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Original contribution

High density of tryptase-positive mast cells in patients with renal cell carcinoma on hemodialysis: correlation with expression of stem cell factor and protease activated receptor-2☆

Shin-ichi Watanabe MD[a], Yasuyoshi Miyata MD, PhD[a,*], Tomohiro Matsuo MD[a], Yasushi Mochizuki MD, PhD[a], Masaharu Nishikido MD, PhD[b], Tomayoshi Hayashi MD, PhD[c], Hideki Sakai MD, PhD[a]

[a]*Department of Nephro-Urology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8591, Japan*
[b]*Department of Hemodialysis and Apheresis, Nagasaki University Hospital, Nagasaki 852-8591, Japan*
[c]*Department of Pathology, Nagasaki University Hospital, Nagasaki 852-8591, Japan*

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Summary Patients on hemodialysis are at higher risk of renal cell carcinoma probably because of inflammatory and immune system disorders. The aim of this study was to clarify the pathologic roles of 2 phenotypes of mast cells, mast cell tryptase and mast cell chymase, and their correlation with stem cell factor and protease-activated receptor 2 in patients with renal cell carcinoma on hemodialysis. The densities of mast cell tryptase and mast cell chymase and expressions of stem cell factor and protease-activated receptor 2 were examined in 35 patients with hemodialysis-renal cell carcinoma and 39 with non–hemodialysis-renal cell carcinoma who were diagnosed and treated in our hospital. Protein expression was examined by immunohistochemistry. The proliferation index represented the number of Ki-67–positive cells. There were no significant differences in clinicopathologic features between the 2 groups. Mast cell tryptase densities in intratumoral (8.3 per high-power field) and peritumoral areas (8.7 per high-power field) were higher in hemodialysis-renal cell carcinoma than non–hemodialysis-renal cell carcinoma (2.7 and 5.3 per high-power field). No such significant correlations were detected in mast cell chymase. In hemodialysis-renal cell carcinoma, intratumoral mast cell tryptase density correlated with the proliferation index (P = .039 and P = .008, respectively) and also with stem cell factor and protease-activated receptor 2 expression. Our results emphasize the important roles of mast cell tryptase in cancer cell proliferation and recurrence in hemodialysis-renal cell carcinoma. Stem cell factor and protease-activated receptor 2 seem to up-regulate mast cell tryptase functions in these patients. The results suggest collaborative effects of stem cell factor, mast cell tryptase, and protease-activated receptor 2 on the malignant potential of hemodialysis-renal cell carcinoma.
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* Corresponding author.
E-mail address: int.doc.miya@m3.dion.ne.jp (Y. Miyata).

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1. Introduction

Chronic inflammation is associated with carcinogenesis and tumor growth, although its role within the tumor microenvironment is not fully understood. Various inflammatory cells, such as macrophages and lymphocytes, are thought to regulate inflammation and the malignant process [1]. Mast cells (MCs) are key components of the immune system and crucial regulators of inflammation and immune response by releasing various bioactive substances [2,3]. Furthermore, MCs are thought to play some role in the carcinogenesis because their accumulation has been recognized in various cancers [1,4,5]. With regard to the pathologic significance of MCs, they are regarded as a “double-edged sword” in cancer-malignant aggressiveness because they can produce both cytokines with antitumoral activity as well as those with protumoral activity [5,6]. In fact, there are 2 conflicting opinions on the prognostic roles of MCs in patients with cancer [4,5,7,8]. One of the reasons for such difference is probably related to the effects of the local microenvironment (eg, cytokines and growth factors) on the differentiation, proliferation, and pathologic roles of MCs [9]. In general, MCs are classified into 2 phenotypes based on their neutral protease composition: MC tryptase (MC-t) (tryptase positive, chymase negative) and MC chymase (MC-tc) (tryptase and chymase positive) [10,11]. Several studies have investigated the clinical significance of MC-t and/or MC-tc in human cancer tissues [12,13]. The results of these studies indicate that the distribution and pathologic roles of MCs in human cancer tissue varies with cancer type [13]. In renal cell carcinoma (RCC), it seems that MCs plays important role in malignant aggressiveness [14,15]. However, there is little or no information on the clinical and pathologic significance of MC-t and MC-tc in RCC.

Patients on hemodialysis (HD) are reported to be at higher risk of malignancies, in particular, they are at approximately 100 times greater risk for RCC than age-matched general population [16]. The reason for the high risk is probably related to the exposure of the kidneys of patients with end-stage renal disease (ESRD) and HD to various protumoral factors, such as suppression of immune response and stimulation of inflammation [17]. However, there is little information on how these factors promote renal carcinogenesis and the malignant potential of RCC in HD patients (HD-RCC). Lacking also is the comparison of the pathologic features of cancer cells and the tumor microenvironment between HD-RCC and normal renal function (non–HD-RCC). With regard to MCs in RCC, MCs are present in renal biopsy tissues from patients with various forms of chronic glomerulonephritides and nephropathy, and their densities are higher than those in controls [17-19]. The recruited MCs probably play important roles in the pathogenesis and progression of chronic glomerulonephritides [18,20]. Based on these facts, MCs are presumed to play some roles in HD-RCC because the biologic activities of MCs are up-regulated in renal dysfunction.

