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Immunohistochemical expression of CD44, matrix metalloproteinase2 and matrix metalloproteinase9 in renal cell carcinomas

Yong-Moon Lee, M.D.[a,1], Jin Man Kim, M.D., Ph.D.[b,1], Hyo Jin Lee, M.D., Ph.D.[c], In-Ock Seong, M.S.[b], Kyung-Hee Kim, M.D., Ph.D.[b,*]

[a] *Department of Pathology, Dankook University, School of Medicine, Cheonan, South Korea*
[b] *Department of Pathology/Medical science, Chungnam National University School of Medicine, Daejeon, South Korea*
[c] *Division of Hematology/Oncology, Department of Internal Medicine, Chungnam National University School of Medicine, Daejeon, South Korea*

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Abstract

Purpose: The aim of our study was to investigate the clinicopathologic values of the expression of CD44, matrix metalloproteinase (MMP)2, and MMP9 in renal cell carcinoma (RCC).

Patients and methods: A total of 107 clear cell RCCs (ccRCCs) and 32 nonclear cell RCCs (non-ccRCCs) were examined for CD44, MMP2, and MMP9 expression by immunohistochemistry. The membrane and cytoplasmic expression levels of the 3 proteins were scored by semiquantitative methods, and the correlations of the 3 proteins with clinicopathological parameters were verified.

Results: The expression levels of CD44, MMP2, and MMP9 were positively correlated with nuclear grade (grade 1−2 vs. grade 3-4) ($P = 0.003$, $P < 0.001$ and $P < 0.001$, respectively) in the ccRCCs, while in the non-ccRCCs, only CD44 expression was correlated with higher nuclear grade (grade 1−3 vs. grade 4) ($P = 0.001$). Furthermore, CD44 expression in ccRCCs and non-ccRCCs was correlated with shorter overall survival in the univariate analyses ($P < 0.001$ and $P = 0.015$, respectively). In the multivariate analysis, which accounted for age, sex, nuclear grade, and pathologic stage, CD44 expression was an independent predictor of shorter overall survival only in ccRCCs. Correlations among the 3 proteins were all positive in ccRCCs, but in non-ccRCCs, only MMP2 and MMP9 were positively correlated.

Conclusion: CD44 expression may play an important role in the progression of both ccRCC and non-ccRCC. CD44 expression in ccRCC may be associated with elevated MMP2 and MMP9 expression levels, which is in contrast to non-ccRCC. The different correlations between CD44, MMP2, and MMP9 in ccRCC and non-ccRCC can be useful in understanding the mechanisms of carcinogenesis and stratifying patients for therapeutic purposes.

Keywords: Renal cell carcinoma; Clear cell renal cell carcinoma; CD44; Matrix Metalloproteinase2; Matrix Metalloproteinase9

1. Introduction

Renal cell carcinoma (RCC) accounts for 2%−3% of all human malignancies and is the second leading cause of death in urologic malignant neoplasms [1]. Unfortunately, approximately 25%−30% of patients present with metastatic disease at the time of diagnosis, eventually dying from it [2]. Thanks to a better understanding of the interactions between tumor initiating cells (also called cancer stem cells [CSCs]) and tumor microenvironment cells (TMEs), technical developments recently applied in the molecular and genomic era have resulted in dramatic achievements; some of these developments include programmed cell death ligand 1 inhibitor, chimeric antigen receptor T cell therapy, and chimeric antigen receptor natural killer cell therapy, which help manage systemic and metastatic diseases previously

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*Corresponding author. Tel: +8-242-580-8238; fax: +8-242-280-7189.
E-mail address: phone330@cnu.ac.kr (K.-H. Kim).

[1]These authors contributed equally to this paper.

