Expression of Heparanase in Renal Cell Carcinomas: Implications for Tumor Invasion and Prognosis

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Abstract

Purpose: Heparanase activity has been detected in many malignant tumors, showing a correlation with the metastatic potential. The present study was undertaken to investigate the expression of heparanase and its prognostic significance in renal cell carcinomas (RCC).

Experimental Design: Nineteen RCCs and 6 nonneoplastic renal tissues were analyzed for heparanase mRNA expression by real-time PCR. Heparanase protein expression was semiquantitatively investigated by immunohistochemistry in 70 RCCs. Involvement of heparanase in the invasiveness of RCC cell lines, 786-O and Caki-2 cells, was examined by down-regulating the gene expression with small interfering RNA (siRNA) using the Matrigel invasion assay.

Results: The expression level of heparanase mRNA was significantly higher in clear cell RCCs than in papillary RCCs, chromophobe RCCs, and nonneoplastic renal tissues. Heparanase was predominantly immunolocalized to cell surface and cytoplasm of clear cell RCCs and mean expression levels of heparanase were significantly higher in clear cell RCCs than in papillary and chromophobe RCCs. The protein expression levels were positively correlated with primary tumor stage, distant metastasis, and histologic grade. Targeting of heparanase mRNA expression in 786-O and Caki-2 cells with siRNA down-regulated the mRNA expression and inhibited the Matrigel invasion by these cells, whereas nonsilencing siRNA showed no effect. Multivariate Cox analysis revealed that elevated heparanase expression was a significant and an independent predictor of disease-specific survival (odds ratio, 8.814; $P = 0.019$).

Conclusions: These data suggest that heparanase plays an important role in invasion and metastasis and silencing of the gene might be a potential therapeutic target in clear cell RCCs.

Renal cell carcinoma (RCC) is the most common malignancy of the kidney (1), and 20% to 30% of the patients who undergo curative surgery will develop metastatic disease during follow-up (2). Tumor metastasis depends on the ability of cancer cells to invade tissue barriers composed of basement membrane and extracellular matrix (3). Matrix metalloproteinases (MMP) may play important roles in tumorigenesis and cancer cell progression because they digest the main components of basement membrane and extracellular matrix such as collagens, proteoglycans, laminin, fibronectin, and vitronectin (4). However, studies on MMP expression in RCCs have been limited and have reported variable findings. Kugler et al. (5) reported a strong correlation between increased MMP2, MMP9 expression, and tumor stage in RCCs. In contrast, Lein et al. (6) reported that only MMP9 was increased in RCCs, but it was not correlated with tumor type, grade, or stage.

Heparan sulfate proteoglycans, in which heparan sulfate chains are covalently attached, are the components of basement membrane (7). Heparanase is the enzyme that degrades heparan sulfate chains of heparan sulfate proteoglycans and its expression was observed in a variety of malignant tumors where the expression level correlated with malignant phenotype (8–11). However, to our knowledge, there was no report on the expression of heparanase in RCC. Here, we show that heparanase is predominantly expressed in clear cell RCCs but not in papillary and chromophobe RCCs, with positive association with primary tumor stage and distant metastasis. Heparanase expression is inversely correlated with disease-free and disease-specific survival. Furthermore, treatments of two RCC cell lines with small interfering RNA (siRNA) for heparanase effectively inhibited the invasion through Matrigel *in vitro*. Our results show that heparanase is highly expressed in clear cell RCCs and suggest that heparanase plays a role in invasion and metastasis of clear cell RCCs.

Materials and Methods

Patients. Seventy patients with histologically verified RCC (55 clear cell, 9 papillary, and 6 chromophobe RCC) were included. Small

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Translational Relevance

Renal cell carcinoma (RCC) is known to have high metastatic potential, but the underlying mechanism has not been fully elucidated yet. Heparanase activity, which degrades heparan sulfate chains of heparan sulfate proteoglycans, had been reported in many metastatic tumor cells, and the activity is ascribed to the heparanase gene. Heparanase inhibitors have been developed by several groups and it has been reported that inhibiting the heparanase activity leads to inhibition of tumor invasion, metastasis, and angiogenesis. Here, we show that heparanase is predominantly expressed in clear cell RCCs compared with non–clear cell RCCs, and its expression level was correlated with advanced primary tumor stage and distant metastasis. Heparanase expression is inversely correlated with disease-free survival and disease-specific survival. Furthermore, treatments of RCC cell lines with small interfering RNA for heparanase effectively inhibited the invasion through Matrigel *in vitro*. We believe that our results have great effect on the research on RCCs because inhibiting heparanase activity can lead to the development of effective therapy for RCCs.

