Int J Clin Exp Med 2017;10(2):3594-3599
www.ijcem.com /ISSN:1940-5901/IJCEM0039177

Original Article

MMP2 rs243865 genotypes are associated with the increased risk of renal cell carcinoma in Chinese Han population

Jingying Wang[1,2], Jinmeng Wang[1,2], Li Zhu[1,2], Wenfeng Li[1,3], Yunsheng Xu[1,2]

[1]Laboratory for Advanced Interdisciplinary Research, Institutes of Translational Medicine, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China; Departments of [2]Dermatovenereology, [3]Radiation Oncology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China

Received August 31, 2016; Accepted November 26, 2016; Epub February 15, 2017; Published February 28, 2017

Abstract: Background: Matrix metalloproteases (MMPs) are proteolytic enzymes that contribute to all stages of malignancy progression. Genetic variants in the MMP genes may influence the biological function of these enzymes and change their role in carcinogenesis and progression. However, there are no data about the role of MMPs polymorphism in development of renal cell carcinoma (RCC). Patients and methods: A hospital-based case-control study was conducted in 250 patients with RCC and 250 healthy controls to investigate the possible association between the MMP2 rs243865 and MMP3 rs3025058 polymorphisms respectively, and the risk of RCC. Results: Significant differences of genotype distribution were observed between RCC cases and controls at the MMP2 rs243865 genotype. Compared with the MMP2 SNP rs243865 homozygote CC, the heterozygous CT genotype was associated with significantly increased risk for RCC (OR = 1.76, 95% CI = 1.48-4.09, P = 0.022); the TT genotype was associated with increased risk for RCC (OR= 1.98, 95% CI = 1.19-3.91, P = 0.015). CT/TT variants were associated with increased risk for RCC compared with the CC genotype (OR = 1.63, 95% CI = 1.21-4.94, P = 0.018). T allele was significantly increased as compared with the C allele (OR = 1.69, 95% CI = 1.25-4.32, P = 0.024). However, the genotype and allele frequencies of MMP3 rs3025058 polymorphisms in RCC patients were not significantly. Conclusion: Our results showed that theMMP2 rs243865 genotype was associated with increased risk for development of RCC in Chinese Han population.

Keywords: MMPs, renal cell carcinoma, single-nucleotide polymorphism, susceptibility

Introduction

Renal cell carcinoma (RCC) is the most common malignancy of the kidney, the frequency of which is increasing in both men and women. The incidence of RCC is two folds more frequent in men than in women, and is observed in the sixth decade [1]. Considerable evidence has been published over the past few years linking increased tumor risk with inflammation, and clinical and experimental studies have associated tumor progression with the upregulation of proinflammatory molecules, especially during late stages of the disease [2, 3]. Chronic inflammation, alongside the intrinsic properties of premalignant cells and other determinants, may be one of the driving forces of tumor initiation and promotion. Genetic polymorphisms indifferent inflammatory cytokine have been associated with cancer risk and the growth or invasiveness of RCC and others types of tumor [4, 5].

Although the exact etiology of RCC remains unclear, studies have shown that it involves environmental and genetic factors. Molecular epidemiology studies suggested that single nucleotide polymorphisms (SNPs) in specific genes and pathways may play an important role in the pathogenesis of RCC. Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that are capable of degrading various components of the extracellular matrix, which is a key event in the invasion and metastasis of most malignancies [6-9]. They are involved in all stages of cancer progression, not only in the

Table 1. Distribution of selected variables between the renal cell carcinoma cases and control subjects