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thought to be incurable [3]. Tumor metastasis involves extensive interactions between the invading cancer cells and TMEs. Such interactions promote degradation of the extracellular matrix (ECM) by specialized proteolytic enzymes, which are produced by cancer cells and TMEs and are likely to affect both primary and metastatic sites. Among these enzymes, urokinase and a variety of matrix metalloproteinases (MMPs), a family of zinc and calcium-dependent proteolytic enzymes, digest various components of ECM, including collagen, laminin, fibronectin, vitronectin, elastin, and proteoglycans. The MMP family consists of at least 23 different members [4] and are classified into collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs, and other MMPs according to their catalytic activity [5]. MMPs play important roles in invasion, migration, metastasis, and tumorigenesis [6,7]. Among the many MMPs, the Type IV collagenases MMP2 (72-kDa gelatinase A) and MMP9 (92-kDa gelatinase B) cleave diverse targets (type IV collagen, gelatin, cytokines, growth factors, chemokines, and cytokine/growth factor receptors) and are associated with cell growth, migration, invasion, inflammation, and angiogenesis [8–10]. Type IV collagen is a major structural protein within the ECM and basement membrane. Many experimental and clinical studies have reported a significant association between tumor aggression and increased levels of MMP2 and MMP9 [9–14].

Gelatinase can interact with CD44, a cell surface integral membrane protein [8]. In studying and treating metastatic disease, one of the promising theories is the CSC concept. CSCs are thought to be responsible for recurrence or distant metastasis as well as for chemo- and radioresistance in many malignancies [15]. As one of the CSC markers, CD44 is the most frequently reported marker in RCC; many studies on this subject demonstrated that CD44 corresponds with a poor prognostic value [16–20]. CD44 is a transmembrane glycoprotein with a number of alternative splice variants and undergoes extensive post-translational modifications [21,22]. The standard form of CD44, the major hyaluronan (HA) receptor, mediates cell-cell and cell-matrix interactions through its affinity for HA [23]. CD44-HA has been known to affect tumor progression, as CSCs are generally capable of self-renewal as well as mediate cancer progression and metastasis [15,24]. Based on these data, we designed a study to investigate the roles of stem cell-like features with MMP activities as prognostic markers in clear cell RCCs (ccRCC) and nonclear cell RCCs (non-ccRCC), data of which are little known.

The aim of this study was to examine the clinicopathological values of the expression of CD44, MMP2, and MMP9 with patient outcomes in ccRCC and non-ccRCC in order to elucidate and compare possible cellular mechanisms relating to aggressive nature regarding tumor cell invasion processes in both cancer types.

2. Patients and methods

2.1. Patients and tissue samples

This study was approved by the Institutional Review Board of Chungnam National University Hospital (CNUH 2018-03-005). Because the immunohistochemical study used formalin-fixed paraffin-embedded tissue and was retrospective in nature, the prerequisite for informed consent was waived. All clinical data were obtained from the National Biobank of Korea at Chungnam National University Hospital.

CD44, MMP2, and MMP9 expression levels were analyzed from 139 patients who underwent surgical resection of RCC at Chungnam National University Hospital in Daejeon, South Korea between 1999 and 2014. The tumor, node, and metastasis (TNM) staging and nuclear histologic grading for RCC were determined at the time of surgical resection and were based on the eighth Edition of the American Joint Committee on Cancer staging system [25]. None of the 139 patients showed distant metastasis. In one case, there was a regional lymph node metastasis in primary tumor category 3 and, in 138 cases, there was no regional lymph node metastasis. RCC recurrence or metastasis was determined via imaging and/or histological findings. Disease-free survival (DFS) was determined as the time interval between the date of initial surgical resection and date of RCC recurrence or metastasis. Overall survival (OS) was defined from the time of initial surgical resection to date of death due to any cause. Without death or recurrence or metastasis confirmation, OS or DFS time was censored at the last known date that the patient was alive. This retrospective cohort consisted of 107 patients with primary ccRCC and 32 with primary non-ccRCC. Of the 32 non-ccRCC cases, 20 were papillary RCCs, 4 were chromophobe RCCs and 8 were sarcomatoid RCCs. None of the patients received preoperative chemotherapy. The 2 most representative viable tumor areas were selected and marked on hematoxylin and eosin-stained slides. Tissue microarrays were created by punching two tissue columns (2.0 mm in diameter) from the original paraffin blocks for each case and inserting the columns into new recipient paraffin blocks.