pieces of tumor tissues (n = 19) and control nonneoplastic renal tissues remote from carcinoma (n = 6) were frozen in liquid nitrogen for reverse transcription-PCR (RT-PCR) analysis. None of the patients received chemotherapy or radiation therapy before surgery. Fifty-six patients were male and 14 patients were female. Their age ranged from 25 to 96 y (mean age 59 y). All patients were nephrectomized at our hospital between 1989 and 2005, and tumors were staged according to the 1997 tumor-node-metastasis staging system (12). Nuclear grading was based on the criteria by Fuhrman et al. (13). All patients were followed with clinical and radiologic examinations. Seventeen patients with clear cell RCC and no patient with papillary and chromophobe RCC had developed metastatic disease. Ten patients with clear cell RCC, a patient with papillary RCC, and no patient with chromophobe RCC had died of the disease. Fifty-nine patients were alive at the end of the follow-up. Patients alive had a median follow-up time of 47 mo (range, 2-74 mo). Informed consent for experimental use of the samples was obtained from the patients according to the hospital ethical guidelines.

Real-time quantitative PCR. Total RNA was extracted from RCC tissues, control nonneoplastic renal tissues, and human RCC cell lines (786-O and Caki-2 cells) using ISOGEN (Nippon Gene) and evaluated by Agilent 2100 Bioanalyzer (Agilent Technologies). The tissue samples, the 28s/18s rRNA ratios of which were larger than 1.0, were used. RNA (1 μg) was reverse transcribed with ReveTra Ace Reverse Transcriptase (TOYOBO Co.) in a 20-μL reaction volume containing Oligo (dT) 12-18 primers. The reaction mixture (1 μL) was then used as template in a TaqMan real-time quantitative PCR assay using ABI Prism 7000 Sequence Detection System (Applied Biosystems) according to the manufacturer's instructions. Cycling conditions were 50°C for 10 min, 95°C for 10 min, and then 40 cycles at 95°C for 15 s and 60°C for 1 min. The primers and TaqMan probes sets (TaqMan Gene Expression Assays, Inventoried) for heparanase (Hs00180737_m1), MMP2 (Hs00234422_m1), MMP7 (Hs01042795_m1), MMP9 (Hs00234579_m1), MMP14 (Hs00237119_m1), and human β-actin endogenous control (4310881E) were purchased from Applied Biosystems (sequences not disclosed). Heparanase/β-actin mRNA and MMPs/β-actin ratios were calculated for each sample to evaluate the relative mRNA expression.

Immunohistochemistry. One representative paraffin block from each case was selected by observing the sections stained with H&E, and paraffin sections (4-μm thick) placed on aminopropyltrimethoxysilane-coated slides were used for immunohistochemistry. After deparaffinization, endogenous peroxidase was quenched by incubation with 0.3% hydrogen peroxide in methanol for 15 min. Nonspecific binding was blocked with 10% normal goat serum. They were then reacted with rabbit polyclonal antiheparanase antibodies (1:200 dilution) in PBS (9, 10) for 60 min at room temperature. For a negative control, the primary antibody was replaced with nonimmune rabbit serum (1:200 dilution). After washing with PBS, the slides were incubated with anti-rabbit IgG conjugated to peroxidase-labeled dextran polymer (no dilution: EnVision + Rabbit; DAKO Japan) for 15 min, and color was developed with 3,3′-diaminobenzamine tetrahydrochloride in 50 mmol/L Tris-HCl (pH 7.5) containing 0.005% hydrogen peroxide. The sections were counterstained with hematoxylin.

Evaluation of immunostaining. To evaluate the staining of heparanase, positively stained carcinoma cells were counted at least 10 representative fields (×400 magnification), and staining was scored according to the method reported previously (14). Namely, a mean percentage of positive cancer cells was calculated and the staining intensity was stratified from 0 to 3 (0, no staining; 1, slight staining; 2, medium staining; 3, strong staining). Heparanase score was measured by applying the following formula: (mean percentage) × (intensity + 1; range, 0-400). The cases with heparanase score ≥200 were defined as high heparanase cases, whereas those with less than heparanase score 200 were defined as low heparanase cases.

siRNA. Human clear cell RCC cell lines, 786-O and Caki-2 cells, were purchased from the American Type Culture Collection and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin, and streptomycin (Sigma-Aldrich) in a well-humidified incubator with 20% O_2 and 5% CO_2 at 37°C. Two predesigned siRNAs for heparanase (i.e., HPSE5 and HPSE6) and nontargeting control siRNA (AllStars Negative Control siRNA) were obtained from Qiagen K.K. Cells (1.5×10^5 per well) were cultured in antibiotic-free medium overnight at 37°C in 5% CO_2 and then transfected with either HPSE5, HPSE6, or nontargeting control, using Lipofectamine 2000 (Invitrogen Co.) as described previously (15). After 24 h, siRNAs were removed by changing the culture medium with fresh RPMI 1640 containing 10% fetal bovine serum and cells were cultured for additional 48 h.