Characteristics	Cases (%) N = 250	Controls (%) N = 250	chi square value	*P* value*
Mean Age (years)	60.5 (±12.3)	59.4 (±11.4)	2.45	0.138
≤ 60	143 (57.2)	131 (52.4)		
> 60	107 (42.8)	119 (47.6)		
Gender			5.43	0.249
Male	186 (74.4)	174 (66.3)		
Female	64 (25.6)	76 (30.4)		
BMI, kg/m^2	23.8 (±2.8)	21.9 (±3.2)	4.32	0.315
≤ 25	137 (54.8)	129 (51.6)		
> 25	113 (45.2)	121 (48.4)		
Smoking status			1.45	0.221
Never	157 (62.8)	139 (55.6)		
Ever	93 (37.2)	111 (44.4)		
Drinking status			3.24	0.303
Never	176 (70.4)	159 (63.6)		
Ever	74 (29.6)	91 (36.4)		
Stage				
Localized (I+II)	198 (79.2)			
Advanced (III+IV)	52 (20.8)			
Grade				
Well (I+II)	176 (70.4)			
Moderately (III)	43 (17.2)			
Poorly (IV)	31 (12.4)			

*Student's t-test for age and BMI distributions between cases and controls.

process of tumor invasion and metastasis, but also in as proliferation, adhesion, migration, differentiation, angiogenesis, senescence, autophagy, apoptosis and evasion of the immune system [10]. The expression of these MMPs by cancer cells may help increase the invasive potential of cancer cells by allowing the remodeling of the extracellular matrix.

MMP2 and MMP3 are the important members of the MMP family. Several polymorphisms in the promoter regions of the MMP2 and MMP3 genes have been well described. Previous researchers reported that these polymorphisms play critical roles in the regulation of MMP gene transcription. However, there is no any report on investigating MMPs polymorphism of RCC patients. The aim of our study was to investigate the possible association between the SNPs of MMPs (MMP2 rs243865 and MMP3 rs3025058) and risk of RCC in Chinese Han population.

Material and methods

Study population

In this study, we enrolled 250 RCC specimens in total from The First Affiliated Hospital of Chongqing Medical University between January 2013 and January 2015. All of them were histologically/pathologically confirmed by two experienced pathologists. The control group comprised 250 healthy volunteers for the general health checkup in our hospital during the same period. Those cases that had previous cancer, metastasized cancer from other or unknown origin and previous radiotherapy or chemotherapy were excluded. The cancer-free control subjects were recruited from those who were seeking health care in the outpatient departments of our hospital. All the healthy controls had been under the health screening, and their clinical characteristics were matched to the sex, agg, BMI, smoking status and drinking status with the RCC cases, as outlined in **Table 1** and Supplementary Table. After obtaining written informed consent, 5 mL of peripheral blood was collected for DNA extraction. Each participant was interviewed using a standard questionnaire by a trained nurse, to collect medical histories, demographic characteristics. The present study was performed with strict protocol under the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University. All the specimens we recruited were of Chinese Han ethnicity and were filtered based on their clinical characteristics. Before the assay, we obtained a written informed consent from each participant in our study. Sex, agBMI, smoking status and drinking status.

DNA extraction and genotyping

The polymorphisms in the promoters of the MMPs genes analyzed in this study are shown in **Table 2**. The polymerase chain reaction (PCR) combined with the restriction fragment length

Table 2. Details of PCR Primer sequences and RFLPs conditions in our study

Gene	Polymorphism	SNP ID	Primer sequence	PCR Conditions
MMP2	-1306 C/T	rs243865	F: GCCATTGTCAATGTTCCCTAAAACA	30 cycles: 95°C 40 s, 54°C 40 s, 68°C 60 s
			R: TGACTTCTGAGCTGAGACCTGAA	
MMP3	-1171 5A/6A	rs3025058	F: TTTCAATCAGGACAAGACgaaGTTT	32 cycles: 94°C 180 s, 62°C 30 s, 72°C 30 s
			R: GATTACAGACATGGGTCACA	