2.2. Immunohistochemical staining analysis

Immunohistochemical staining of the tissue sections from the tissue microarray paraffin blocks was performed with DISCOVERY UltraMap-HRP detection and ChromoMap DAB detection using a Ventana automated immunostainer Discovery XT (Ventana Medical Systems Inc. Tucson, AZ). A primary mouse monoclonal antibody to human CD44 (product # 3570, diluted 1:50; cell signaling technology, Danvers, MA), a primary rabbit polyclonal antibody to human MMP2 (product # ab37150, diluted 1:100; Abcam, Cambridge, UK) and a primary rabbit

polyclonal antibody to human MMP9 (product # ab38898, diluted 1:100; Abcam, Cambridge, UK) were used. These antibodies were incubated with the sections at 31˚C for 32 minutes.

The modified Allred et al. method was used to evaluate both the intensity of immunohistochemical staining and the proportion of RCC cells with cytoplasmic or membrane staining in each case [26]. The proportion scores ranged from 0 to 5 (0, no staining; 1, up to 1/100; 2, 1/100 to 1/10; 3, 1/10 to 1/3; 4, 1/3 to 2/3; 5, >2/3 to 1), and the intensity scores ranged from 0 to 3 (0, negative; 1, weak; 2, moderate; 3, strong). To generate the total immunohistochemical score, the intensity and proportional scores were multiplied for each specimen (range, 0−15) [27]. The results were examined separately and scored by YML and JMK, who were blinded to patients' clinicopathological details. Discrepancies in the scores were discussed to obtain a consensus.

2.3. Statistical analyses

The relationships between the expression of CD44, MMP2, and MMP9 and the clinicopathological parameters were evaluated using the Mann-Whitney *U* test. The strengths of the association between CD44 and both MMP2 and MMP9 expression were assessed via Spearman's coefficient of rank correlation. OS and DFS were determined using the Cox proportional hazards model for univariate and multivariate survival analyses. Statistical significance was set at $P < 0.05$ (SPSS v.24; SPSS Inc., Chicago, IL).

3. Results

3.1. Correlation of clinicopathological features with the expression patterns of CD44, MMP2, and MMP9 in ccRCC and non-ccRCC

A total of 139 cases (107 ccRCC cases and 32 non-ccRCC cases) were evaluated immunohistochemically for CD44, MMP2, and MMP9 expression in tumor cells (Fig. 1). Correlations between CD44, MMP2 and/or MMP3 expression and gender, age, histologic nuclear grade, T (tumor) stage, nodal status, and pTNM staging are summarized in Tables 1 and 2 [25].

In the 107 ccRCC cases, the expression levels of CD44, MMP2, and MMP9 were all positively correlated with higher histologic nuclear grade (grade 1−2 vs. grade 3-4; $P = 0.003$, $P < 0.001$, and $P < 0.001$, respectively), while in the 32 non-ccRCC cases, only CD44 expression was positively correlated with higher histologic nuclear grade (grade 1−3 vs. grade 4) and pathologic stage (stages I−II vs. stages III−IV) ($P = 0.001$ and $P = 0.042$, respectively). In the 32 non-ccRCC cases, the ratio of grade 1/2 to Grade 3/4 was 2:30; therefore, the cases were divided into grade 1−3 vs. grade 4 for analysis.