Matrigel invasion assay. Invasion of tumor cells was accessed by counting the number of cells that migrated through transwell inserts with a polyethylene terephthalate membrane (8-μm pore size) coated with a uniform layer of BD Matrigel Basement Membrane Matrix (BD Biosciences), according to the protocol recommended by the manufacturer. Briefly, tumor cells (1×10^4 in 100 μL RPMI 1640) were seeded

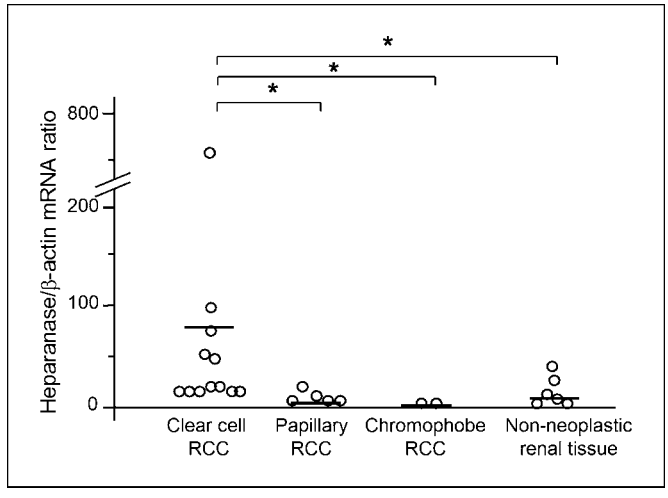

Fig. 1. Association between heparanase/β-actin mRNA ratio, histologic subtypes of RCCs, and nonneoplastic renal tissues. Bars, mean heparanase/β-actin mRNA ratio. *, $P < 0.05$.