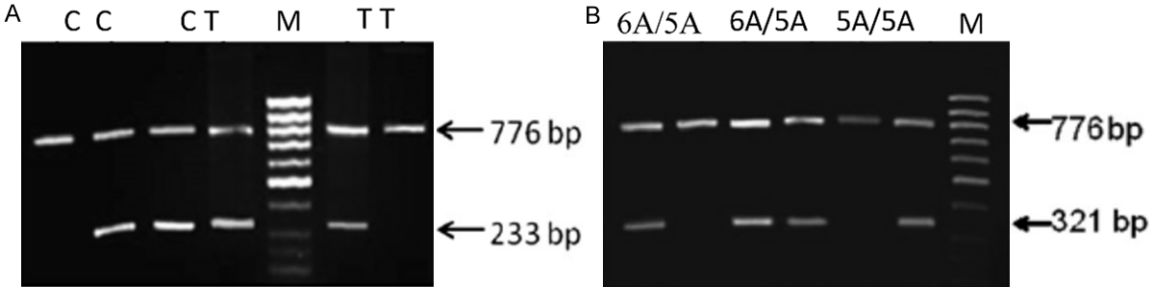

Figure 1. Representative gel pictures of MMP2 rs243865 and MMP3 rs3025058 gene polymorphisms. A: Gel pictures of MMP2 rs243865 polymorphism. B: Schematic gel pictures of MMP3 rs3025058 polymorphism.

polymorphism (RFLP) was used to determine the MMPs genotypes. Representative gel pictures of MMP2 rs243865 and MMP3 rs3025058 gene polymorphisms were shown in **Figure 1**. Genomic DNA used for the assay was extracted from peripheral blood samples (96.5% of total samples) or exfoliated buccal cells (3.5% of total samples) as previously described [11]. For quality control, genotyping was repeated randomly in at least 5% of the samples, and two of the authors independently reviewed all results. PCR reactions were carried out in a total volume 10 μl containing 20 ng of genomic DNA, 0.25 mM of each Dntp (Ecogen, Biologia Molecular S.L.), 0.2 units of Taq polymerase (Biotools, Inc.) and 2.5 pmol of each primer in a 1×PCR buffer (Sigma-Aldrich Co.). The details of the primers and PCR conditions used for the amplification of MMPs are shown in **Table 2**.

Statistical analysis

During the analysis, student t-test and chi-square (χ^2) test were performed to analysis the differences in the distribution of various considered characteristics as well as the differences of genotype frequencies between the RCC patients and the healthy controls, as appropriate. Similarly, the Hardy-Weinberg equilibrium (HWE) of each subject was examined by implying a two-sided chi-square (χ^2) test which was performed by comparison of observed and expected genotype frequencies. The MMP2 and 3 SNP genotypes related RCC risk was assessed by odds ratio (OR) and their corresponding respective confidence intervals 95% (CIs) value of the univariate and multivariate logistic regression, for both combined and respective genotype. We managed all the statistical analysis with the SPSS software version 19.0. A two-sided *P* value less than 0.05 was considered to be statistically significant for all the analyses.

Results

Clinical characteristics

This study included 250 RCC patients and 250 healthy controls, their age, gender, BMI, smoking status, drinking status, stage and grade were summarized in **Table 1**. The mean age (±SD) for case and control groups was 60.5 (12.3) and 59.4 (11.4) years, respectively. Our study included 250 RCC cases, including 186 males and 64 females, and 250 healthy controls, including 174 males and 76 females. No significant difference was detected in the age, gender, BMI, smoking status, drinking status distribution between two groups ($P > 0.05$). Regarding the clinical stage, 79.2% of patients were in stage I and II, and 20.8% were in stage III and IV. The control population was consistent with the Hardy-Weinberg Equilibrium (HWE) for

Table 3. Association between two genotypes of SNPs (MMP2 rs243865 and MMP3 rs3025058) and risk of RCC patients in multivariate regression