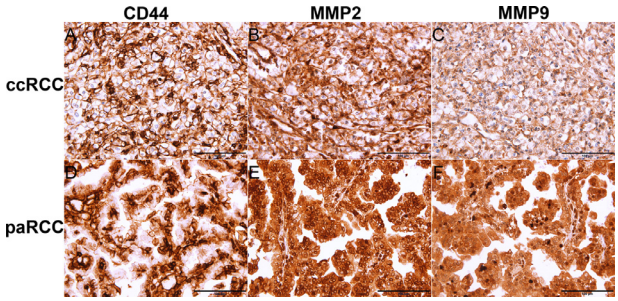

Fig. 1. Representative photographs of positive CD44, MMP2, and MMP9 immunohistochemical staining in a clear cell renal cell carcinoma (ccRCC) and a papillary renal cell carcinoma (paRCC). (A-C, Case no. 8) CD44, MMP2, and MMP9 expression in ccRCC, (D-F, Case no. 134) CD44, MMP2, and MMP9 expression in papillaryRCC (scale bar = 100 μm).

Univariate and multivariate analyses using Cox's proportional hazard regression model for OS and DFS were performed for the ccRCC and non-ccRCC groups. In the ccRCC group, univariate, and multivariate analyses showed that increased CD44 expression was an independent predictor of shorter OS ($P < 0.001$ and $P = 0.036$; Tables 3 and 4). In the non-ccRCC group, univariate analysis showed a positive correlation between CD44 expression and shorter OS, but multivariate analysis did not reach a statistically significant result (Supplementary Tables 1 and 2). Furthermore, MMP2 and MMP9 expression did not show an association with survival in the univariate or multivariate analyses in either the ccRCC or non-ccRCC groups.

3.2. The expression correlations between CD44, MMP2, and MMP9

Positive correlations between CD44 expression and MMP2 expression and MMP9 expression attained in the ccRCC group. However, in the non-ccRCC group, CD44 expression did not correlate with either MMP2 expression or MMP9 expression, while MMP2 and MMP9 showed a positive correlation with each other (Table 5).

4. Discussion

The expression levels of CD44, MMP2, and MMP9 were positively correlated with nuclear grade in the ccRCC group, while only CD44 expression was correlated with higher nuclear grade in the non-ccRCC group. CD44 expression in ccRCC and non-ccRCC was correlated with shorter OS by univariate analysis and was an independent predictor of shorter OS in the ccRCC group by multivariate analysis. Furthermore, CD44 expression was positively correlated with MMP2 and MMP9 expression in the ccRCC group but not in the non-ccRCC group.

CSCs are capable of self-renewal and contributing to cancer heterogeneity and are responsible for cancer initiation, progression, and eventual recurrence of cancer [15,28]. CSCs are frequently identified by surface protein markers, such as CD44, CD105, CD133, and CXCR4

Table 1
Summary of clinicopathological characteristics of patients with clear cell renal cell carcinoma ($n = 107$)

Characteristics	No.	CD44		MMP2		MMP9	
		Mean ± SD	P^a	Mean ± SD	P^a	Mean ± SD	P^a
Gender			0.767		0.780		0.489
Female	31	5.86 ± 3.564		7.13 ± 3.828		7.82 ± 3.662	
Male	76	5.87 ± 4.080		7.26 ± 3.559		7.24 ± 3.560	
Age at surgery			0.197		0.615		0.701
≤65	55	5.26 ± 3.599		7.08 ± 3.473		7.57 ± 3.615	
>65	52	6.50 ± 4.176		7.38 ± 3.799		7.23 ± 3.575	
Nuclear grade			0.003		<0.001		<0.001
1&2	72	5.05 ± 3.547		5.88 ± 3.103		6.56 ± 3.313	
3&4	35	7.54 ± 4.166		9.99 ± 3.016		9.16 ± 3.523	
Pathologic stage			0.148		0.105		0.735
I	45	4.97 ± 2.929		7.97 ± 3.822		7.57 ± 3.674	
II–IV	62	6.52 ± 4.416		6.69 ± 3.398		7.29 ± 3.541	
Pathologic stage			0.524		0.154		0.808
I–II	62	5.57 ± 3.597		7.70 ± 3.617		7.44 ± 3.551	
III–IV	45	6.28 ± 4.336		6.57 ± 3.561		7.37 ± 3.666	

[a] Mann-Whitney *U* test.

