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Expression profile of N-cadherin differs from other classical cadherins as a prognostic marker in renal cell carcinoma

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Received October 24, 2005; Accepted December 21, 2005

Abstract. Renal cell carcinomas (RCCs) primarily express cadherin-6 and N-cadherin rather than E-cadherin, and the aberrant expression of cadherin-6 correlates with a poor prognosis in patients with E-cadherin-absent RCC. However, the role of N-cadherin in RCC has not yet been elucidated. In this study, we analyze the expression pattern of N-cadherin in surgical RCC specimens and discuss the function of N-cadherin in RCC together with a re-evaluation of our previous study. Forty-six surgically resected RCC specimens were used in this study. The expression and localization of E-cadherin and N-cadherin in surgical specimens were demonstrated by immunohistochemistry. Correlations between the expression pattern of each cadherin, pathological parameters and patient survival were analyzed. RCC specimens expressed N-cadherin as a normal pattern despite the elevation of tumor grade, and patients with N-cadherin-normal RCC had a poorer prognosis than those with N-cadherin-abnormal RCC. Since our previous study showed that N-cadherin only functioned in cell attachment when E-cadherin and cadherin-6 were impaired, N-cadherin plays a different role from E-cadherin or cadherin-6 in RCC and may be associated with the aggressiveness and malignant potential of RCC.

Introduction

Renal cell carcinoma, the most common neoplasm arising from renal tubular epithelium, is usually treated by radical nephrectomy. After surgery, however, metastasis via the blood circulation can be observed, depending on the tumor stage, grade and/or aggressiveness. We previously reported that disruption of cadherin-mediated cell-cell adhesion was a risk factor for future metastasis, and the cadherin expression pattern might be indicative of the prognosis of a patient with RCC (1). RCCs primarily express cadherin-6 and N-cadherin rather than E-cadherin at the cell-cell contact site (2,3). Cadherin-6 functions as a metastasis- or invasion-suppressor molecule in RCC, whereas the role of N-cadherin is still unknown in the metastatic cascade of RCC (1,4).

We previously reported that N-cadherin acts as a cell-cell adhesion molecule when the function of cadherin-6 and E-cadherin is impaired in RCC cell lines (5). This phenomenon indicates that N-cadherin does not work as a metastasis suppressor but facilitates metastasis and/or invasion in aggressive RCCs, which frequently express abnormal patterns of cadherin-6 or E-cadherin. To evaluate the significance of N-cadherin expression in RCC, we clinically investigated the correlation between N-cadherin expression pattern and the survival of patients with RCC.

Materials and methods

Patients with RCC. Formalin-fixed paraffin-embedded sections of 46 patients with RCC who underwent nephrectomy at the Tsukuba University Hospital were studied after informed consent was obtained from each patient. The age of patients at the time of surgery ranged from 40 to 78 years (average, 59.2 years), and the male to female ratio was 32:14. The follow-up period ranged from 6 to 141 months with an average of 84.2 months. Each sample included a tumor and corresponding normal kidney specimen. Histopathological evaluation was performed on hematoxylin and eosin-stained sections, and tumors were divided into local and advanced groups according to the pathological TNM classification criteria of the UICC, 6th edition (6). Histological cell types were evaluated based on the WHO classification (7) with 41 samples of clear cell type, 4 papillary type, 1 spindle cell type and none of the chromophobe type.

Immunohistochemical staining. Formalin-fixed paraffin-embedded sections were autoclaved for antigen retrieval, and the primary antibody was applied. The antibodies used were N-cadherin (diluted 1/100, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for N-cadherin and HECD-1 for E-cadherin (diluted 1/100; Takara, Tokyo), and normal mouse serum was used as a control for primary antibodies. Immunohistochemistry was performed using a biotinylated anti-mouse antibody and streptavidin-biotin peroxidase complex (Vector Laboratories, Burlingame, CA, USA)

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Key words: N-cadherin, cell adhesion, metastasis, invasion

