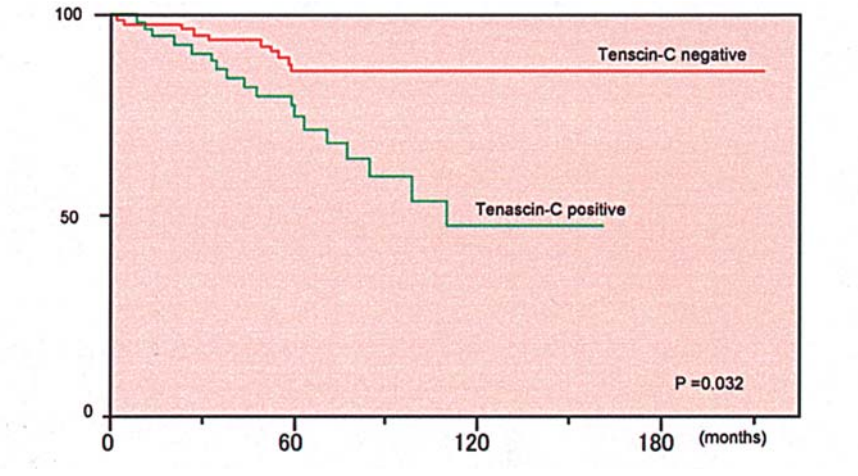

Figure 3. Cancer-specific survival in clear cell RCC. Survival rates were estimated using the Kaplan-Meier method. Statistical significance was assessed using the log-rank test. Patients with tenascin-C-positive tumors exhibited a better prognosis than those with tenascin-C-negative tumors (P=0.032).

(1 case), and ovary (1 case). Multiple organs were affected in 6 cases. The metastasis-free rates were 88.5% at 5 years and 82.1 at 10 years in patients with tenascin-C-negative tumors. In contrast, the metastasis-free rates in patients with tenascin-C-positive tumors were 67.8% at 5 years and 53.4% at 10 years. These rates were significantly lower than those in patients with tenascin-C-negative primary tumors (P=0.0038; Fig. 4). A univariate analysis showed that the tumor stage, nuclear grade and tenascin-C expression are significant predictors of metastasis. The Cox regression analysis also demonstrated that tenascin-C expression is an independent predictor of metastasis (P=0.0345; Table III).

Discussion

In this study, we analyzed the tenascin-C expression in clear cell RCC. Tenascin-C was expressed in 55 (40.1%) of the 137 clear cell RCC sections, and its expression clearly correlated with a higher nuclear grade and advanced stage. Patients with tenascin-C-positive tumors exhibited a poor prognosis. In particular, tenascin-C expression was an independent predictor of metastasis in patients with stage 1-3 disease.

Tenascin-C is a large (~300 kDa) hexameric extracellular matrix glycoprotein that is involved in tumor growth, metastasis, angiogenesis and immunosuppression (6). Tenascin-C

Metastasis-free rate in patients with stage 1-3 disease.

	Metastasis-free (%)		Likelihood ratio		
	5-year	10-year	Log-rank test	Chi-square test	Cox
T stage					
1	89.1	84.3	0.0017	3.52001	0.1720
2	81.8	49.1			
3	55.1	48.2			
Grade					
1	90.7	83.0	0.0031	2.27898	0.3200
2	80.7	69.2			
3 + 4	45.5	45.5			
Microscopic venous invasion					
Negative	85.5	80.4	0.1533		
Positive	80.0	64.3			
Tenascin-C expression					
Negative	88.5	82.1	0.0038	4.46966	0.0345
Positive	67.8	53.4			