Polymorphisms	Cases (%)	Controls (%)	HWE-*P* Value	OR (95% CI)	*P*-value*
MMP2 rs243865					
CC	158 (63.2)	195 (78)	0.324	1	
CT	61 (24.4)	40 (16)		1.76 (1.48-4.09)	0.022
TT	31 (12.4)	15 (6)		1.98 (1.19-3.91)	0.015
CT+TT	92 (36.8)	55 (22)		1.63 (1.25-4.94)	0.018
C	377 (73.3)	430 (81.7)		1	
T	123 (26.7)	70 (18.3)		1.69 (1.25-4.32)	0.024
MMP3 rs3025058					
6A/6A	76 (30.3)	95 (37.9)	0.153	1	
6A/5A	125 (50.2)	103 (41.4)		1.43 (0.79-3.46)	0.275
5A/5A	49 (19.5)	52 (20.7)		1.81 (0.95-3.83)	0.246
6A/5A+5A/5A	174 (69.7)	156 (62.1)		1.78 (0.86-4.08)	0.143
6A	276 (55.4)	293 (58.6)		1	
5A	223 (44.6)	207 (41.4)		1.68 (0.87-4.19)	0.131

OR, odds ratio; CI, confidence interval. *Multiple logistic regression models calculated by c2 statistics or Fisher's exact test. Bold numbers indicate that the *P*-value is < 0.05.

Table 4. Association between two genotypes of SNPs (MMP2 rs243865 and MMP3 rs3025058) and risk of RCC patients in univariate regression

Polymorphisms	Cases (%)	Controls (%)	HWE-*P* Value	OR (95% CI)	*P*-value*
MMP2 rs243865					
CC	158 (63.2)	195 (78)	0.324	1	
CT	61 (24.4)	40 (16)		2.36 (1.63-4.62)	0.016
TT	31 (12.4)	15 (6)		2.38 (1.32-4.57)	0.027
CT+TT	92 (36.8)	55 (22)		2.14 (1.51-4.35)	0.021
C	377 (73.3)	430 (81.7)		1	
T	123 (26.7)	70 (18.3)		1.62 (1.36-4.61)	0.041
MMP3 rs3025058					
6A/6A	76 (30.3)	95 (37.9)	0.153	1	
6A/5A	125 (50.2)	103 (41.4)		1.61 (0.52-4.34)	0.174
5A/5A	49 (19.5)	52 (20.7)		2.7 (0.57-4.33)	0.422
6A/5A+5A/5A	174 (69.7)	156 (62.1)		2.85 (0.66-6.88)	0.332
6A	276 (55.4)	293 (58.6)		1	
5A	223 (44.6)	207 (41.4)		2.15 (0.74-5.92)	0.314

OR, odds ratio; CI, confidence interval. *Univariatelogistic regression models calculated by c2 statistics or Fisher's exact test. Bold numbers indicate that the *P*-value is < 0.05.

the polymorphisms inMMP2 rs243865 and MMP3 rs3025058.

Distributions of MMP2 rs243865 and MMP3 rs3025058 genotypes and risk of RCC

The two genotypes of SNPs (MMP2 rs243865 and MMP3 rs3025058) were examined in this study. The genotype and allele frequencies and the Hardy-Weinberg equilibrium (HWE) of these gene polymorphisms among the cases and controls are shown in **Tables 3**, **4**.

There were significant differences in the genotype and allele frequencies of rs243865 of MMP2 genotypes between RCC cases and controls by multivariate (**Table 3**) and univariate (**Table 4**) regression. Compared with the MMP2 SNP rs243865 homozygote CC, the heterozygous CT genotype was associated with signifi-

cantly increased risk for RCC (OR = 1.76, 95% CI = (1.48-4.09), P = 0.022); the TT genotype was associated with increased risk for RCC (OR = 1.98, 95% CI = 1.19-3.91, P = 0.015). CT/TT variants were associated with increased risk for RCC compared with the CC genotype (OR = 1.63, 95% CI = 1.25-4.94, P = 0.018). T allele was significantly increased as compared with the C allele (OR = 1.69, 95% CI = 1.25-4.32, P = 0.024). However, the genotype and allele frequencies of MMP3 rs3025058 polymorphisms in RCC patients were not significantly different from controls (P > 0.05) as shown in **Tables 3**, **4**.