MC development is regulated by various factors. Specifically, stem cell factor (SCF) is associated with cell proliferation, migration, and secretion of MCs under various pathologic conditions [21,22]. SCF expression is up-regulated in kidney tissues of chronic nephritis [18,19], and its serum levels in patients with ESRD are higher than those in healthy control [23]. Based on this background, we hypothesized that SCF expression is up-regulated in HD-RCC tissues and that it is involved in tumorigenesis.

Protease-activated receptors (PARs) constitute a unique branch of G protein-coupled receptor super family. PAR-2 was originally reported as a trypsin receptor; however, it is also known to be activated by other serine proteases including tryptase produced by MCs [24,25]. One of the main biologic roles of PAR-2 signaling of MC-t is the regulation of cell proliferation under physiologic and pathologic conditions including cancers [7,26]. Thus, PAR-2 is speculated to be involved in human cancers. In fact, PAR-2 immunoreactivities are higher in various cancer tissues than normal ones [7]. On the other hand, PAR-2 expression correlates with serum creatinine levels in patients with nephropathy and is mainly localized within proximal tubular cells in immunoglobin A nephropathy [19,27]. Thus, it is possible that PAR-2 expression in proximal tubular cells is modulated in the presence of renal diseases and that its overexpression could be associated with the malignant potential of RCC.

The main aim of the present study was to clarify the clinical and pathologic roles of MC-t and MC-tc in patients with HD-RCC. In addition, we estimated the densities of these phenotypes of MCs within and around the tumoral area in the same tissues. Based on the results, we also examined the relationship between SCF expression and the density of MC-t and between PAR-2 expression and cancer cell proliferation in HD-RCC tissues. Our results should be useful to our understanding of the pathologic characteristics of HD-RCC.

2. Materials and methods

2.1. Patients and tumor samples

The study subjects were patients with HD-RCC who underwent nephrectomy at our hospital between 1992 and 2009. Patients with HD-RCC with more than pT3 and/or metastasis were excluded because of the small number of such patients (n = 3). Thus, the study specimens (n = 35) were obtained from 26 patients with conventional RCC (74.3%), 6 with papillary RCC (17.2%), and 3 with chromophobe RCC (8.6%). Pathology-matched non–HD-RCC specimens were selected at random from patients with RCC with normal serum creatinine level (<1.1 mg/dL). We excluded from the control those patients with pT3 and pT4 tumors, metastasis, and elderly patients (>75 years) to match clinicopathologic features. Their clinicopathologic features are shown in Table 1. The study also included normal control specimens,

Table 1 Patients' characteristics

	HD (n = 35)	Non-HD (n = 39)	*P*
Age at operation, y	54 (48-61)	67 (52-73)	.053
Male (%)/female	25/10 (71.4)	23/16 (59.0)	.263
pT stage			.213
T1a	25 (71.4)	22 (56.4)	
T1b	7 (20.0)	15 (38.5)	
T2	3 (8.6)	2 (5.1)	
Grade			.299
G1	15 (42.9)	20 (51.2)	
G2	13 (37.1)	16 (41.0)	
G3/4	7 (20.0)	3 (7.7)	

representing 23 kidney tissues free of hydronephrosis that were obtained by surgery from patients with ureter tumors. All patients were evaluated by chest x-ray, ultrasonography, and computed tomography of the abdomen. Tumors were staged according to the 2004 TNM classification, and the grade was determined using the criteria of Fuhrman et al. [28]. The study protocol met the ethical standard of the human ethics review committee of Nagasaki University Hospital.

2.2. Immunohistochemistry and terminal deoxynucleotidyl transferase-mediated nick end labeling

We used antibody for MC-t (NeoMarkers, Fremont, CA), MC chymase (NeoMarkers), SCF (Immuno-Biological Laboratories Co, Gunma, Japan), PAR-2 (Santa Cruz Biotechnology, Santa Cruz, CA), Ki-67 (Dako, Carpinteria, CA), cleaved caspase-3 (R&D Systems, Inc, Abingdon, UK), and CD68 (Novocastra Laboratories, Newcastle, UK). Five-micrometer-thick sections were deparaffinized in xylene and rehydrated in ethanol. Antigen retrieval was performed, and then the sections were immersed in 3% hydrogen peroxide for 30 minutes. Sections were incubated overnight with the primary antibody at 4°C and then were washed in 0.05% Tween-20 in phosphate-buffered saline. The sections were then incubated with peroxidase using the labeled polymer method with Dako EnVision + Peroxidase (Dako) for 60 minutes. The peroxidase reaction was visualized with the liquid 3,3-diaminobenzidine tetrahydrochloride substrate kit (Zymed Laboratories, San Francisco, CA). Sections were counterstained with hematoxylin. Positive controls were tonsil for Ki-67, MC, and macrophage, liver cancer for SCF, and colon cancer for PAR-2. A consecutive section from each sample processed without the primary antibody was used as a negative control. In situ labeling for detection of apoptotic cells was performed as described previously [29]. We used the Apop Tag In Situ Apoptosis Detection Kit (Intergen Company, Purchase, NY) based on terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL).