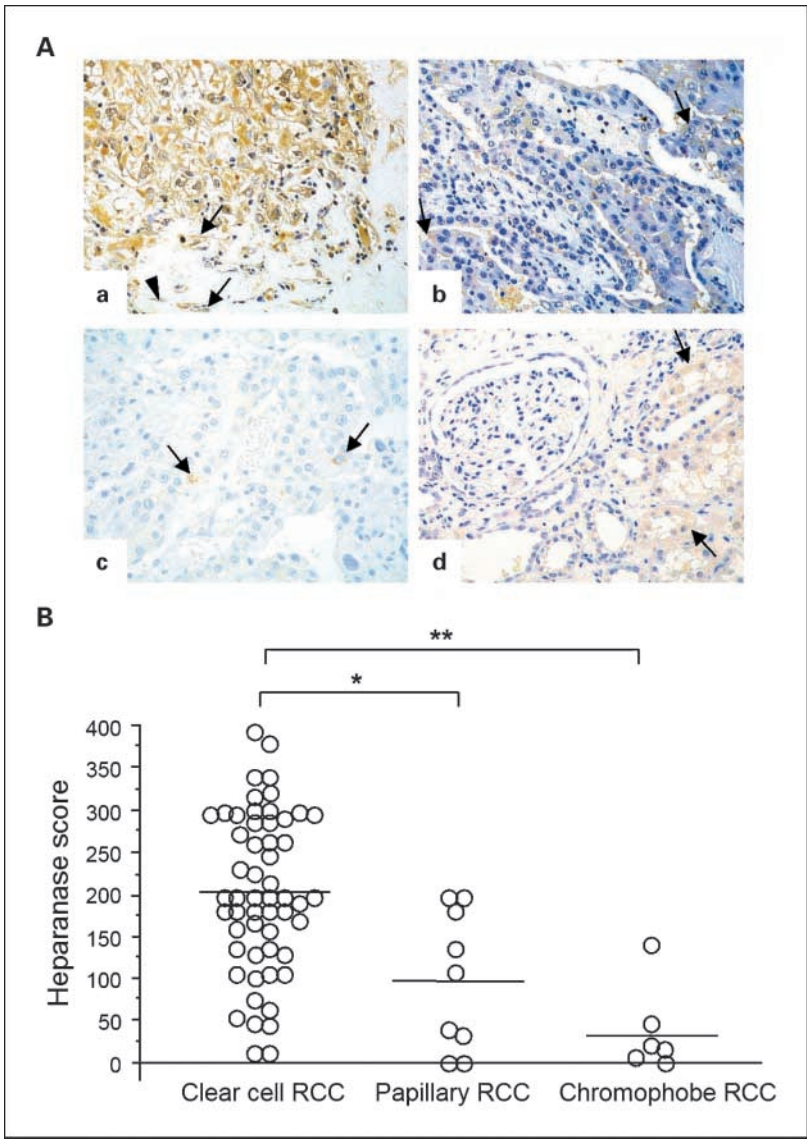

Fig. 2. *A,* immunostaining of heparanase in clear cell RCC (*a*), papillary RCC (*b*), chromophobe RCC (*c*), and nonneoplastic renal tissues (*d*). *a,* strong heparanase staining was observed in clear cell RCCs. Note that fibroblast (*arrowhead*) and endothelial cells (*arrows*) were also positive for heparanase. *b,* heparanase staining was weak and focal in papillary RCCs (*arrows*). *c,* weak perinuclear heparanase staining was observed in chromophobe RCCs (*arrows*). *d,* very weak cytoplasmic staining was observed in renal tubules (*arrows*). *B,* association between heparanase score and histologic subtypes of RCCs. Bars, mean heparanase score. *, $P = 0.0065$; **, $P = 0.0006$.

on top the transwell inserts, whereas the lower chamber was filled with 0.6 mL RPMI 1640 with 1% fetal bovine serum as chemoattractants. Cells were cultured for 24 h at 37°C in a well-humidified incubator before the nonmigrating cells in the inserts were scraped off; membranes were fixed and stained using Diff-Quik stain kit (Sysmex Co.). The cells that had migrated through the membranes were quantified by determination of the cell number in three randomly chosen visual fields at ×200 magnification.

Statistical analysis. Mann-Whitney's *U* test was used to analyze the relationship between heparanase score and clinicopathologic variables. The log-rank test and Kaplan-Meier method were used for survival analyses. Univariate and multivariate analyses were done according to Cox proportional hazard model. *P* values below 0.05 were considered to be significant. Statistical analyses were done using the statistical package software StatView for Windows (version 5.0; Abacus Concepts, Inc.).

Results

Expression of heparanase in RCCs and nonneoplastic renal tissues. Real-time quantitative PCR indicated that the mean expression levels of heparanase mRNA are significantly higher in clear cell RCC (87 ± 210; mean ± SD) than in papillary RCCs (3 ± 6), chromophobe RCCs (0.1 ± 0.1), and nonneoplastic renal tissues (6 ± 8; Fig. 1; $P < 0.05$). In all 55 clear cell RCCs, heparanase staining was observed on the cell surface and in cytoplasm of the carcinoma cells (Fig. 2A-a). Heparanase was also positive in the endothelial cells, fibroblasts, and inflammatory cells adjacent to the carcinoma cell nests (Fig. 2A-a). However, no staining was observed in stromal cells or inflammatory cells distant from carcinoma cell nests (data not shown). In papillary RCCs, only weak and focal heparanase staining was observed in the cytoplasm of cancer cells in 7 of 9 cases (Fig. 2A-b). A small number of carcinoma cells showed weak perinuclear staining in 5 of 6 chromophobe RCCs (Fig. 2A-c). No heparanase staining was obtained in the renal glomeruli, but weak and diffuse heparanase staining was observed in renal tubules (Fig. 2A-d).

Mean score of heparanase immunohistochemistry in clear cell RCCs (203 ± 95; mean ± SD) was significantly higher than that in papillary RCCs (98 ± 82; $P = 0.0065$) or chromophobe

Table 1. Relationship between heparanase score and clinicopathologic variables

	No. patients	Heparanase score (mean ± SD)
Gender		
Male	56	174 ± 108
Female	14	181 ± 94
P		0.692
Age (y)		
Median or less (≤60)	35	163 ± 110
Over median (>60)	35	188 ± 99
P		0.226
Pathologic tumor stage		
$pT_{1,2}$	50	154 ± 100
$pT_{3,4}$	20	228 ± 101
P		0.005
Lymph node metastasis		
Negative	64	172 ± 102
Positive	6	209 ± 137
P		0.522
Distant metastasis		
pM_0	61	166 ± 100
pM_1	9	239 ± 120
P		0.042
Histologic grade		
$G_{1,2}$	44	150 ± 90
$G_{3,4}$	26	218 ± 116
P		0.006
Venous invasion		
Negative	47	164 ± 97
Positive	23	198 ± 119
P		0.209

RCCs (38 ± 52; $P = 0.0006$; Fig. 2B). Association between heparanase score and clinicopathologic variables was summarized in Table 1. Heparanase score was positively correlated with primary tumor stage, distant metastasis, and histologic grade (Table 1).