(SDF-1) [29]. Among these surface markers, CD44 has a suggested positive correlation with aggressive behavior in RCC [30,31]. CD44-HA promotes the migration of tumor cells [32] and activates the ankyrin-based cytoskeleton and various Rho GTPase signaling pathways during tumor progression [33]. The mRNA expression of CD44 was higher in RCC tissues than in normal kidney [34] and was significantly associated with metastasis [35]. These studies indicate that CD44 is a prognostic predictor for RCC. The present study was conducted to evaluate the expression and prognostic significance of CD44 in ccRCC and non-ccRCC. In both groups, CD44 expression was associated with higher histologic nuclear grade. In addition, CD44 expression was an independent prognostic factor indicative of short OS in the ccRCC group, but not in the non-ccRCC group. Our study showed that CD44 expression was more pronounced in ccRCC than in non-ccRCC [31].

Metastasis, a complex multistep process comprising the detachment of cancer cells from the primary tumor, disruption of the basement membrane, subsequent invasion into the surrounding stroma, cancer cell entry into the vascular or lymphatic system and transport to distal sites, is responsible for a majority of cancer-related deaths [10]. Cell invasion through basement membrane barriers is the definitive initiation of metastatic disease, which includes degradation of the ECM, a complex network of extracellular macromolecules such as collagen, proteoglycans, fibronectin, laminin and

Table 2
Summary of clinicopathological characteristics of patients with non-clear cell renal cell carcinoma ($n = 32$)

Characteristics	No.	CD44		MMP2		MMP9	
		Mean ± SD	P^a	Mean ± SD	P^a	Mean ± SD	P^a
Gender			0.001		0.313		0.949
Female	8	3.56 ± 0.979		12.63 ± 3.159		12.88 ± 3.917	
Male	24	9.79 ± 4.869		13.75 ± 2.355		13.67 ± 2.099	
Age at surgery			0.551		0.433		0.602
≤65	15	8.73 ± 5.216		13.77 ± 2.528		13.90 ± 1.919	
>65	17	7.79 ± 4.981		13.21 ± 2.658		13.09 ± 3.119	
Nuclear grade			0.001		0.779		0.206
1–3	18	5.83 ± 4.318		13.64 ± 2.388		13.89 ± 2.720	
4	14	11.32 ± 4.214		13.25 ± 2.867		12.93 ± 2.472	
Pathologic stage			0.165		0.737		0.331
I	17	6.94 ± 4.433		13.71 ± 2.346		14.03 ± 1.980	
II–IV	15	9.70 ± 5.411		13.20 ± 2.865		12.83 ± 3.143	
Pathologic stage			0.042		0.506		0.639
I–II	21	6.86 ± 4.385		13.26 ± 2.759		13.48 ± 2.926	
III–IV	11	10.86 ± 5.334		13.86 ± 2.237		13.46 ± 2.031	

[a] Mann-Whitney *U* test.

Table 3
Univariate analysis of overall survival and disease-free survival in 107 patients with clear cell renal cell carcinoma

	Overall survival			Disease-free survival		
	P	HR	95% CI	*P*	HR	95% CI
CD44	<0.001	1.257	1.115−1.418	0.593	1.039	0.904−1.194
MMP2	0.721	1.026	0.893−1.178	0.093	0.862	0.725−1.025
MMP9	0.611	1.037	0.902−1.192	0.635	0.964	0.826−1.123
Age at surgery	0.074	1.045	0.996−1.096	0.372	1.021	0.975−1.070
Sex (Female vs. Male)	0.396	1.729	0.488−6.127	0.906	1.072	0.336−3.421
Nuclear grade[a]	0.031	3.133	1.113−8.817	0.777	1.171	0.392−3.499
Pathologic stage	0.004			0.253		
I		1 (reference)			1 (reference)	
II	0.066	4.070	0.910−18.208	0.429	2.059	0.344−12.335
III	0.146	2.734	0.704−10.616	0.047	3.794	1.019−14.130
IV	<0.001	116.324	8.529−1586.6	0.989	0.000	0.000−0.000

CI = confidence interval; HR = hazard ratio.
[a] 1&2 vs. 3&4.