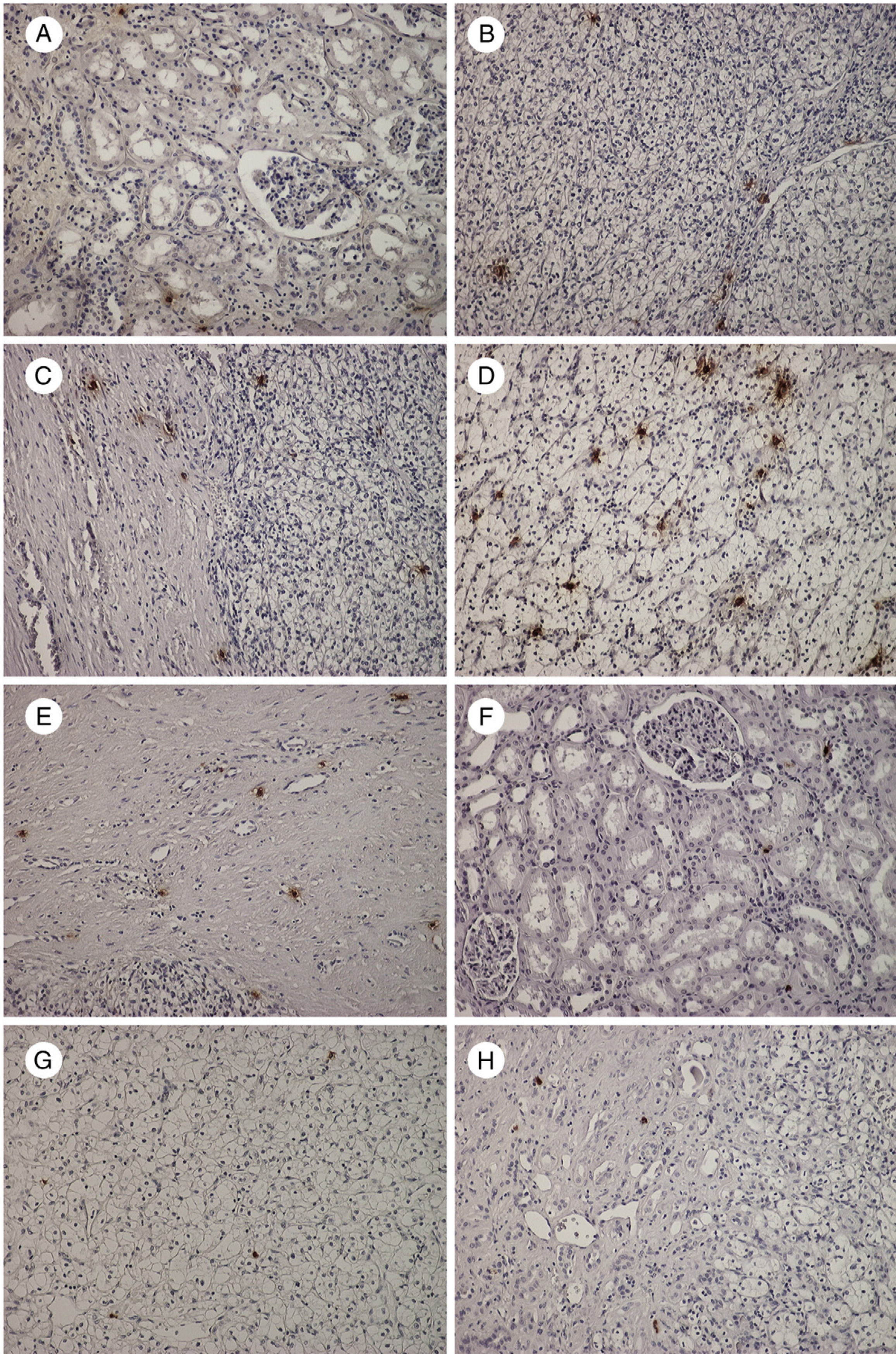
2.3. Evaluation

The number of MCs was counted in each case in 5 of intratumoral and peritumoral areas at high-power magnification of ×200, and the mean count of positive cells per high-power field (HPF) was calculated. Evaluation of expression of all molecules was assessed semiquantitatively, taking into account the percentage of positively stained cancer cells (at least 500 cells). In this study, SCF expression was considered positive if staining intensity was strong or moderate. The percentage of positively stained cancer cells was determined using a continuous scale. PAR-2 expression was quantified by the immunoreactive score (IRS) system, where IRS = staining intensity × percentage of positive cells. Staining intensity was determined as follows: 0, negative; 1, weak; 2, moderate; and 3, strong. The percentage of positive cells was defined as follows: 0, negative; 1, 1% to 20%; 2, 21% to 50%; and 3, 51% to 100% positive cells. In addition, for statistical analysis, patients were divided into 2 groups based on the IRS, that is, negative and positive; those with IRS above the median level were considered the positive group. Similarly, patients were divided according to age at operation, taking the median age as the cutoff value. To evaluate the apoptotic cells, we used 2 parameters: the proportions of cleaved caspase-3–positive and TUNEL-positive cells [29]. All specimens were examined using a Nikon E-400 microscope, and digital images were captured (model DU100, Nikon, Tokyo, Japan). In addition, we used a computer-aided image analysis system (Win ROOF, version 5.0; MITANI, Fukui, Japan) to calculate the statistical variables. Slides were blindly evaluated twice at different times by 2 investigators (YM and SW) who were blinded to the clinical and pathologic data.

