INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY Vol. 20, no. 4, 697-705 (2007)

QUANTITATIVE IMMUNOHISTOCHEMICAL ANALYSIS OF MATRILYSIN 1 (MMP-7) IN VARIOUS RENAL CELL CARCINOMA SUBTYPES

E. PATRAKI and M.R. CARDILLO

Department of Experimental Medicine and Pathology (Section of Pathologic Anatomy-Uropathology Unit), University "La Sapienza" of Rome, Rome, Italy

Received February 2, 2007 - Accepted May 29, 2007

The role of matrilysin 1 or matrix metalloproteinase-7 (MMP-7) in cancer is extremely complex and poorly understood. In this study we investigated differential expression of MMP-7 in the epithelium and stroma of 95 paraffin-embedded renal tumor samples by immunohistochemistry and compared tumoral with normal peritumoral renal tissue. We also determined a possible correlation of the immunohistochemical findings with histological subtype, tumor grade and stage of RCC. In all areas examined MMP-7 protein expression was significantly higher in epithelium than in stroma (P<.01). MMP-7 was more less expressed in peritumoral normal areas than in benign epithelial neoplasias (renal papillary and oncocytomas) and RCC carcinomas, reaching the highest immunopositive reaction in chromophobe RCC subtypes, followed by conventional clear-cell and chromophilic-papillary RCC histological subtypes and the lowest levels in more aggressive RCC histotypes (spindle-cell and collecting-duct RCCs). MMP-7 reached their highest levels in high-grade and high-stage RCCs. Our observation suggests an important role of MMP-7 in the development and progression of renal cancer. The differential expression of MMP-7 in the various histological RCC subtypes may reflect the malignant phenotype and more aggressive behavior of RCCs.

Matrix metalloproteinases (MMPs) play a critical role in tumor invasiveness and metastasis (1-3). A positive correlation between tumor progression and increased expression of MMPs has been described in various human cancers, including colon (4), breast (5), ovary (6), prostate tumors (7), and renal cell carcinoma (RCC) (8). In the kidney, some evidence suggests that MMP expression is correlated not only with the aggressiveness of RCCs, but also with the histological type tumor (9). The association between MMPs, cancer progression and metastasis has raised increasing interest because MMP proteins are an attractive target for the development of new therapeutic strategies using drugs aimed at inhibiting MMP activity (10). One of the smallest (28 kDa) known members of the MMP family is MMP-7, also known as matrilysin 1. MMP-7 resembles the stromelysins in its substrate specificity, but differs structurally because it contains only the minimal number of domains required for secretion and activity, a pre-domain (cleaved during secretion), a latency or pro-domain and a catalytic domain that mediates enzymatic activity (11). MMP-7 is secreted by cells as an inactive proenzyme, is activated by proteinases, and indirectly activates the latent forms of other MMPs, such as MMP-2 and several other

Key words: metalloproteinases-7, renal cell carcinoma, immunohistochemistry, computer-assisted image analysis

Mailing address: Maria Rosaria Cardillo, M.D., F.I.A.C.,
Department of Experimental Medicine and Pathology,
Histopathology Unit,
University "La Sapienza", Policlinico Umberto I,
324 Viale Regina Elena 00161 Rome, Italy
Tel. ++39 06 49972417 Fax. ++39 06 4454820
e-mail: mariarosaria.cardillo@uniroma1.it

0394-6320 (2007)

proteins including urokinase-type plasminogen activator (uPA) (10). MMP-7 has been detected in various tumors (13-17) and is an important marker in human cancer progression, in tumor generation and growth (18-26) as well as in many other fundamental processes, such as epithelial repair and host defense (27). The expression of MMP-7 has been poorly investigated in human RCC (28-29). In their study describing the expression of MMPs in various RCC subtypes, Hagemann et al (8) described neither the distribution nor the localization of MMP-7.

In this study we therefore investigated the localization, distribution and intensity of MMP-7 levels in the epithelium and stroma of normal peritumoral and neoplastic areas of 95 human renal epithelial tumors by immunohistochemistry, assessing MMP-7 protein levels by computer-assisted image analysis in the various RCC histological subtypes, and investigated the association of MMP-7 protein levels with histological grade and tumor-node-metastasis (TNM) pathological stage.