Discussion

In current hospital based case-control study, we assessed the association between the polymorphisms of three SNPs of MMPs (MMP2 rs243865 and MMP3 rs3025058) and risk of RCC in Chinese Han population and found the significant association betweenMMP2 rs243865 polymorphisms and risk of RCC. The genotype and allele distribution of polymorphisms rs243865 of MMP2 genotypes were significantly different between case and control groups, indicating that this SNP might be related to RCC development. To the best of our knowledge, our study is the first report to describe the possible role of MMP2 and 3 gene polymorphisms as a risk factor for RCC and found that MMP2 rs243865 genotype variations do influence susceptibility to RCC development in the Chinese Han population.

The MMP family comprises 23 human enzymes that traditionally have long been associated with cancer invasion and metastasis because of their ability to degrade the extracellular matrix. However, recent studies have showed that the roles of MMPs in tumor development and metastasis are much more complex than was originally envisioned. Several studies have demonstrated that MMPs are also the key mediators of growth factor activation, bioavailability and receptor signalling, cell adhesion and motility, apoptosis and survival mechanisms, angiogenesis, and inflammatory responses and immune surveillance [12]. In this sense, high levels of MMP2 and MMP3 proteins have been implicated in several malignancies including oral, oesophageal, colorectal, NSCLC, renal, head and neck, melanomas and breast cancer [13-20].

Previous studies showed that the MMP2 SNP C-T transition at -1306 is reported to interrupt the Sp1 type promoter site, causing lower promoter activity and also reducing the transcriptional activity [21]. In 2001 [22], the rs243865 -1306C/T of MMP2 promoter polymorphisms was identified, it was likely that the -1306CC genotype may be associated with a high transcription level and enzyme activity of MMP2 and it might affect individual susceptibilities to cancers.

In spite of interesting findings on the association of MMP2 rs243865 polymorphisms with RCC risk, there were several limitations that need to be addressed regarding the present study. We did not collect lifestyle data for individual participants, e.g. on local environmental factors, diet, or level of physical activity, which potentially could interact with genetic variations in influencing overall risk of developing RCC. Besides, the relative small sample size might hide some weak gene-disease association and gene-environment interactions. Studies need to be performed in larger study groups to confirm our preliminary results.

In conclusion, our study provided the evidence of association between MMP2 rs243865 and MMP3 rs3025058 polymorphisms and the risk of RCC and found the MMP2 rs243865 genotypes were associated with greater susceptibility for developing RCC. Because this is the first report concerning the MMPs polymorphism and the risk of RCC in the literature, studies with larger sample size and further investigations into the mechanism are warranted to clarify and validate.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 81571395, 81371748 and 81373075).

Disclosure of conflict of interest

None.

Address correspondence to: Yunsheng Xu, Laboratory for Advanced Interdisciplinary Research, Institutes of Translational Medicine, Department of Dermatovenereology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China. Tel: +86-577-63310987; Fax: +86-577-63310987; E-mail: wanogewva001@sina.com

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Nelson D, Ganss R. Tumor growth or regression: powered by inflammation. J Leukoc Biol 2006; 80: 685-90.

[3] Coussens LM, Werb Z. Inflammation and cancer. Nature 2002; 420: 860-7.

[4] Romero JM, Sáenz-López P, Cózar JM, Carretero R, Canton J, Vazquez F, Concha A, Tallada M, Garrido F, Ruiz-Cabello F. A polymorphism in the interleukin-10 promoter affects the course of disease in patients with clear-cell renal carcinoma. Hum Immunol 2009; 70: 60-4.

[5] Kawai Y, Sakano S, Korenaga Y, Eguchi S, Naito K. Associations of single nucleotide polymorphisms in the vascular endothelial growth factor gene with the characteristics and prognosis of renal cell carcinomas. Eur Urol 2007; 52: 1147-55.