2.4. Statistical analysis

All data were expressed as median and interquartile range (IQR) based on the skewed distribution. The Mann-Whitney *U* test was used for comparisons of continuous variables. The χ^2 and Fisher exact tests were used for categorical

Fig. 1 A to C, Representative examples of MC-t in non–HD-RCC. MC-t in the normal kidney (A), intratumoral area (B), and peritumoral area (C). D to E Representative examples of MC-t in HD-RCC. MC-t in the intratumoral (D) and peritumoral areas (E). MC-t density in the intratumoral area of HD-RCC (D) is higher than that of non–HD-RCC (B). F to J, Representative examples of MC-tc. Normal kidney (F), intratumoral area of non–HD-RCC (G), peritumoral area of non–HD-RCC (H), intratumoral area of HD-RCC (I), and peritumoral area of HD-RCC (J). K to M, Representative examples of PAR-2 expression. Normal kidney tissue (K), tumor area of non–HD-RCC (L), and tumor area of HD-RCC (M). N to P, Representative examples of SCF. Normal kidney (N), non–HD-RCC (O), and HD-RCC (P) (original magnification ×200).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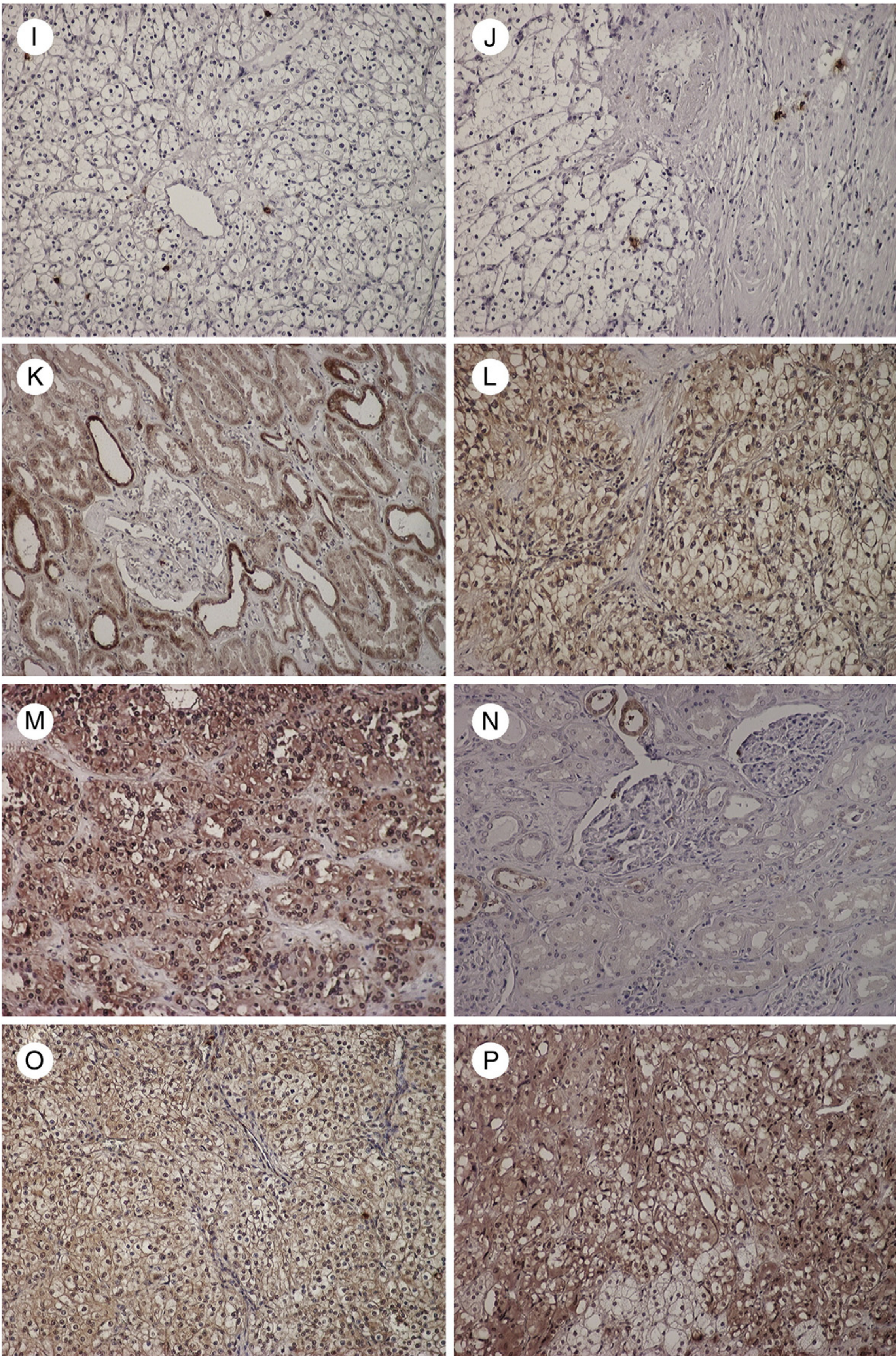