MATERIALS AND METHODS

Tumor Samples

Ninety-five formalin-fixed, paraffin-embedded renal specimens were obtained from 95 patients (age range 51 to 88 years, mean age 68.11 years) who had undergone total nephrectomy for primary RCC in the Department of Urology "U. Bracci", University "La Sapienza", Rome (Italy) from 1997 to 2003. Tumors were classified, graded and staged according to the RCC classification of the American Joint Committee on Cancer 2002 (30) and the WHO 2004 (31). Of the 95 tumors studied, 10 (10.52%) tumors showed the morphological features of benign epithelial tumors (4 papillary adenomas and 6 oncocytomas). The remaining 85 tumors (94.45%) were RCCs: 56 (65.88%) were clear-cell, 13 (15.29%) chromophilic-papillary renal cell, 9 (10.58%) chromophobe-cell, 2 (2.35%) spindle-cell and 5 (5.88%) collecting-duct carcinoma subtypes. According to histological grading (32), 23/85 (27.05%) were grade I, 37/85 (43.52%) grade II and 25/85 (29.41%) grade III/IV. According to TNM pathological staging 32/85 (37.64%) tumors were pT1, 14/ 85 (16.47%) pT2, and 39/85 (45.88%) pT3.

Immunohistochemical (IHC) analysis

Two serial sections (3-μm thick) from renal blocks were cut onto slides coated with 3-aminopropyl-ethyl-xylene (Sigma-Aldrich, S.R.L, Milan, Italy), microwaved for 5 minutes at 750 W (two cycles) and stained using the primary mouse monoclonal antibody anti-MMP-7 (Matilysin/PUMP-1)(clone ID-2) IgG_{2b} fraction (supplied by Chemicon International, Inc), which reacts with a proactive and active form of human MMP-7, at 1:100 dilution and 4°C overnight incubation by the the avidin-biotin-peroxidase complex (ABC) method (reagents from Dako S.p.A., Milan, Italy), as previously reported (7). All reactions included appropriate positive controls (breast tissue) and negative controls (the primary antibody was replaced by normal swine serum).

Immunohistochemical evaluation by computer-assisted image analysis (IHC)

To quantify MMP-7 immunoreactivity in tissue sections, and reduce operator subjectivity, we used computer-assisted image analysis. Three representative areas of each sample (assessed by IHC) were randomly selected for each tumor tissue sample from the normal tissue adjacent to tumoral areas and RCC under a light microscope (Olympus Uplan FI [20x objective]), and captured with a digital camera (Nikon). Areas of interest were quantified with IMAGE-Pro Plus 4.5 software (Media Cybernetics, Silver Spring, MD, USA) using the histogram function of the software, as already described (7). For each field, the number of positive areas was expressed as a fraction of the epithelium-stroma area (positive areas divided by the overall field area). For each slide, the mean+SE of colorPercent area (ColorPercent=ColorArea/tissueAreax100) was calculated.

Statistical analysis

The results are reported as mean ± SE and expressed in arbitrary densitometric units. The data were analyzed by Multistat program (Biosoft, Cambridge, 1988). Chi-square analysis and Student's *t* test were used to test differences in the percentage of tumors expressing MMP-7 in epithelium and stroma of normal peritumoral and tumoral areas. Chi-square analysis and Student's *t* test were also used to determine the association of MMP ligand levels with histological grading and TNM pathological stages. P values less than 0.05 were considered to indicate statistical significance.

RESULTS

In the 95 renal tumors examined, MMP-7 protein was significantly more strongly expressed in the epithelium than in the stroma of normal tissue adjacent to tumoral areas and tumoral tissue (P=0.0022 and P=0.0001 by chi-square test) (Table I). Comparing benign tumors (adenomas or