***Effect of siRNA for heparanase on Matrigel invasion by RCC cell lines* in vitro.** The expression level of heparanase mRNA is 3-fold higher in 786-O cells than in Caki-2 cells (Fig. 3A). Transfection of two different siRNA for heparanase (HPSE5 and HPSE6) reduced the mRNA expression level of heparanase by 50% and 42% in 786-O cells ($P < 0.05$), whereas no different expression was observed by transfection with nonsilencing siRNA (NTC; Fig. 3A). Similarly, transfection of HPSE5 and HPSE6 to Caki-2 cells reduced the level by 51% and 77% (Fig. 3A; $P < 0.05$). On the other hand, siRNA for heparanase did not down-regulate the mRNA expression of MMP2, MMP7, MMP9, and MMP14 in 786-O and Caki-2 cells (data not shown). Immunoblot analysis, however, showed negligible bands probably because of relatively lower expression level of heparanase in these cell lines compared with cells transfected with heparanase cDNA, in which an immunoreactive band was observed (data not shown; refs. 10, 16).

Quantitative analysis of cell invasion showed that 786-O cells are more invasive than Caki-2 cells (20.3 ± 3.1 versus 6.7 ± 1.5; Fig. 3B and C). 786-O cells transfected with HPSE5 or HPSE6 exhibited decreased invasion activity (7.3 ± 2.5 or 5.3 ± 1.5) compared with those transfected without siRNA or with NTC (20.3 ± 3.1 or 20.3 ± 2.3; $P < 0.05$). Similarly, transfection of Caki-2 cells with HPSE5 or HPSE6 decreased invasion activity (1.0 ± 1.0 or 0.7 ± 0.6) compared with cells without siRNA or cells transfected with NTC (6.7 ± 1.5 or 5.0 ± 1.0; $P < 0.05$).

Heparanase and survival of the patients. Patients with low heparanase tumor had significantly longer disease-free survival than those with high heparanase tumor ($P < 0.0005$; Fig. 4A). Pathologic tumor stage, distant metastasis, histologic grade, venous invasion, and heparanase score were the prognostic factors for the disease-free survival in univariate analysis (Table 2A). By multivariate analysis, pathologic tumor stage ($P = 0.0020$), histologic grade ($P = 0.0185$), and heparanase score ($P = 0.0012$) were the independent prognostic factors for the disease-free survival (Table 2A).

Patients with low heparanase tumor had significantly longer disease-specific survival than those with high heparanase tumor ($P < 0.001$; Fig. 4B). Pathologic tumor stage, distant metastasis, histologic grade, venous invasion, and heparanase score were the prognostic factors for the disease-specific survival in univariate analysis (Table 2B). By multivariate analysis, pathologic tumor stage ($P = 0.0165$) and heparanase score ($P = 0.0185$) were the independent prognostic factors for the disease-free survival.

Discussion

In the present study, we have provided the first evidence that heparanase is immunolocalized predominantly to carcinoma cells in the tumor masses of clear cell RCCs with a correlation with pT, pM, and histologic grade, suggesting that overexpression of heparanase is associated with tumor aggressiveness of clear cell RCCs. Furthermore, elevated heparanase expression was identified as an independent worse prognostic factor for both disease-free survival and disease-specific survival in RCCs. These findings are in agreement with enhanced metastatic spread and reduced

postoperative survival noted for several other human cancers overexpressing heparanase (8, 9).

Patients with clear cell RCCs have a poorer prognosis compared with those with papillary or chromophobe RCCs, and this difference in outcome is observed even after stratifying by tumor stage and nuclear grade (17). Because heparanase was predominantly expressed in clear cell RCCs compared with papillary and chromophobe RCCs and the immunohistochemical scores correlated with the pathologic variables, heparanase may be associated with the malignant potential of clear cell RCCs. Furthermore, in clear cell RCC, strong heparanase staining was observed on the cell surface as well as in the cytoplasm; however, in papillary and chromophobe RCCs, only weak staining was observed in the cytoplasm of cancer cells. Because the predominant function of heparanase is commonly attributed to degradation of heparan sulfate chains on the cell surface and in the extracellular matrix (18), heparanase may function well in clear cell RCC compared with papillary and chromophobe RCC.

It was reported that inhibition of MMP14 expression in a RCC cell line, WT8, by siRNA resulted in suppression of tumor cell invasion through transwell membrane coated with type 1 collagen (19). However, the degradation of only type 1 collagen is not sufficient to invade through the basement membrane and extracellular matrix. Therefore, we used membranes coated with reconstituted basement membrane (Matrigel) in the present study and showed that inhibition of heparanase expression of RCC cell lines by siRNA may be a beneficial method to prevent cancer cell invasion through Matrigel. MMP2, MMP7, MMP9, and MMP14 were expressed in RCCs (5, 6, 19, 20), and it is well-known that these MMPs cleave major components of the basement membrane. However, we think that the reduced invasiveness of the cell lines through Matrigel may be due to the down-regulated expression of heparanase by siRNA, because siRNA for heparanase did not down-regulate these MMPs.