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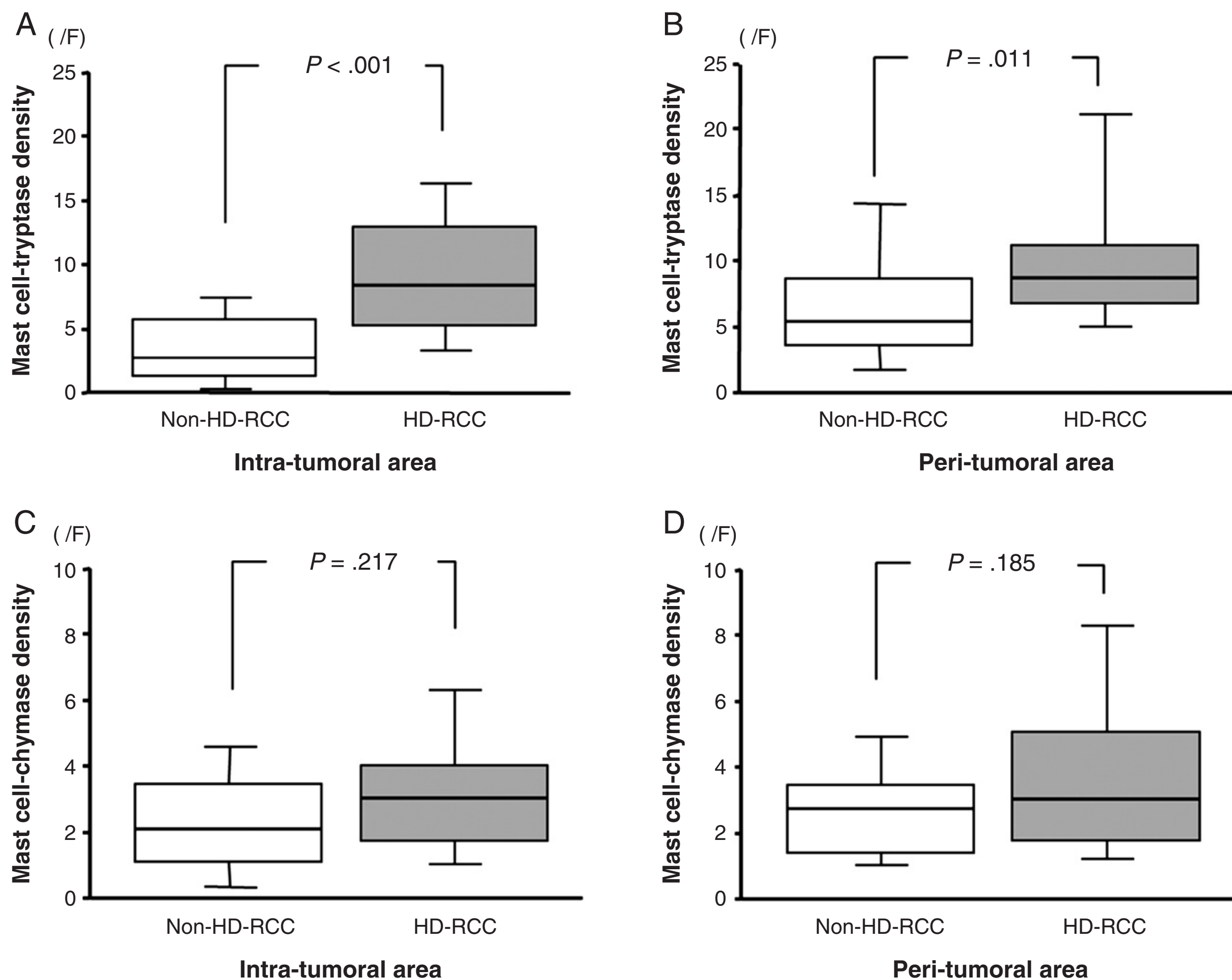

Fig. 2 Box-and-whisker plots of MC-t density (A and B) and MC-chymase density (C and D) in intratumoral and peritumoral areas of non–HD-RCC and HD-RCC. In these plots, lines within the boxes represent median values; the upper and lower lines of the boxes, the 25th and 75th percentiles, respectively; and the upper and lower bars outside the boxes, the 90th and 10th percentiles, respectively.

comparison of the data. The Scheffé test was used for multiple comparisons of the data. Spearman correlation coefficient was used to determine the association between 2 continuous variables. The disease-free survival time was compared with Kaplan-Meier survival curve and log-rank test. All statistical analyses were 2 sided, and significance was defined as $P < .050$. All statistical analyses were performed on a personal computer with the statistical package StatView for Windows (version 5.0; Abacus Concept, Inc, Berkeley, CA).