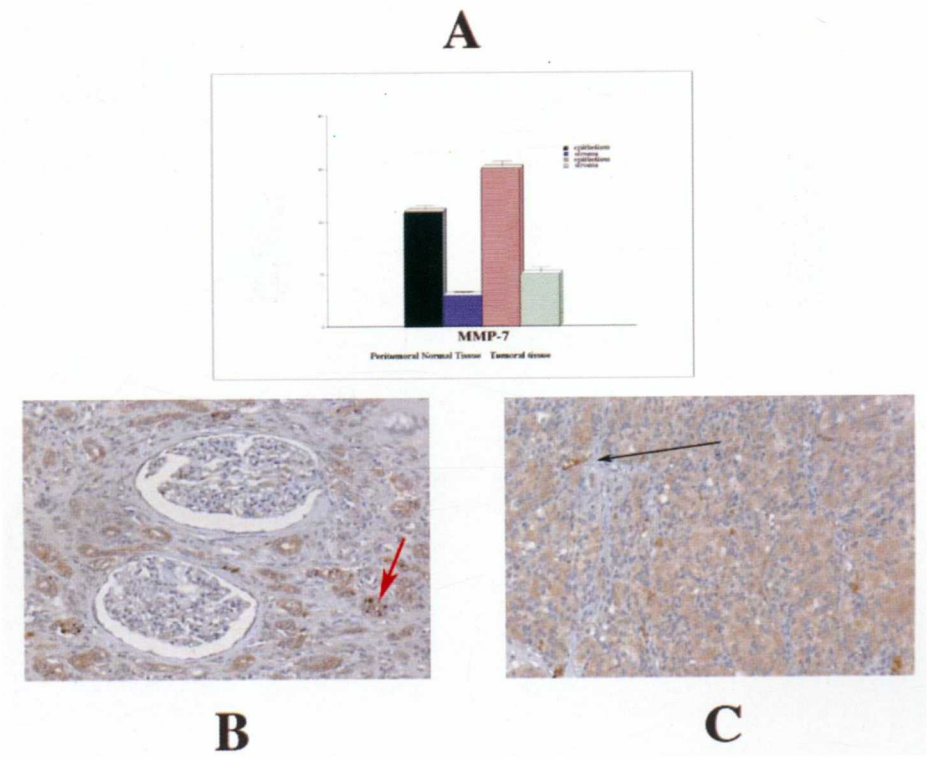

Fig. 1. *Matrix metalloproteinase-7 (MMP-7) immunoreactivity in 95 nephrectomies. A) comparison between epithelium and stroma of normal and tumoral tissues. In the epithelium and stroma MMP-7 expression was higher in tumoral than in normal renal tissue. In the tumors, MMP-7 epithelial levels were significantly higher than stromal levels (P<.01, by chi-square test). B) peritumoral normal renal parenchyma showing focal and moderate-to-strong MMP-7 immunoreactivity in the cytoplasm of renal epithelial proximal tubules (red arrows), patchy immunostaining in renal corpuscles (mesangial cells and podocytes) and scarce and very weak MMP-7 immunostaining in the stroma. C) oncocytoma showing diffuse moderate MMP-7 immunoreactivity, localized in the cytoplasm of oncocytic cells, and scarce immunoreactivity in a loose hypocellular fibrous stroma, but strong in endothelial cells (black arrow) (ABCX400).*

oncocytomas) with RCCs, we found significantly increased MMP-7 levels only in the epithelial compartment of RCCs (33.59±0.99 vs 22.19±0.81; P=0.0314 by Student's *t* tests) (Fig. 1A). Epithelial and stromal MMP-7 expression was significantly higher in renal tumoral tissue (33.59±0.99 and 10.09±0.90) than in peritumoral normal kidney (22.19±0.81 and 6.18±0.50) (P=0.0001 and P=0.0026 by chi-square test) (Fig 1A). Dysplastic changes in normal peritumoral tubules were identified in 10 of the 95 (9.5%) samples investigated.

MMP-7 showed cytoplasmic immunoreactivity. Immunostaining patterns of expression differed in normal peritumoral tissue and kidney tumor. In normal peritumoral kidney, immunoreactivity for MMP-7 was distributed in renal tubules and corpuscles. Renal tubules showed diffuse, moderate to weak MMP-7 immunoreactivity, localized in the cytoplasm of the epithelium lining the proximal and distal convoluted tubules and collecting duct. In renal corpuscles, the distribution was patchy and MMP-7 was weakly expressed in the mesangial cells, podocytes and Bowman's capsule of glomerules (Fig. 1B). In the few areas of dysplastic tubules adjacent to RCC and in benign epithelial renal tumors (renal papillary and oncocytomas), MMP-7 protein levels were higher than in the normal peritumoral tissue, reaching significance only in the stroma of tumoral tissue (P=0.006, by chi-square test) (Table II; Fig 1A).

In malignant epithelial renal tumors, the immunohistochemical analysis of RCCs showed