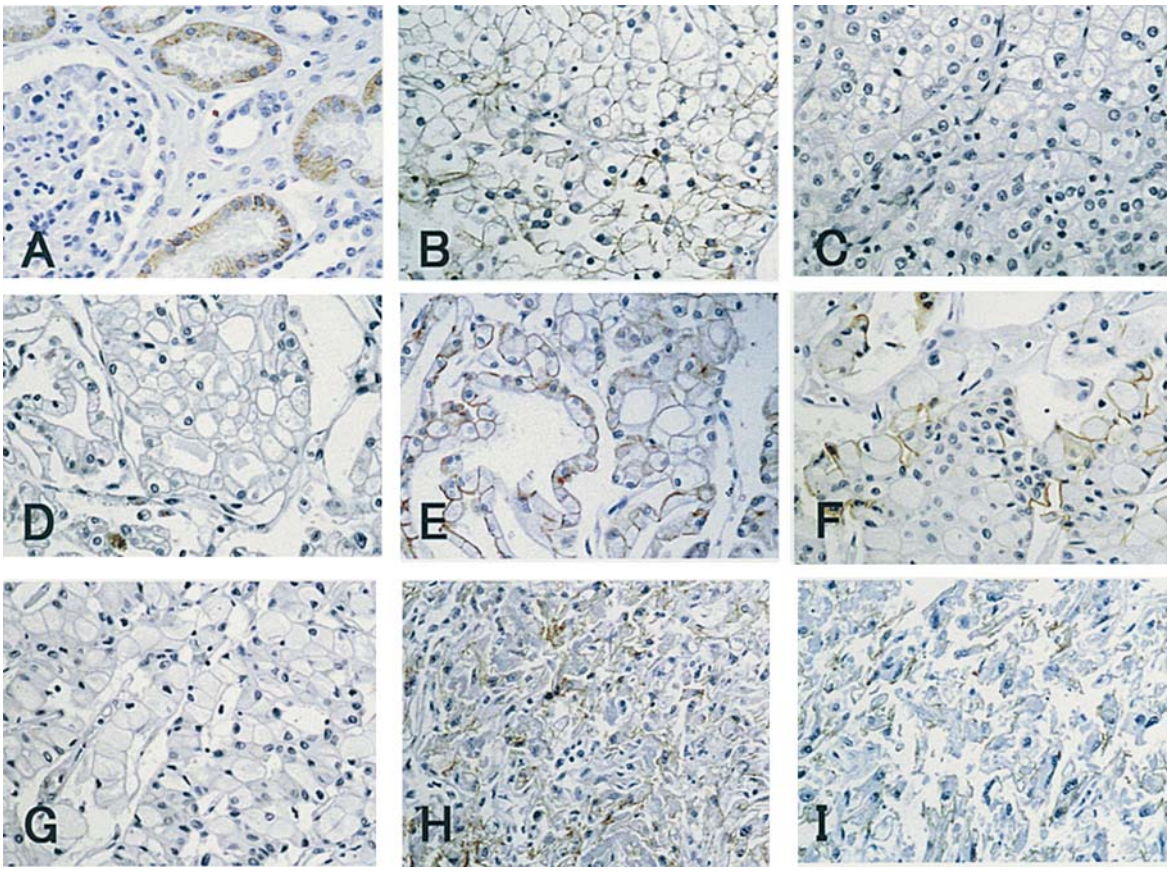

Figure 1. N-cadherin expression in a normal kidney is observed in renal proximal tubular epithelial cells, which are recognized by the presence of brush border cells, but not in distal tubular epithelial cells (A). In renal cell carcinoma (RCC), B and C, D and F, and G and H show different staining in the same specimen. N-cadherin staining is shown in B, D, F, and H, while E-cadherin staining is shown in C, E, G and I. Correlation between the expression patterns of N-cadherin and E-cadherin in RCC is shown as homogeneous (B) and absent (C), absent (D) and homogeneous (E), heterogeneous (F) and absent (G), and heterogeneous (H) and heterogeneous (I). In clear cell carcinoma (B and C, D and E, and F and G), E-cadherin expression is frequently absent, and various patterns of N-cadherin expression are observed, most likely according to tumor grade. The sarcomatoid tumor expresses both cadherins abnormally (H-I).

Table I. Correlation between the expression patterns of N-cadherin and E-cadherin in RCC.

N-cadherin		Abnormal		
	Normal	Heterogeneous	Absent	Total
E-cadherin				
Normal	2	0	2	4
Abnormal				
Heterogeneous	0	0	0	0
Absent	19	10	13	42
Total	21	10	15	46

Correlation between expression patterns of N- and E-cadherin (χ^2=0.03, p=0.99).

according to the manufacturer's instructions. The color reaction was developed with 3,3'-diaminobenzidine tetrahydrochloride/0.03% H_2O_2, counterstained with hematoxylin and mounted.