3. Results

3.1. Densities of MC-t and MC-tc and expression levels of SCF and PAR-2

Figs. 1A to E shows representative examples of MC-t in normal kidney, non–HD RCC, and HD-RCC. The density of MC-t in the intratumoral area of HD-RCC (median, 8.3; IQR, 5.3-12.9 per HPF) was significantly higher than in the non–HD-RCC (2.7, 1.3-5.7 per HPF, $P < .001$, Fig. 2A). Likewise, the MC-t density in the peritumoral area was significantly higher in the HD-RCC (8.7, 6.8-11.2 per HPF) than in the non–HD-RCC (5.3, 3.5-8.6 per HPF, $P = .011$, Fig. 2B). In addition, MC-t density in each area was significantly higher than in the normal kidney (1.3, 0.4-2.8).

Figs. 1F, G, H, I, and J show representative examples of MC-tc in the normal kidney, intratumoral area of non–HD-RCC, peritumoral area of non–HD-RCC, intratumoral area of HD-RCC, and peritumoral area of HD-RCC, respectively. In contrast to MC-t density, there were no significant differences in MC-tc density between non–HD-RCC and HD-RCC in intratumoral ($P = .217$, Fig. 2C) and peritumoral areas ($P = .185$, Fig. 2D). On the other hand, there was no specific or characteristic distribution pattern for MC-tc based on the area of the tumor or histopathologic type. Finally, the MC-t densities in both intratumoral and peritumoral areas were significantly

Table 2 Relationship between MC densities and pathologic features in HD-RCC

	No. of patients	MC-t density (IQR)			
		Intratumoral	*P*	Peritumoral	*P*
pT stage					
T1a	25	9.1(5.1-12.8)	] 0.757	8.0(6.6-10.8)	] 0.575
T1b	7	10.3(5.7-16.0)	] 0.472	9.7(7.0-20.8)	] 0.750
T2	3	7.5(4.9- 7.9)		10.7(8.2-11.1)	
Grade					
G1	15	8.3(3.5-12.4)	] 0.378	9.7(7.6-10.7)	] 0.587
G2	13	9.7(6.7-16.3)	] 0.771	9.0(6.2-17.9)	] 0.712
G3/4	7	8.0(5.9-12.3)		7.3(6.4-10.6)	
ACDK					
Without	10	11.0(5.2-16.3)	] 0.228	8.9(7.6-10.7)	] 0.812
With	25	7.7(5.1-11.7)		8.3(6.6-11.3)	

HD-RCC: renal cell carcinoma with hemodialysis, ACDK: acquired cystic kidney disease.

higher than MC-tc densities in the corresponding areas in both non–HD-RCC and HD-RCC (Fig. 2).

Figs. 1K, L, and M show representative examples of PAR-2 expression in normal kidney tissue, non–HD-RCC, and HD-RCC, respectively. PAR-2 was detected in parts of the normal tubular cells, and some tubules showed strong expression. However, PAR-2 was only weakly expressed in almost all tubules, and no strong expression was detected in normal cells. On the other hand, in cancer cells, moderate to strong expression was often found. Finally, in non–HD-RCC, 13 (33.3%) of 39 specimens were judged positive for PAR-2 expression. On the other hand, the proportion of PAR-2–positive cancer cells was 68.6% in HD-RCC. The proportion of PAR-2–stained cells was significantly higher in HD-RCC than in non–HD-RCC (P = .003).

Figs. 1N, O, and P provide representative examples of SCF in normal kidney, non–HD-RCC, and HD-RCC, respectively. SCF was mainly detected in the cell cytoplasm and part of the cell membrane. The proportion of SCF-positive cells was significantly higher in HD-RCC cells (62.9%) than non–HD-RCC (33.3%, P = .035). However, moderate or strong expression was relatively rare in normal renal tubular cells.

3.2. Clinical and pathologic significance of MC-t and PAR-2 expression

Table 2 summarizes the relationships between pathologic features and MC-t density in intratumoral and peritumoral areas in HD-RCC. There was no significant relationship among these factors and pT stage or grade. In addition, MC-t density in intratumoral and peritumoral areas did not correlate with sex (P = .273 or .841) or age (P = .463 or

Table 3 Relationship between PAR-2 and SCF and pathologic features in HD-RCC

	PAR-2 expression			SCF expression		
	Negative	Positive	*P*	Negative	Positive	*P*
pT stage			.983			.937
T1a	8 (72.7)	17 (70.8)		9 (69.2)	16 (72.7)	
T1b	2 (18.2)	5 (20.8)		3 (23.2)	4 (18.2)	
T2	1 (9.1)	2 (8.3)		1 (9.2)	2 (9.1)	
Grade			.203			.905
G1	7 (63.6)	8 (33.3)		5 (38.5)	10 (45.5)	
G2	2 (18.2)	11 (45.8)		5 (38.5)	8 (36.4)	
G3/4	2 (18.2)	5 (20.8)		3 (23.1)	4 (18.2)	
ACDK			.447			.055
Without	2 (18.2)	8 (33.3)		1 (7.7)	9 (40.9)	
With	9 (81.7)	16 (66.7)		12 (92.7)	13 (59.1)	