Table 4
Multivariate analysis of overall survival and disease-free survival in 107 patients with clear cell renal cell carcinoma

	Overall survival			Disease-free survival		
	P	HR	95% CI	*P*	HR	95% CI
CD44	0.036	1.157	1.009−1.327	0.941	1.006	0.864−1.170
Age at surgery	0.630	1014	0.958−1.073	0.479	1.019	0.968−1.073
Sex (Female vs. Male)	0.203	2.503	0.610−10.265	0.730	1.240	0.365−4.210
Nuclear grade[a]	0.125	2.520	0.774−8.200	0.679	1.290	0.386−4.304
Pathologic stage	0189			0.252		
I		1 (reference)			1 (reference)	
II	0.135	3.437	0.680−17.371	0.430	2.076	0.338−12.742
III	0.153	2.851	0.678−11.990	0.047	3.885	1.018−14.835
IV	0.043	17.542	1.090−282.213	0.988	0.000	0.000−0.000

CI = confidence interval; HR = hazard ratio.
[a] 1&2 vs. 3&4.

Table 5
Correlation between CD44, MMP2, and MMP9 expression by immunohistochemical staining of clear cell renal cell carcinoma and nonclear cell renal cell carcinoma

Spearman's rho		Clear cell renal cell carcinoma			Nonclear cell renal cell carcinoma		
		CD44	MMP2	MMP9	CD44	MMP2	MMP9
CD44	Correlation coefficient	1.000	0.402**	0.406**	1.000	0.016	−0.012
	Sig. (2-tailed)	.	<0.001	<0.001	.	0.929	0.949
	No.	107	107	107	32	32	32
MMP2	Correlation coefficient	0.402**	1.000	0.640**	0.016	1.000	0.551**
	Sig. (2-tailed)	< 0.001	.	0.000	0.929	.	0.001
	No.	107	107	107	32	32	32
MMP9	Correlation coefficient	0.406**	0.640**	1.000	−0.012	0.551**	1.000
	Sig. (2-tailed)	<0.001	< 0.001	.	0.949	0.001	.
	No.	107	107	107	32	32	32

*$P < 0.05$; **$P < 0.01$.

many other glycoproteins that acts as a barrier against the spread of cancer cells to distal sites, by MMPs [6,7]. The roles of MMPs have been implicated in various cancers to affect multiple signaling pathways as well as ECM degradation [36]. Unlike other MMPs, MMP2, and MMP9, also known as gelatinases, have a collagen-binding domain (CBD) that binds collagenous substrates, elastin, fatty acid, and thrombospondin [37]. Increased gelatinase activity has been observed in a variety of physiological and pathological conditions, including reproduction, growth and development,

inflammation, infective diseases, degenerative diseases of the brain and vascular diseases, and cancer [37]. Increased gelatinase expression in various cancers, including breast cancer, brain cancer, ovarian cancer, pancreatic cancer, colorectal cancer, bladder cancer, prostate cancer, lung cancer and melanoma, is often accompanied by increased invasiveness and metastasis [8].

Gelatinases have been shown to bind to integral membrane proteins, for example, MMP2/integrin $\alpha V\beta 3$ in melanoma, MMP9/DNA repair protein Ku in acute myeloid leukemia, and MMP9/CD44 in chronic lymphocytic leukemia [8]. The cell surface hyaluronan receptor CD44 mediates tumor invasion by recruiting MMP9 to the cell surface [38]. Gelatinases have been recognized as major contributors to the proteolytic activity at the cell surface, which is greatly involved in cell migration and invasion [37]. A previous study showed that CD44 expression was a predictive factor of more aggressive behavior, tumor progression, and worse prognosis in ccRCC but not in the papillary and chromophobe RCC subtypes [31]. The present immunohistochemical study showed that CD44 expression correlated with higher pathologic nuclear grade in both ccRCC and non-ccRCC. Specifically, CD44 expression in the ccRCC group independently correlated with shorter OS and showed a positive correlation with both MMP2 and MMP9 expression, while in non-ccRCC, CD44 expression was not correlated with either MMP2 or MMP9 expression.