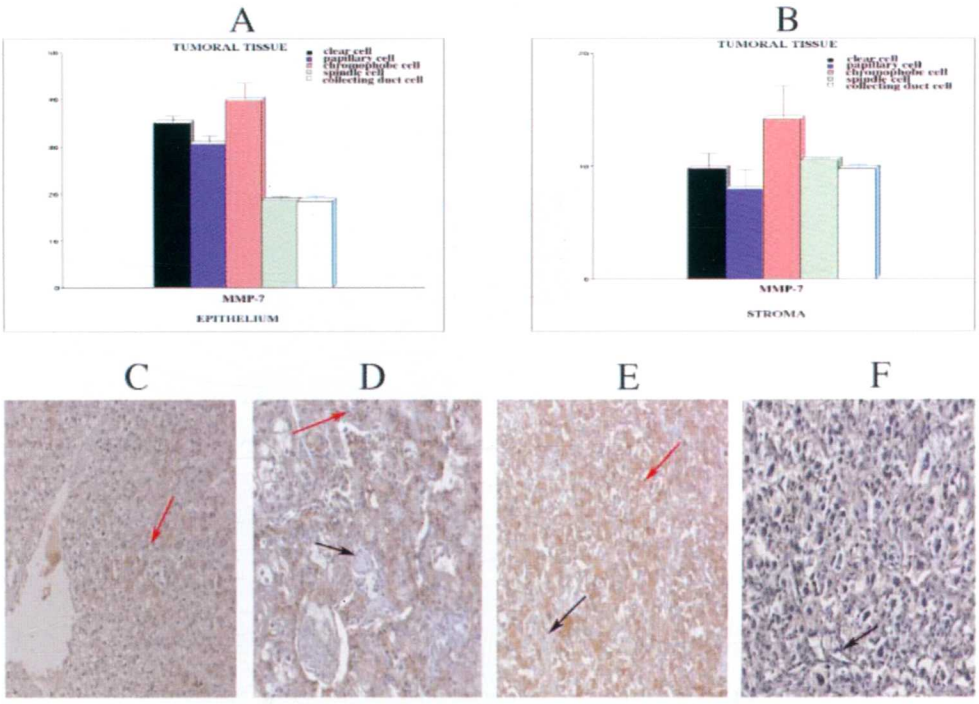

Fig. 2. *Matrix metalloproteinase-7 (MMP-7) expression in some histologic subtypes of renal-cell carcinomas.* ***A)*** *Distribution of MMP-7 protein in epithelium and* ***B)*** *in the stroma of the various histologic subtypes of renal-cell carcinomas. MMP-7 levels are higher in the epithelium than in the stroma of the clear, chromophilic-papillary and chromophobe-cell types, in contrast, MMP-7 levels are higher in the stroma than in the epithelium of spindle-cell and collecting-duct cell types.* ***C)*** *areas of clear-cell renal carcinoma showing moderate-to-strong diffuse immunoreactivity for MMP-7 in epithelial neoplastic cells.* ***D)*** *areas of a papillary renal carcinoma type I showing moderate-to-strong MMP-7immunoreactivity in the cytoplasm of malignant epithelial cells and very patchy and weak in stromal cells (macrophages and lymphocytes) (black arrow);* ***E)*** *areas of a chromophobe renal-cell carcinoma, showing diffuse and very strong MMP-7 immunoreactivity in the epithelium and focal and very weak MMP-7 immunoreactivity in the stromal cells.* ***F)*** *areas of a renal cell carcinoma spindle-cell subtype showing weaker and more focal MMP-7 immunoreactivity in epithelial cells (red arrow) than in stromal cells (black arrow)(ABC X400).*

diffuse, moderate-to-strong cytoplasmic staining for MMP-7 in accordance with the histological RCC subtypes (Fig. 2C, D, E, F). Whereas there was no significant difference in MMP-7 expression between clear-cell, papillary-chromophilic or chromophobic RCCs, the percentage area stained for MMP-7 was significantly lower in spindle cell and collecting-duct than in clear-cell, chromophilic and chromophobic RCC subtypes either in tumoral or peritumoral normal areas. Clear-cell and chromophobic RCC subtypes contained significantly higher MMP-7 levels than spindle-cell and collecting-duct subtypes in the epithelium of malignant ($P<.01$) and surrounding non-malignant tissue ($P\leq.05$). Papillary-chromophilic and collecting-duct RCC subtypes contained higher MMP-7 levels than spindle-cell RCC only in perineoplastic normal tissue ($P<.05$) (Table II and Fig. 2A and B).

MMP-7 expression in epithelial and interstitial renal tissue reached significantly higher levels in high-grade (grade III/IV) than in lower-grade (grade II or I) RCC ($P=0.024$ and $P= 0.00001$ by Student's *t* test). The epithelium of advanced stage (pT3) tumors contained significantly higher levels of MMP-7 than lower stage (pT1) tumors ($P=0.0010$ by Student *t* test) (Table III).

DISCUSSION

In this *in vivo* immunohistochemical study we

Table I. *Evaluation of matrix metalloproteinase-7 (MMP-7) protein expression in epithelium and stroma of 95 renal epithelial neoplasias by computer-assisted quantitative image analysis. The percentage of MMP-7 positive cells was significantly higher in the epithelium than in the stroma of benign tumors (P=0.0022 and P=0.0001) by chi-square test and malignant renal tumors (P=0.0026 and P=0.001). The epithelial compartment of renal cell carcinoma contained significantly higher MMP-7 levels than epithelial normal tissue adjacent to cancer areas (P=0.0314 by Student's t*