Evaluation of immunohistochemical staining. Tumor sections were scored by light microscopy without prior knowledge of the patient's profile. If the staining in cancer cells was exclusively at the cell-cell border, the antigen expression was scored as normal. If reactivity was absent, i.e. a complete absence of immunoreactivity, or heterogeneous, i.e. the tumor was composed of positive and negative areas, the antigen expression was scored as abnormal.

Statistical analyses. Correlations between the expression patterns of cadherin and pathological parameters were analyzed using the χ^2 test. Survival curves were constructed using the Kaplan-Meier method, and the difference between the curves was analyzed with a log-rank test.

Results

Cadherin expression in normal kidney. N-cadherin expression in the normal kidney is localized to the cell-cell contact and restricted to proximal tubules (Fig. 1A), and E-cadherin expression to distal tubules and collecting ducts.

Cadherin expression in RCC. Various patterns of N-cadherin expression were observed in 46 specimens of RCC (Fig. 1B-I).

Correlation between cadherin expression and cal factors in RCC.

	N-cadherin		E-cadherin	
	N	A	N	A
Local	9	14	4	19
Advanced	12	11	0	23
χ^2/p-value	0.789/0.55		4.38/0.11	
Grade 1	4	3	3	4
Grade 2	13	16	1	28
Grade 3	2	4	0	6
χ^2/p-value	0.746/0.13		10.9/0.51	

N, normal; A, abnormal; local, stage I and II; advanced, stage III and IV.

Normal expression of N-cadherin was seen in 21 of 46 RCC tissues, whereas only 4 of 46 RCCs expressed E-cadherin molecules in a normal pattern (Table I). In clear cell carcinoma, E-cadherin expression is frequently absent. In terms of histological cell type, no statistical correlation with cadherin expression was observed, since a majority of samples were clear cell carcinoma. No correlation between the expression patterns of N-cadherin and E-cadherin was observed in the 46 RCCs of this study (p=0.99, Table I). In addition, there was no significant correlation between the N-cadherin expression pattern and pathological parameters, i.e. stage and grade (p=0.55 and 0.13, respectively) (Table II).

Correlation between cadherin expression pattern and prognosis of RCC patients. Survival analysis showed that patients with E-cadherin-normal RCC seemed to have a better prognosis (Fig. 2A), but the difference in survival according to E-cadherin expression pattern was not statistically significant because the absence rate of E-cadherin in RCC was high (Table I). Patients with N-cadherin-normal RCC had a poorer prognosis than those with N-cadherin-abnormal RCC (p=0.048, Fig. 2B).

Table III. Expression of cadherin and function for cell-cell adhesion as assessed by spheroid blocking assay in RCC cell lines (5).

	Expression			Function		
SKRC	E	N	6	E	N	6
6	+	+(w)	-	+	-	-
35	-	+(w)	+	-	-	+
52	-	+	-	-	+(w)	-
59	+	+	+	+	-	+

E, E-cadherin; N, N-cadherin; 6, cadherin-6; +, w, and -, positive, weak, and absent expression or function, respectively.

Discussion

Most carcinomas derived from epithelial tissues express E-cadherin at the cell-cell contact site, and this molecule is important to the preservation of tissue integrity and polarity of the cells (8,9). Therefore, disruption of the E-cadherin-mediated cell-adhesion system is reasoned to be an initial phase in the metastasis and invasion cascade of cancer (10-12). However, in RCCs, E-cadherin is expressed at a relatively low rate (approximately 20% to 30%) because expression of E-cadherin is absent in renal proximal tubules, which is presumably the origin of most RCCs. We previously reported that cadherin-6 and N-cadherin were frequently expressed in RCC instead of E-cadherin (2). Cadherin-6 was expressed in 80% of RCCs, and its abnormal expression was correlated with a poor prognosis in patients with RCC (2,13,14). On the other hand, it is not yet clear whether N-cadherin plays a role as a cell-cell adhesion molecule or if its expression is a predictor of patient prognosis. Tani *et al* have reported that N-cadherin expression does not correlate with the tumor grade of RCC in surgically resected specimens (4). We reanalyzed our previous assay (5) in the four RCC cell lines of SKRC-6, -35, -52 and -59, and the cadherin expression pattern of each cell line is summarized in Table III. N-cadherin expression was not associated with the cellular morphology and characteristics of RCC cell lines, and functional analysis