Heparanase is a potential target for development of anti-metastatic drugs, and several heparanase inhibitors have been prepared (21, 22). PI-88 is a representative of heparanase

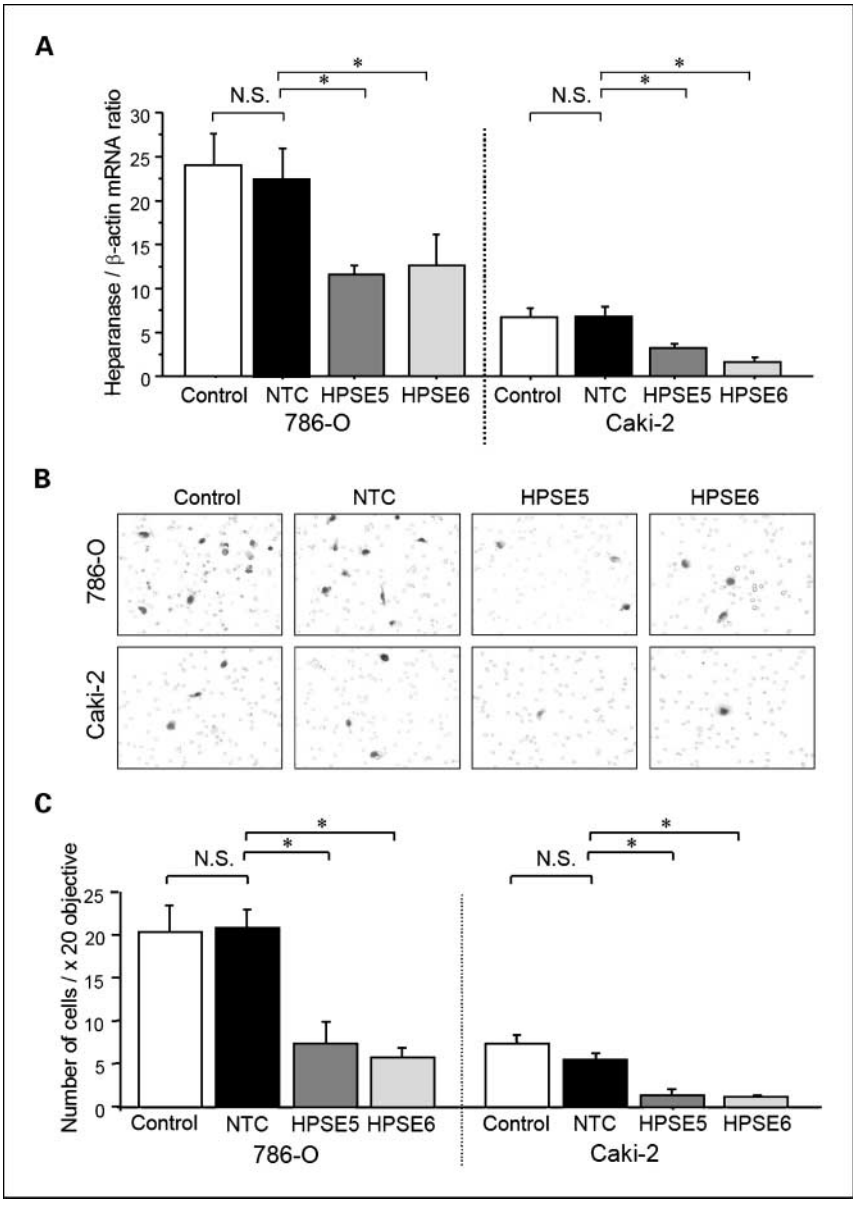

Fig. 3. *A,* expression of heparanase mRNA in 786-O and Caki-2 cells with or without siRNA targeting heparanase. siRNA targeting heparanase (HPSE5, HPSE6) down-regulates heparanase mRNA expression of 786-O and Caki-2 cells compared with that of cells with nontargeting siRNA and cells without siRNA. Columns, mean; bars, SD. *B,* the cells invaded through the BD Matrigel Basement Membrane Matrix were stained with Diff-Quik. *C,* the number of cells invaded through the BD Matrigel Basement Membrane Matrix was quantified as described in Materials and Methods. Columns, mean; bars, SD. *, $P < 0.05$; N.S., not significant; Control, cells without siRNA; NTC, nontargeting siRNA; HPSE5 and HPSE6, siRNA targeting heparanase.