Our study determined significant positive correlations of MMP2, MMP9, and CD44 expression to nuclear grade in ccRCC, indicating that all these markers may contribute to the aggressiveness of ccRCC, but only CD44 showed a profound positive correlation to nuclear grade and pathologic stage in non-ccRCC, indicating a dominant role of CSCs in non-ccRCC. Based on these findings, we reasoned that non-ccRCC, which has more aggressive clinical behavior than ccRCC, seems to be more dependent on CSCs than on MMPs activity. Recently, Kelley et al. used *C. elegans* to genetically remove MMPs and observed that cell invasion is delayed but still persists through an adaptive cell response requiring increased F-actin protrusive force and localized ATP production by mitochondria [13]. Therefore, it can be speculated that highly aggressive non-ccRCC activates different mechanisms than ccRCC in terms of invasion.

Secondly, we performed the univariate and multivariate analyses for OS and DFS in 107 patients with ccRCC, and, out of the 3 proteins, CD44 was an independent poor prognostic factor of OS in both the univariate and multivariate analyses. In non-ccRCC cases, only CD44 was a factor of poor prognosis in the univariate analysis of OS, and none of the 3 proteins showed statistically significant results as an independent prognostic factor in the multivariate analysis. Herein, we speculate the presence of different tumorigenic mechanisms between ccRCC and non-ccRCC. These findings suggest that MMP2 and MMP9 may play roles in a specific, restricted aspect of tumor progression and do not influence independent long-term adverse events, such as shortened OS and DFS, in both ccRCC and non-ccRCC. In contrast to MMP2 and MMP9, CD44, one of the CSC markers, seems to be an independent factor of poor prognosis in ccRCC.

Finally, we found a strong correlation among CD44, MMP2, and MMP9 expression by immunohistochemical staining of ccRCC, but only a strong correlation between MMP2 and MMP9 in non-ccRCC (Table 5). The MMP2 and MMP9 correlation was previously identified by gelatinase zymogram using the RCC cell line (RCC-786-0) [10]. These findings suggest that ccRCC may exert multiple mechanisms to invade the ECM as a different strategy compared to highly aggressive non-ccRCC. Through the large-scale efforts of The Cancer Genome Atlas and other international collaborative groups, it has been begun to elicit the molecular biology that underlies these classic subtypes. The results of these studies have demonstrated that the ccRCC and non-ccRCC are not only different histologically but are unique in their molecular profiles, little of which the molecular and genetic characteristics among the RCC subtypes are overlapped. The ccRCC has somatic mutation such as *VHL, PBRM1, SETD2, BAP1, KDM5C*, and *mTOR*, while non-ccRCC has exclusive somatic mutation such as *MET, SETD2, NF2, KDM6A, SMARCB1, FH, CDKN2A, TP53*, and *PTEN* [39].

In conclusion, the results suggest that in ccRCC, CD44, MMP2, and MMP9 expression levels are closely correlated with each other and correlated with higher pathologic nuclear grade, one of the relatively well-known indicators of poor prognosis, and CD44 can be an independent indicator of shorter OS. Although MMP2 and MMP9 did not show statistically significant values as independent prognostic markers, they are worth noting with regard to specific critical roles during ECM invasion in certain phases of tumor progression. The different correlations between CD44, MMP2, and MMP9 in ccRCC and non-ccRCC can be useful in understanding the mechanisms of carcinogenesis and therapeutic stratification, and further studies in a larger cohort of non-ccRCC patients are needed.

Conflict of interest

No potential conflicts of interest relevant to this article are reported.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2019.04.017.

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