Table 4 PI in patients with HD

	PI (%)	P
Intratumoral MC-t		
Low	5.5 (4.6-9.3)	.039
High	10.4 (6.8-15.7)	
Peritumoral MC-t		
Low	9.3 (5.7-10.4)	.476
High	9.5 (5.3-15.3)	
PAR-2 expression		
Negative	5.5 (4.4-7.3)	.008
Positive	11.2 (6.9-16.0)	
SCF expression		
Negative	9.3 (5.4-11.9)	.573
Positive	8.9 (5.0-15.5)	

Data were shown as median (IQR).

.689). The duration of HD (median, 141; IQR, 74-213 months) did not correlate with MC-t in intratumoral (r = 0.04, P = .815) and peritumoral areas (r = 0.23, P = .201). The density of MC-t did not correlate with acquired cystic kidney disease (ACDK) (Table 2) or with pathologic type. Furthermore, the expression of both PAR-2 and SCF in HD-RCC cells did not correlate with various pathologic parameters (Table 3). On the other hand, the presence of ACDK tended to correlate with SCF expression, albeit insignificantly (P = .055, Table 3).

We also investigated the pathologic role of the above factors and proliferation index (PI) in HD-RCC (Table 4). PI correlated with MC-t density in the intratumoral area (P = .039) and PAR-2 expression (P = .008) but not with MC-t in the peritumoral area or SCF expression (Table 4). In addition, for the intratumoral area, PI was significantly higher in specimens showing PAR-2–positive and high–MC-t density (median, 12.9; IQR, 9.6-18.0 per HPF) than in those with PAR-2–positive and low–MC-t density or PAR-2–negative and high–MC-t density (6.8, 4.5-9.8, P = .011) and those with PAR-2 negative and low MC-t (5.0, 3.8-5.4, P = .037). To clarify in more detail the activities of MC-t in intratumoral area and PAR-2 expression, we investigated the relationship among these parameters and apoptosis. With regard to MC-t in intratumoral area, the proportion of TUNEL-positive cells in specimens with high–MC-t density (1.4, 1.1-2.4) was significantly (P = .014) lower than in those with low–MC-t density (2.3, 1.9-3.1). In addition, a similar trend was found in cleaved caspase-3–positive cancer cells (P = .181). On the other hand, there was no significant relationship between PAR-2 expression and TUNEL-positive (P = .776) or cleaved caspase-3–positive cells (P = .790).

Recurrence occurred in 4 patients with HD-RCC and 1 patient with non–HD-RCC. The disease-free survival was worse in HD-RCC (log-rank P = .034). In addition, among the 4 patients with HD-RCC who developed recurrence, the MC-t density in intratumoral area was high in 3 patients.

3.3. Relationship among MC-t density and expression of PAR and SCF in HD-RCC

Based on the above results, we further investigated the relationships among MC-t density in intratumoral area and the expression of SCF and PAR-2 in cancer cells in HD-RCC. MC-t density in SCF-positive specimens (median, 5.0; IQR, 10.0-15.0 per HPF) was significantly higher (P = .039) than in SCF-negative ones (7, 3.6-8.7 per HPF). In addition to SCF, MC-t density correlated positively with the density of macrophages in intratumoral area (r = 0.462, P = .005). On the other hand, SCF expression also correlated with PAR-2 expression (P = .356). Furthermore, the IRS of PAR-2 correlated with MC-t density (r = 0.378, P = .025).

4. Discussion

One of the unique results of our study is that MC-t density in HD-RCC was significantly higher than in non–HD-RCC, but no such difference was found in MC-tc. In addition, in HD-RCC, the MC-t densities in both intratumoral and peritumoral areas were significantly higher than MC-tc densities in similar areas. Based on these results, we speculate the dominance of MC-t relative to MC-tc and that MC-t plays a more important role in HD-RCC. Our findings add support to those of previous studies that showed a significantly higher MC-t density than that of MC-tc in non–small cell lung [13] and gastric cancers [30]. In this regard, a previous study using in situ staining reported the presence of tryptase and only low or undetectable amounts of chymase in human renal MCs derived from human renal tumors tissues [31]. These studies add support to the notion that MC-t may have a more significant contribution to the malignant potential compared with MC-tc under the pathologic conditions in RCC. In fact, our results showed that MC-t was associated with poor outcome in HD-RCC.