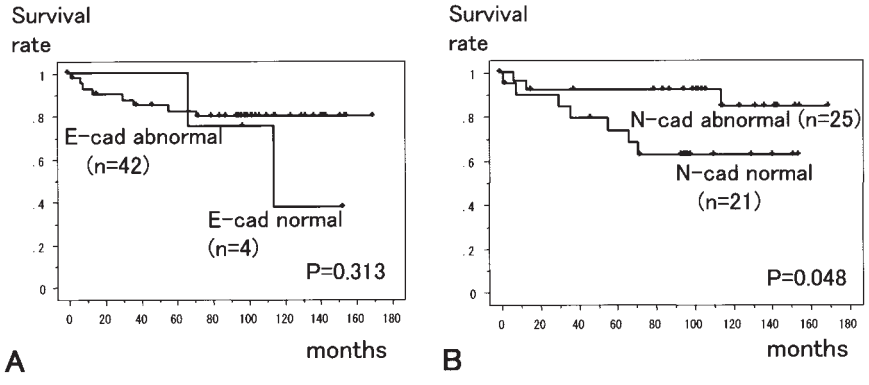

Figure 2. Survival curves demonstrate no significant difference in the survival of patients with RCC according to the E-cadherin expression pattern (p=0.31), as E-cadherin-normal RCC can be considered a minority (n=4) (A). RCC patients with normal N-cadherin expression have a poorer prognosis than those with abnormal N-cadherin expression (p=0.048) (B).

of cadherin molecules showed that N-cadherin seemed to function as a cell adhesion molecule in RCC when other cadherins, i.e. cadherin-6 and E-cadherin, were impaired (5). This indicates that the expression of N-cadherin might remain normal despite the elevation of tumor aggressiveness. Otherwise, N-cadherin expression may increase along with tumor aggressiveness in RCC, in contrast to the E-cadherin expression profile in most carcinomas.

In the present clinical analysis, N-cadherin expression did not correlate with tumor grade, stage or the E-cadherin expression pattern in RCC. In addition, normal N-cadherin expression was associated with poor prognosis in patients with RCC, suggesting that N-cadherin might facilitate the tumor progression of RCC in invasion and metastasis. It has been reported that N-cadherin is expressed instead of E-cadherin, the so-called 'cadherin switching,' in bladder tumor cell lines with a more aggressive phenotype (15). In a previous study, Rieger-Christ *et al* reported that the Akt signaling pathway is implicated in N-cadherin-mediated invasion in bladder tumor cell lines (16). Tomita *et al* also reported that a loss of E-cadherin expression accompanied N-cadherin up-regulation in prostate cancer (17).

In malignancies other than urological cancers, a correlation of N-cadherin expression with tissue invasion or tumor aggressiveness was observed in esophageal cancer (18) and brain gliomas (19). In esophageal cancer, high N-cadherin expression at the mRNA level in patients was correlated with poorer prognosis more than low N-cadherin expression, suggesting that N-cadherin expression may be associated with depth of invasion and poor prognosis (18). Asano *et al* reported that the expression of N-cadherin correlated with a dramatic decrease in invasive behavior of high-grade gliomas in an extracellular matrix invasion assay (19). This correlation may suggest that N-cadherin is a key player in the preservation of tissue integrity in brain tumors as tissues surrounding gliomas primarily expressed N-cadherin.

In conclusion, N-cadherin expression might be considered a promoter of invasion and a marker of poor prognosis among the complex set of cadherins (e.g. E-cadherin, cadherin-6, N-cadherin, and cadherin-11) that are expressed in RCC (1). To determine the correlation of N-cadherin with the invasive and metastatic potential of RCC, the expression level and pattern of cadherin-6 should also be taken into consideration. Unfortunately, an anti-cadherin-6 antibody is unavailable for use on formalin-fixed paraffin-embedded sections. Therefore, in the next stage, we plan to investigate the prospective clinical analysis of complex cadherin expression profiles and mechanisms using fresh frozen RCC specimens.

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