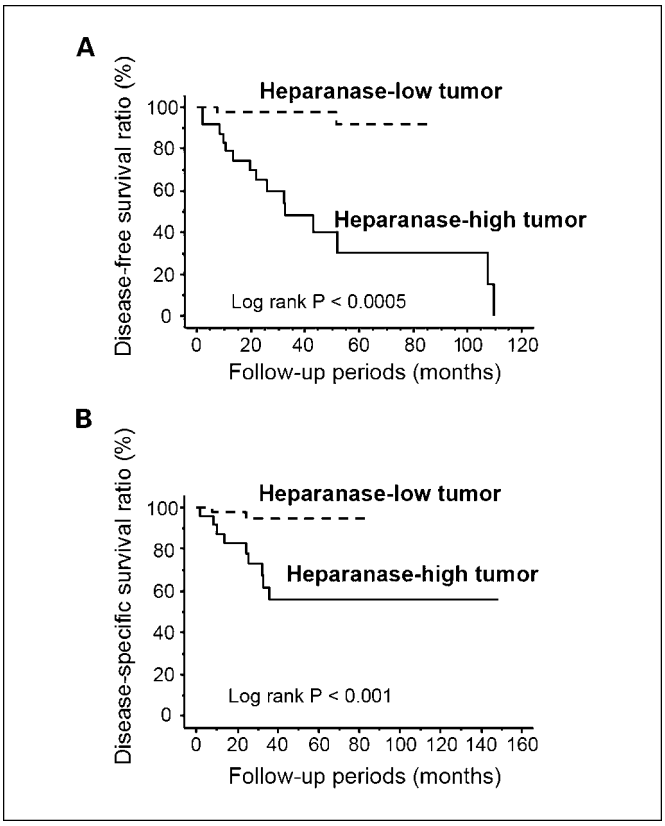

Fig. 4. Kaplan-Meier curves of disease-free survival (*A*) and disease-specific survival (*B*) according to heparanase expression in 70 patients with RCC.

inhibitors and is being evaluated in a phase II clinical trial (23). However, due to the toxicity consisted of thrombocytopenia and pulmonary embolism, clinical trials using heparanase inhibitors require further investigations (24). Therefore, a blockade of the gene expression of heparanase by siRNA may be a promising alternative strategy. In the present study, we have provided the first evidence that siRNA-mediated inhibition of heparanase expression in clear cell RCC cell lines results in decreased invasive properties. This is a strictly *in vitro* observation because 786-O cells do not metastasize in nude mice. However, we think that down-regulation of heparanase by siRNA is a potential remedy for clear cell RCCs, because elevated heparanase expression was a significant and independent predictor of disease-free and disease-specific survivals of patients with RCCs.

In summary, we have shown the predominant heparanase expression in clear cell RCC compared with papillary and chromophobe RCC. Expression of heparanase was found to be associated with the malignant potential of RCC, and heparanase expression was inversely correlated with the prognosis. Furthermore, we have provided the data that down-regulating of the heparanase expression can efficiently inhibit the invasion of RCC cell lines through Matrigel *in vitro*. It is likely that the inhibition of heparanase probably depresses the degradation of heparan sulfate proteoglycans in the basement membrane, leading to inhibition of the invasion of these cell lines. Accordingly, targeting the inhibition of heparanase activity may be a beneficial antitumor therapy for RCCs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Table 2. Cox regression analysis for progression-free survival and disease-specific survival

Risk factors	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
A. Progression-free survival				
Pathologic tumor stage				
$pT_{3,4}$	8.585 (2.877-25.622)	0.0001	12.450 (2.524-61.413)	0.0020
Distant metastasis				
Positive	4.929 (1.174-14.175)	0.0031	0.842 (0.224-3.174)	0.7998
Histologic grade				
$G_{3,4}$	25.910 (3.404-197.205)	0.0017	13.355 (1.544-115.538)	0.0185
Venous invasion				
Positive	5.081 (1.728-14.941)	0.0031	1.135 (0.298-4.322)	0.8529
Heparanase score				
High	16.478 (3.696-73.467)	0.0002	19.846 (3.247-121.290)	0.0012
B. Disease-specific survival				
Pathologic tumor stage				
$pT_{3,4}$	8.773 (2.314-33.266)	0.0014	7.742 (1.454-41.230)	0.0165
Distant metastasis				
Positive	4.187 (1.220-14.363)	0.0228	0.248 (0.045-1.380)	0.1115
Histologic grade				
$G_{3,4}$	17.818 (2.280-139.271)	0.0060	6.791 (0.690-66.853)	0.1006
Venous invasion				
Positive	10.743 (2.315-49.844)	0.0024	4.340 (0.743-25.345)	0.1030
Heparanase				
High	9.096 (1.964-42.124)	0.0048	8.814 (1.440-53.925)	0.0185

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.