		PERITUMORAL NORMAL					TUMORAL TISSUE		
		Epithelium		Stroma		Epithelium		Stroma	
		total area	PerArea (%)	total area	PerArea (%)	total area	PerArea (%)	total area	PerArea (%)
	N° of		positive cells		positive cells		positive cells		positive cells
	samples	mean±SE	mean±SE	mean±SE	mean±SE	mean±SE	mean±SE	mean±SE	mean±SE
MMP-7	**95**	**26139.75±699.57**	**22.01±0.77**	**11567.50±1973.43**	**5.96±0.48**	**39589.27±502.07**	**33.08±0.97**	**3560.84±501.07**	**9.91±0.87**
Benign tumors	10	22031.59±545.22	18.97±0.27	12517.20±238.33	2.25±0.09	39857.39±1626.66	24.43±3.22	4025.39±1010.38	6.91±3.26
Renal cell carcinomas	85	26381.41±731.91	22.19±0.81	11519.74±2090.03	6.18±0.50	39573.50±524.71	33.59±0.99	3533.51±527.87	10.09±0.90

Table II. *Matrix metalloproteinase-7 (MMP-7) expression in histological subtypes of renal cell carcinomas: comparison between epithelium and stroma of normal and neoplastic tissue. All data are expressed as means ± SE.*

			MMP-7		
	N.°of	**peritumoral normal tissue**		**tumoral tissue**	
	cases	epithelium	stroma	epithelium	stroma
		mean+SE	mean+SE	mean+SE	mean+SE
Renal cell tumors	95	positive cells(%)	positive cells(%)	positive cells(%)	positive cells(%)
Benign Tumors	***10***	***18.97+0.27***	***2.25+0.09***	***24.43+3.22***	***6.91+3.26***
papillary adenoma	4	18.59+0.54	2.03+0.02	20.60+1.34	2.51+0.24
oncocytoma	6	19.23+0.26	2.40+0.05	26.99+5.09	9.85+4.97
Renal carcinomas	***85***	***22.19+0.81***	***6.18+0.50***	***33.59+0.99***	***10.09+0.90***
clear cell	56	21.99+0.94	6.62+0.66	35.02+1.07	9.81+1.22
papillary cell	12	26.03+2.21	6.26+0.79	30.62+1.47	8.01+1.58
chromophobe cell	10	23.75+0.87	5.43+1.83	39.76+3.40	14.21+2.72
spindle cell	2	11.17+0.72	2.29+0.59	18.60+0.59	10.56+0.05
collecting duct cell	5	11.66+0.49	4.12+0.87	18.45+0.76	9.79+0.23

found strong MMP-7 expression in the epithelium and weaker expression in the stroma of normal peritumoral and neoplastic areas of the human renal epithelial tumors studied. Of the 95 RCCs investigated, 93 expressed MMP-7.

In line with previous data on the localization of MMP-7 expression in human (7) and mouse tissue (28, 33), we detected MMP-7 staining mainly in the cytoplasm of the epithelial cancer cells and less in interstitial or inflammatory cells. We also detected significantly higher–levels in the epithelial tissue of malignant and non-malignant perineoplastic areas than in the stroma. This localization agrees with previous immunohistochemical and *in situ* hybridization studies in RCCs (9-10) and other tumors (35), including breast (13), describing MMP-7 localized specifically in the tumor cells and less in the stroma, but disagrees with Heppner *et al* (36) who found MMP-7 focally localized in the fibroblasts of breast tumor cells. A possible reason why MMP-7 tissue localization differs might be RCC heterogeneity. The divergent findings in the various studies may also be partly explained by differences between the antibodies used in immunohistochemistry. In this study, we used an antibody against the MMP-7 proform, recognizing both the proactive (inactive) and active forms of MMP-7. The intracellular and extracellular localization of MMP-7 we describe in human RCCs suggests that the secretory protein MMP-7 is

Table III. *Matrix metalloproteinase-7 (MMP-7) expression according Gleason histological grade and TNM stage of renal-cell carcinomas: comparison between epithelium and stroma. The percentage of MMP-7 positive cells was significantly higher in epithelium and stroma of grade III than in grade II renal-cell carcinoma and in T3 than in T1 stage(p<.01 by Student's t test).*

		MMP-7	
No of cases	**85**		
grade score		***epithelium***	***stroma***
grade I	23	32.85±1.29	5.43±1.33
grade II	37	31.24±1.41	8.35±1.25
grade III/IV	25	37.02±2.33	16.94±1.32
TNM			
T1	32	31.46±1.11	3.86±0.88
T2	14	31.59±2.25	8.28±1.90
T3	39	36.05±1.73	15.85±1.10

produced in epithelial cells and then secreted into the extracellular matrix.

An interesting finding on the distribution of MMP-7 expression in the renal epithelial tumor samples evaluated in this immunohistochemical study was that MMP-7 levels were higher in dysplastic tubules and in benign epithelial neoplasias (renal papillary and oncocytomas) than in the surrounding normal renal tissue. This specific distribution pattern suggests that MMP-7 is produced by normal renal epithelial cells and kept by these cells at low basal levels. In line with other reports in prostatic neoplasia (7, 29) and in gastric cancer (25), in RCCs, we found that MMP-7 was expressed not only in cancer cells, but also in dysplastic and benign lesions, indicating that MMP-7 was already apparent as a precancerous event. This constitutive pattern of expression indicates that rather than intervening merely in the remodelling process, as well as having a well-characterized role in tumor invasion, MMP-7, probably as in prostate cancer (7), may also be important in initiating or promoting RCCs.