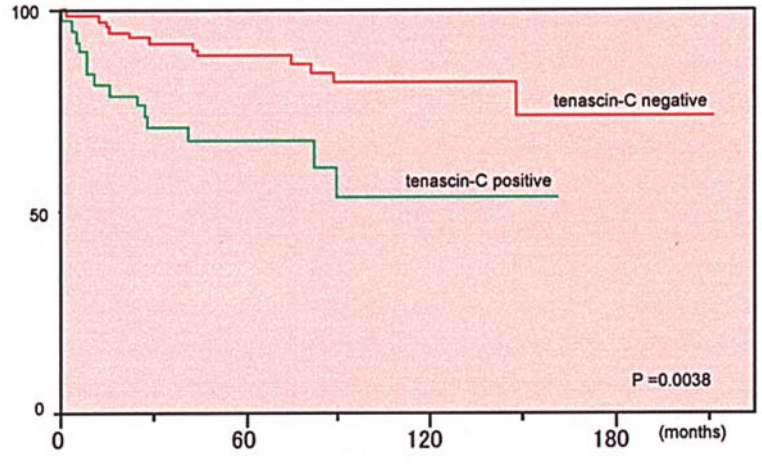

Figure 4. Metastasis-free rate in patients with stage 1-3 disease. Metastasis-free rates were estimated using the Kaplan-Meier method. Statistical significance was assessed using the log-rank test.

is strongly expressed in various types of cancers such as that of the breast, lung, stomach, liver, pancreas, colorectum, kidney, bladder and prostate (7-14). The prognostic significance of tenascin-C expression remains controversial. Earlier studies on tenascin-C expression in colorectal and breast cancers showed that a positive stromal expression is associated with a favorable prognosis (11,14). However, Jahkola *et al* demonstrated that the stromal tenascin-C expression at the invasion border of breast cancer is a predictor of local and distant recurrences (15). In colorectal cancer, Kressner *et al* demonstrated that a diffuse stromal tenascin-C expression was associated with a shorter survival time (16). In addition, other studies demonstrated tenascin-C production in cancer cells, and that tenascin-C expression in cancer cells correlates with metastasis and a poor prognosis (17,18). Therefore, it is important to consider the cellular source of tenascin-C because a functional difference in tenascin-C may exist between the stromal and cancer cells. Tenascin-C has many isoforms, each performing different functions (19). There is also evidence that specific tenascin-C isoforms are expressed in invasive breast cancers (20). Tenascin-C observed in stromal lesions may function in inhibiting metastasis or in promoting cancer cell invasion via its anti-adhesive effect depending on the condition. Moreover, tenascin-C detected in cancer cell cytoplasm may function as a factor promoting cancer cell growth and exerting an anti-adhesive effect in areas surrounding the tumor.

Only one report has addressed tenascin expression in RCC. Using surgical specimens, Lohi *et al* demonstrated stromal staining for tenascin in less-differentiated tumors

(10). They did not report on the correlation between the tenascin expression and prognosis. Although our cytoplasmic and membranous staining for tenascin-C was not consistent with their results, they had reported on the production of tenascin in the renal cancer cell lines, CAKI-1, ACHN and A498. This may imply that RCC produces tenascin identical to breast cancer. We also observed expression of tenascin-C mRNA in recently established renal cancer cell lines (data not shown). The function of tenascin-C in renal cancer biology, however, remains unclear. Tenascin-C expression near the pseudocapsule of the tumor and around the tumor vessels observed in the present study indicates that tenascin-C may function to promote cell proliferation and/or angiogenesis.

Many prognostic factors of RCC have been identified, such as anatomical (e.g., tumor size, lymph node involvement and distant metastases), histological (e.g., tumor grade, histological subtype and sarcomatoid feature), clinical (e.g., performance status and hematuria), and molecular factors (e.g., vascular endothelial growth factor and cadherin-6) (21). Recent microarray and proteomic analyses have enabled the screening of thousands of candidate molecular markers for various types of cancer (22,23). It is believed that the process of metastasis involves a series of steps consisting of proliferation, local invasion, spread through the vascular system, extravasation, and progressive growth in distant organs (24). We can plausibly use the numerous molecules involved in these steps as prognostic markers in addition to using molecular targets for anticancer therapies. In this study, we analyzed the tenascin-C expression in clear cell RCC because DNA microarray has demonstrated that tenascin-C is one of the differentially expressed genes in the RCC cell line, which we recently established. There was a significant correlation between tenascin-C expression in cancer cells and the metastatic development of clear cell RCC. Although its role remains to be investigated, tenascin-C expression in clear cell RCC may provide additional prognostic information.

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