References

1. Eble JN, Sauter G, Epstein JI, Sesterhenn IA, editors. World Health Organization classification of tumors. Pathology and genetics of tumors of the urinary system and male genital organs. Lyon: IARC Press; 2005.
2. Zisman A, Pantuck AJ, Wieder J, et al. Risk group assessment and clinical outcome algorithm to predict the natural history of patients with surgically resected renal cell carcinoma. J Clin Oncol 2002;20:4559–66.
3. Engbring JA, Kleinman HK. The basement membrane matrix in malignancy. J Pathol 2003;200:465–70.
4. Chambers AF, Matrisian LM. Changing views of the role of matrix metalloproteinases in metastasis. J Natl Cancer Inst 1997;89:1260–70.
5. Kugler A, Hemmerlein B, Thelen P, et al. Expression of metalloproteinase 2 and 9 and their inhibitors in renal cell carcinoma. J Urol 1998;160:1914–8.
6. Lein M, Jung K, Laube C, et al. Matrix-metalloproteinases and their inhibitors in plasma and tumor tissue of patients with renal cell carcinoma. Int J Cancer 2000;85:801–4.
7. Timar J, Lapis K, Dudas J, et al. Proteoglycans and tumor progression: Janus-faced molecules with contradictory functions in cancer. Semin Cancer Biol 2002;12:173–86.
8. El-Assal ON, Yamanoi A, Ono T, et al. The clinicopathological significance of heparanase and basic fibroblast growth factor expressions in hepatocellular carcinoma. Clin Cancer Res 2001;7:1299–305.
9. Gohji K, Okamoto M, Kitazawa S, et al. Heparanase protein and gene expression in bladder cancer. J Urol 2001;166:1286–90.
10. Mikami S, Ohashi K, Usui Y, et al. Loss of syndecan-1 and increased expression of heparanase in invasive esophageal carcinomas. Jpn J Cancer Res 2001;92:1062–73.
11. Vlodavsky I, Friedmann Y, Elkin M, et al. Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. Nat Med 1999;5:793–802.
12. (UICC) IUAC. TNM classification of malignant tumours. In: Sobin L, Wittekind C, editors. New York: Wiley-Liss; 1997.
13. Fuhrman SA, Lasky LC, Limas C. Prognostic significance of morphologic parameters in renal cell carcinoma. Am J Surg Pathol 1982;6:655–63.
14. Mikami S, Oya M, Mizuno R, et al. Expression of Ets-1 in human clear cell renal cell carcinomas: implications for angiogenesis. Cancer Sci 2006;97:875–82.
15. Wang Z, Banerjee S, Li Y, et al. Down-regulation of notch-1 inhibits invasion by inactivation of nuclear factor-κB, vascular endothelial growth factor, and matrix metalloproteinase-9 in pancreatic cancer cells. Cancer Res 2006;66:2778–84.
16. Uno F, Fujiwara T, Takata Y, et al. Antisense-mediated suppression of human heparanase gene expression inhibits pleural dissemination of human cancer cells. Cancer Res 2001;61:7855–60.
17. Cheville JC, Lohse CM, Zincke H, et al. Comparisons of outcome and prognostic features among histologic subtypes of renal cell carcinoma. Am J Surg Pathol 2003;27:612–24.
18. Vreys V, David G. Mammalian heparanase: what is the message? J Cell Mol Med 2007;11:427–52.
19. Petrella BL, Brinckerhoff CE. Tumor cell invasion of von Hippel Lindau renal cell carcinoma cells is mediated by membrane type-1 matrix metalloproteinase. Mol Cancer 2006;5:66.
20. Miyata Y, Iwata T, Ohba K, et al. Expression of matrix metalloproteinase-7 on cancer cells and tissue endothelial cells in renal cell carcinoma: prognostic implications and clinical significance for invasion and metastasis. Clin Cancer Res 2006;12:6998–7003.
21. Vlodavsky I, Friedmann Y. Molecular properties and involvement of heparanase in cancer metastasis and angiogenesis. J Clin Invest 2001;108:341–7.
22. Sasisekharan R, Shriver Z, Venkataraman G, et al. Roles of heparan-sulphate glycosaminoglycans in cancer. Nat Rev Cancer 2002;2:521–8.
23. Parish CR, Freeman C, Brown KJ, et al. Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel *in vitro* assays for angiogenesis and heparanase activity. Cancer Res 1999;59:3433–41.
24. Basche M, Gustafson DL, Holden SN, et al. A phase I biological and pharmacologic study of the heparanase inhibitor PI-88 in patients with advanced solid tumors. Clin Cancer Res 2006;12:5471–80.