MMP-7 staining differed significantly in intensity among the various RCC histological subtypes. We detected the highest MMP-7 protein levels in the chromophobic RCC subtypes, followed by the conventional clear-cell and chromophilic-papillary RCC subtypes, and the lowest levels in the highly aggressive spindle-cell and collecting-duct RCC types studied. Whereas we found no significant difference in MMP-7 expression between clear-cell and chromophilic-papillary or chromophobic RCC subtypes, the percentage area stained for MMP-7 was significantly lower in spindle-cell and collecting-duct than in clear-cell, chromophilic and chromophobic RCC subtypes both in tumoral and peritumoral normal areas (P<01 by chi-square test). In a study investigating mRNA expression of MMPs in RCC subtypes, Hagemann et al (8) showed stronger expression of most MMPs in clear-cell than in the papillary RCC, with the exception of MMP-2 and MMP-14 for which they found no detectable differences. In the same study, clear-cell RCCs were associated with a high mRNA MMP-9 concentration, whereas chromophilic-papillary-cell RCCs exhibited higher mRNA MMP-1 expression in malignant and non-malignant tissues. These different results may be explained by the different techniques used. Unlike the immunohistochemical technique we used, the quantitative RT-PCR technique Hagermann et al used for measuring mRNA expression, provided no information on the amount of protein expressed, in particular of MMP-7 protein. Studies conducted by others (9-10) reported that chromophilic-papillary RCC showed significantly stronger MMP-2 expression than clear-cell RCC, whereas the highly aggressive sarcomatoid RCC displayed the least immunoreactivity among the studied types.

The higher MMP-7 protein expression we found in the chromophobic RCC than in clear-cell and chromophilic-papillary RCC subtypes confirms that the chromophobic RCC type is the least aggressive among all RCC types. In contrast, the lower MMP-7 protein expression we found in spindle-cell and collecting-duct RCC subtypes indicate that these RCCs are more aggressive than the other types. Hence, our findings along with current knowledge suggest that MMP-7 is associated with the malignant RCC phenotype and indicate that MMP-7 may be a marker of better differentiation and a relatively favorable prognosis. Whether it acts to inhibit tumor spreading is an interesting question for future studies to clarify. Our study agrees with Leinonen et al (37), who founded high MMP-7 expression in less aggressive non small cell lung cancer.

In accordance with the only two similar previous

studies (8, 29), we detected a significant correlation between high histological grade and advanced tumor stages. This increased expression of MMP-7 in high-grade and advanced-stage RCC, as reported in many other tumors including astrocytoma (17), gastric (14, 24-25, 38), colon (26), esophageal (23), ovarian (20), squamous cell (19) and prostate (7, 14, 20) and lung (37, 39) cancers, may be associated with increased depth of tumor invasion and metastasis.

In conclusion, MMP-7 expression in human RCCs differs according to the histological type, grade and stage, suggesting that MMP-7 may be associated with the histomorphological features of RCC subtypes and their respective biological behavior.

ACKNOWLEDGEMENTS

This work was supported by research grants from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (M.U.R.S.T.), University of Rome "La Sapienza" (Progetto Ateneo- Facoltà 2004 e 2005). We thank L. Luci for technical assistance.