[6] Freije JM, Balbin M, Pendas AM, Sanchez LM, Puente XS. Matrix metalloproteinases and tumor progression. Adv Exp Med Biol 2003; 532: 91-107.

[7] Stetler-Stevenson WG, Yu AE. Proteases in invasion: matrix metalloproteinases. Semin Cancer Biol 2001; 11: 143-52.

[8] Nagase H, Woessner JF. Matrix metalloproteinases. J Biol Chem 1999; 274: 21491-21494.

[9] Stetler-Stevenson WG, Liotta LA, Kleiner DE. Extra cellular matrix 6: role of matrix metalloproteinases in tumor invasion and metastasis. FASEB J 1993; 7: 1434-1441.

[10] Deryugina EI, Quigley JP. Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev 2006; 25: 9-34.

[11] Miller DP, Anderson RE, de Pablo JJ. Stabilization of lactate dehydrogenase following freeze thawing and vacuum-drying in the presence of trehalose and borate. Pharm Res 1998; 15: 1215-1221.

[12] Deryugina EI, Quigley JP. Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev 2006; 25: 9-34.

[13] Ohashi K, Nemoto T, Nakamura K, Nemori R. Increased expression of matrix metalloproteinase 7 and 9 and membrane type 1-matrix metalloproteinase in esophageal squamous cell carcinomas. Cancer 2000; 88: 2201-2209.

[14] Kim TD, Song KS, Li G, Choi H, Park HD, Lim K, Hwang BD, Yoon WH. Activity and expression of urokinase-type plasminogen activator and matrix metalloproteinases in human colorectal cancer. BMC Cancer 2006; 6: 211.

[15] Franchi A, Santucci M, Masini E, Sardi I, Paglierani M, Gallo O. Expression of matrix metalloproteinase 1, matrix metalloproteinase 2, and matrix metalloproteinase 9 in carcinoma of the head and neck. Cancer 2002; 95: 1902-1910.

[16] Duffy MJ, Maguire TM, Hill A, McDermott E, O'Higgins N. Metalloproteinases: role in breast carcinogenesis, invasion and metastasis. Breast Cancer Res 2000; 2: 252-257.

[17] Lin TS, Chiou SH, Wang LS, Huang HH, Chiang SF, Shih AY, Chen YL, Chen CY, Hsu CP, Hsu NY, Chou MC, Kuo SJ, Chow KC. Expression spectra of matrix metalloproteinases in metastatic non-small cell lung cancer. Oncol Rep 2004; 12: 717-723.

[18] Tsai CH, Hsieh YS, Yang SF, Chou MY, Chang YC. Matrix metalloproteinase 2 and matrix metalloproteinase 9 expression in human oral squamous cell carcinoma and the effect of protein kinase C inhibitors: preliminary observations. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 95: 710-716.

[19] Shellman YG, Makela M, Norris DA. Induction of secreted matrix metalloproteinase-9 activity in human melanoma cells by extracellular matrix proteins and cytokines. Melanoma Res 2006; 16: 207-211.

[20] Kallakury BV, Karikehalli S, Haholu A, Sheehan CE, Azumi N, Ross JS. Increased expression of matrix metalloproteinases 2 and 9 and tissue inhibitors of metalloproteinases 1 and 2 correlate with poor prognostic variables in renal cell carcinoma. Clin Cancer Res 2001; 7: 3113-3119.

[21] Xu E, Lai M, Lv B, Xing X, Huang Q, Xia X. A single nucleotide polymorphism in the matrix metalloproteinase-2 promoter is associated with colorectal cancer. Biochem Biophys Res Commun 2004; 324: 999-1003.

[22] Price SJ, Greaves DR, Watkins H. Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene: role of Sp1 in allele-specific transcriptional regulation. J Biol Chem 2001; 276: 7549-58.