MCs have 2 paradoxical actions (procancer and anticancer) within the tumor microenvironment [5,6]. In the present study, the intratumoral density of MC-t correlated with cancer cell proliferation in HD-RCC. The involvement of tryptase secreted from MCs in cell proliferation has been reported also in various cancers such as colon and pancreas cancers [7,8]. On the other hand, our results showed no relation between MC-t and MC-tc and any of the pathologic parameters examined in patients with HD-RCC, in agreement with the results of previous reports on patients with RCC [14,15]. Although further studies are needed to examine the clinical significance of MCs in these patients, it should be noted that all patients of this study underwent ultrasonography and/or computed tomography of the kidney at 1 to 3 times every year. Thus, 25 (71.4%) of 35 patients with HD-RCC were diagnosed with pT1a. Tuna et al [14] analyzed the number of MC in 71 RCC patients and reported a pT1a rate of 23.9%. In addition, Mohseni et al [15] also

conducted a similar study in 40 patients, including those with 4 (10%) pT1a tumors. Thus, in our study population, tumors were detected in early stage, and there is a possibility that MC-t density was not associated with the pathologic features because of such high frequency of low-stage cancer. Interestingly, in tissues of 3 patients with HD-RCC who developed local invasion and/or metastasis, the MC-t density in the intratumoral area was markedly high (17.2, 20.0-21.0 per HPF). In addition, our study population was relatively small because it was performed in a single hospital.

Contrary to HD-RCC, a significant relation between MC-t and cancer cell proliferation was not found in non–HD-RCC. Our result also showed a significantly higher PI in HD-RCC than in non–HD-RCC (data not shown). This result suggests that the pathologic role of MC-t in HD-RCC is different from that in non–HD-RCC. To further clarify the role of MC-t in HD-RCC, we investigated PAR-2 expression in HD-RCC tissues because the biologic activities of PAR-2 are mediated by tryptase from MCs, and PAR-2 is known to be involved in the initiation of cell proliferation in various cancers [7,25,26,32]. Although PAR gene expression was previously reported in kidney cancer cell line (A-498) by reverse transcriptase–polymerase chain reaction [33], there is no information on PAR-2 protein expression in human RCC tissues. In our study, PAR-2–positive cells were rarely found in normal renal tubules, adding support to the results of a previous report [27]. On the other hand, PAR-2 expression was detected in approximately one third of the non–HD-RCC tissues. Thus, we speculate that PAR-2 expression might be up-regulated by the carcinogenic process of RCC. In addition, our results also showed the expression of PAR-2 in approximately two thirds of HD-RCC tissues and that it was significantly higher than in non–HD-RCC. Although the in vivo physiologic and pathologic roles of PAR-2 remain poorly understood, previous studies reported that PAR-2 expression correlated with serum creatinine levels and tubulointerstitial fibrosis in various types of nephropathies [20,27]. Furthermore, PAR-2 is mainly expressed within the proximal tubular cells in specimens of nephropathy [27]. Considered together, the above results suggest that PAR-2 expression is up-regulated by progression of nephropathy. On the other hand, 1 study reported that MCs-released tryptase stimulated PAR-2 expression in colon cancer cells in a paracrine manner [7]. Our results also showed that MC-t density correlated significantly with PAR-2 expression in HD-RCC. Thus, it is possible that trypsin released from MC-t stimulates PAR-2 expression in HD-RCC in a paracrine manner similar to colon cancer. On the other hand, our results showed that MC-t density, but not PAR-2 expression, correlated negatively with apoptosis in HD-RCC. Although we did not explore the main reason for such difference, it seems that the apoptotic function of MC-t in HD-RCC is modulated by PAR-2–independent pathway.

Another key factor investigated in the present study is SCF. This factor is essential in almost all biologic functions of MCs including cell differentiation, proliferation, survival, recruitment, and secretion under various pathologic conditions including malignancies [18,22]. Our results showed a higher SCF expression in HD-RCC tissues compared with non–HD-RCC. This result may explain the finding of significantly higher MC-t density in HD-RCC relative to non–HD-RCC. What is the mechanism underlying SCF up-regulation in HD-RCC compared with non–HD-RCC? Although the exact mechanism remains obscure, we speculate that 2 different mechanisms may affect the expression of SCF in HD-RCC tumors. First, the progression of nephropathy and worsening of renal dysfunction correlates with SCF function [19,27]. For example, SCF expression was found to correlate with serum creatinine level and renal tubular fibrosis. Furthermore, serum SCF levels were reported to be 5-fold higher in patients with ESRD than in healthy controls [23]. Second, cancer cells and/or stromal cells may enhance SCF expression by changing the tumor microenvironment. This notion is based on 2 facts: cultured RCC cells (A-498, BFTC-909, CAKI-1, CAKI-2, and CAL-54 cell lines) did not secrete SCF when incubated in standard media [34] and hypoxia in the local tumor microenvironment and hypoxia-inducible factor can directly promote SCF expression in breast cancer cells [35].

The biologic functions and pathologic roles of MC vary with changes in the microenvironment by inflammatory-associated factors including cytokines and growth factors [3]. Likewise, the kidney of patients with HD is also exposed to these inflammatory-associated factors. Our study showed a significantly higher density of macrophages in HD-RCC relative to that in non–HD-RCC (data not shown). Thus, the pathogenesis and malignant aggressiveness of RCC in patients on HD seem to be regulated by complex mechanisms. The pathologic significance and function of MCs in HD-RCC tumors is also considered to be regulated by a panel of different and complex mechanisms. Further studies, including cell culture experiments, are needed to determine the clinical significance and define the regulatory mechanism(s) that control MCs in patients with RCC with HD.

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