REFERENCES

1. **McCawley L.J. and L.M. Matrisian.** 2000. Matrix metalloproteinases: multifunctional contributor to tumor progression. *Mol. Med. Today 6(4):149.*
2. **Coussens L.M., B. Fingleton and L.M. Matrisian.** 2002. Matrix metalloproteinases, inhibitors and cancer: trials and tribulations. *Science 295:2387.*
3. **Egeblab M. and Z. Werb.** 2002. New functions for the matrix metalloproteinases in cancer progression. *Nat. Rev. Cancer 2:161.*
4. **Zucker S. and J Vacirca.** 2004. Role of matrix metalloproteinases (MMPs) in colorectal cancer. *Cancer and metastasis Reviews 23:101.*
5. **Li H.C., D.C. Cao, Y. Liu, Y.F. Hou, J. Wu, J.S. Lu, G.H. Di, G. Liu, F.M. Li, Z.L. Ou, C. Jie, Z.Z. Shen and Z.M. Shao.** 2004. Prognostic value of matrix metalloproteinases (MMP-2 and MMP-9) in patients with lymph node-negative breast carcinoma. *Breast Cancer Research and Treatment 88:75.*
6. **Sood A.K., M.S. Fletcher, J.E. Coffin, M.Yang, E.A. Seftor, L.M. Gruman, D.M. Gershenson and M.J.C. Hendrix.** 2004. Functional role of matrix metalloproteinases in ovarian tumor cell plasticity. *Am J. Obstet. Gynaec. 190:899.*
7. **Cardillo M.R., F. Di Silverio and V. Gentile.** 2006. Quantitative immunohistochemical and in situ hybridization analysis of metalloproteinases in prostate cancer. *Anticancer Research 26(2A):973.*
8. **Hagemann T., B. Gunawan, M. Schulz, L. Fuzebi, and C. Binder.** 2001. mRNA expression of matrix metalloproteinases and their inhibitors differs in subtypes of renal cell carcinomas. *European J. Cancer 37:1839.*
9. **Kallakury B.V.S., S. Karikehalli, A. Haholu, E. Christine, C.E. Sheehan, N. Azumi and J.S. Ross.** 2001. Increased expression of matrix metalloproteinase 2 and 9 and tissue inhibitors of metalloproteinases-1 and 2 correlate with poor prognostic variables in renal cell carcinoma. *Clin. Cancer Research 7:3113.*
10. **Abdel-Wahed M.M., N.Y. Asaad and M. Aleskandarany.** 2004. Expression of matrix metalloproteinase-2 in renal cell carcinoma. *J. of the Egyptian Nat. Cancer Inst. 16(3):168.*
11. **Wilson C.L. and L.M. Matrisian.** 1996. Matrilysin: An epithelial matrix metalloproteinase with potentially novel functions. *Int. J. Biochem. Cell Biol. 28:123.*
12. **Andreasen P.A., L. Kjoller, L. Christensen and M.I. Duffy.** 1997. The urokinase-type plasminogen activator system in cancer metastasis: a review. *Int. J. Cancer 72:1.*
13. **Mylona E., A. Kapranou, J. Mavrommatis, S. Markaki, A. Keramopoulos and L. Nakopoulou.** 2005. The multifunctional role of the immunohistochemical expression of MMP-7 in invasive breast cancer. *APMIS 113:246.*
14. **Sillanpaa S.M., M.A. Anttila, K.A. Voutilainen, K.M. Ropponen, R.K. Sironen, S.V. Saarikoski and V.M. Kosma.** 2006. Prognostic significance of matrix metalloproteinase-7 in epithelial ovarian cancer and its relation to β-catenin expression. *Int. J. Cancer 119:1792.*
15. **Aihara R., E. Mochiki, Y. Kamiyama, T. Ohno, T. Asao and H. Kuwano.** 2006. Matrilysin expression is a useful marker of submucosal invasion and lymph node metastasis in early stage signet ring cell carcinoma of the stomach. *J. of Surgical Oncology 93:491.*

16. **Lee K.H. S.J. Shin, K.O. Kim, M.K. Kim, M.S. Hyun, T.N. Kim, B.I. Jang, S.W. Kim, S.K. Song, H.S. Kim, S.H. Bae and H.M. Ryoo.** 2006. Relationship between E-cadherin, matrix metalloproteinase-7 gene expression and clinicopathological features in gastric carcinoma. *Oncol. Rep. 16(4):823.*

17. **Lu Z., Y. Wang, Q. Zhang, X. Zang, S. Wang, H. Xie, Y. Li, B. Jiao and J. Zhang.** 2006. Association between the functional polymorphism in the matrix metalloproteinase-7 and susceptibility to adult astrocytoma. *Brain Research 1118:6.*

18. **Li M., H. Yamamoto, Y. Adachi, Y. Maruyama and Y. Shinomura.** 2006. Role of matrix metalloproteinase-7 (matrylisin-1) in human cancer and angiogenesis. *Exp. Biol. Med. 231:20.*

19. **Impola U., V.J. Uitto, J. Hietanen, L. Hakkinen, L. Zhang, H. Larjava, K. Isaka and U. Saarialho-Kere.** 2004. Differential expression of matrilysin-I (MMP-7), 92kD gelatinase (MMP-9), and metalloelastase (MMP-12) in oral verrucous and squamous cell cancer. *J. Pathol. 202:14.*

20. **Ouyang X.S., X. Wang, D.T.W. Lee, S.W. Tsao and Y.C. Wong.** 2001. Up-regulation of TRPM-2, MMP-7 and ID-1 during sex hormone-induced prostate carcinogenesis in Noble rat. *Carcinogenesis 22(6): 965.*

21. **Miyamoto S., K. Yano, S. Sugimoto, G. Ishii, T. Hasebe, Y. Endoh, K. Kodama, M. Goya, T. Chiba and A. Ochiai.** 2004. Matrix metalloproteinase-7 facilitates insulin-like growth factor bioavailability through its proteinase activity on insulin-like growth factor binding protein 3. *Cancer Res. 64:665.*

22. **Wang F.-Q., J. So, S. Reierstad and D.A. Fishman.** 2005. Matrilysin (MMP-7) promotes invasion of ovarian cancer by activation of progelatinase. *Int. J. Cancer 114(1):19.*

23. **Tanioka Y., T. Yoshida, T. Yagawa, Y. Saiki, S. Takeo, T. Harada, T. Okazawa, H. Yanai and K. Okita.** 2003. Matrix metalloproteinase-7 and matrix metalloproteinase-9 are associated with unfavourable prognosis in superficial oesophageal cancer. *Br. J. Cancer 89:2116.*

24. **Saiton Y., H. Yanai, S. Higaki, H. Nohara, T. Yoshida and K. Okita.** 2004. Relationship between matrix metalloproteinase-7 and pit pattern in early stage colorectal cancer. *Gastrointest. Endosc. 59(3):385.*

25. **Kiton T., H. Yanai, Y. Saitoh, Y. Nakamura, Y. Matsubara, H. Kitoh, T. Yoshida and K. Okita.** 2004. Increased expression of matrix metalloproteinase-7 in invasive early gastric cancer. *J. Gastroenterol. 39:434.*

26. **Shiomi T. and Y. Okada.** 2003. MT1-MMP and MMP-7 in invasion and metastasis of human cancer. *Cancer Metastasis Rev. 22:145.*

27. **Wielockx B., C. Libert and C. Wilson.** 2004. Matrilysin (matrix metalloproteinase-7): a new promoting drug target in cancer and inflammation?. *Cytokine Growth Factor Rev. 15:111.*

28. **Surendran K., T.C. Simon, H. Liapis and J.K. McGuire.** 2004. Matrilysin (MMP-7) expression in renal tubular damage: Association with *Wnt4*. *Kidney Int. 65:2212.*

29. **Sumi T., T. Nakatani, H. Yoshida, Y. Hyun, T. Yasui, Y. Matsumoto, F. Nakagawa, K. Sugimura, H. Kawashima and O. Ishlkn.** 2003. Expression of matrix metalloproteinases 7 and 2 in human renal cell carcinoma. *Oncol. Rep. 10(3):567.*

30. **American Joint Committee on Cancer**. *Cancer Staging Manual 6th ed. 2002,* Lippincott-Raven, Philadelphia, p. 1.

31. **Eble J.N., G. Sauter, J.I. Epstein and I.A. Sesterhenn.** 2004. World Health Organization (WHO) classification of tumours: pathology and genetics of tumours of the urinary system and male genital organs. *Lyons: IARC Press.*

32. **Fuhrmann S.A., L.C. Lasky and C. Limas.** 1982. Prognostic significance of morphologic parameters in renal cell carcinoma. *Am. J. Surg. Pathol. 6:655.*

33. **Ouyang X.S., D.T.W. Lee, S.W. Tsao and Y.C. Wong.** 2001. Up-regulation of TRPM-7, MMP-7 and ID-1 during sex hormone-induced prostate carcinogenesis in the noble rat. *Carcinogenesis 22(6):965.*

34. **Cardillo M.R. and F. Ippoliti.** 2006. IL-6, IL-10 and HSP-90 expression in tissue microarrays from human prostate cancer assessed by computer-assisted image analysis. *Anticancer Research 26:3409.*

35. **Tuszynski J.A.** 2001. The role of matrix metalloproteinases in tumor angiogenesis and tumor metastasis. *Pathol. Oncol. Res. 7:14.*

36. **Heppner K.J., L.M. Matrisian, R.A. Jensen and**

W.H. Rodgers. 1996. Expression of most matrix metalloproteinase family members in breast cancer represents a tumor-induced host response. *Am. J. Pathology 149:273.*

37. **Leinonen T., R. Pirinen, J. Bohm, R. Johansson, K. Ropponen and V.M. Kosma.** 2006. Expression of matrix metalloproteinase 7 and 9 in non-small cell lung cancer. Relation to clinicopathological factors, beta-catenin, and prognosis. *Lung Cancer 51 (3):313.*
38. **Ajisaka H., Y. Yonemura and K. Miwa.** 2004. Correlation of lymph-node metastases and expression of matrix metalloprotreinase-7 in patients with gastric cancer. *Hepato-Gastroenterology 51:900.*
39. **Kren L., V.N. Goncharuk, Z. Krenova, D. Stratil, M. Hermanova, J. Skrickova, C.E. Sheehan and J.S. Ross.** 2006. Expression of matrix metalloproteinases 3, 10 and 11 (stromelysins 1, 2 and 3) and matrix metalloproteinase (matrilysin) by cancer cells in non-small cell lung neoplasms. Clinicopathologic studies. *Cesk Patol. 42(1):16.*