

## Expression of Matrix Metalloproteinase-2 in Renal Cell Carcinoma

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### ABSTRACT

**Background:** Renal cell carcinoma (RCC) is a family of carcinomas arising from the renal tubular epithelium through different genetic lesions. The most common characteristics of RCC are hypervascularity, tendency to metastasize widely before giving rise to any symptoms or signs, and poor prognosis. It has been reported that matrix metalloproteinases (MMPs) are a family of endopeptidases implicated in tissue remodeling and cancer invasion. Therefore, the aim of the present study was to investigate the expression of MMP-2 in RCC and correlate its expression with the clinicopathologic prognostic parameters.

**Material and Methods:** Fifty cases of primary RCC comprised the material of this study. Formalin-fixed paraffin-embedded tissues were stained with MMP-2 immunostaining, using improved streptavidin-biotin amplified system. The intensity of staining and percentage of positive cells were assessed.

**Results:** MMP-2 was expressed as diffuse brown cytoplasmic staining in positive cells. The non-tumorous renal tissue showed negative staining whereas the tumor cells, fibroblasts and vascular endothelial cells showed immunoreactivity in positive cases. 43/50 RCC cases studied were positive for MMP-2. A positive correlation was observed between MMP-2 expression and tumor size, histologic type, capsular & vascular invasion and high levels of cellular proliferation.

**Conclusion:** It seems that MMP-2 is involved in tumor expansion phenomena associated with tumor progression, invasion of the microvasculature and distant metastasis of RCC.

**Key words:** Renal cell carcinoma - Matrix metalloproteinase-2 - Immunohistochemistry.

### INTRODUCTION

The kidney is the site for both benign and malignant tumors. With the exception of oncocytoma, benign tumors are incidental findings at autopsy and are rarely of clinical significance. On the other hand, malignant tumors are of

great clinical importance. By far, the most common of these tumors is renal cell carcinoma (RCC) [1]. It represents about 1-3% of all visceral cancers; however, it accounts for 85-90% of all renal tumors in adults [2].

The classification of RCC has been revised recently, based on correlative cytogenetic, genetic and histologic studies of both familial and sporadic tumors [3]. The major types in descending order of frequency are conventional (clear cell) renal cell carcinoma, papillary renal cell carcinoma, chromophobe renal cell carcinoma and collecting duct carcinoma. Sarcomatoid changes arise infrequently in all types of RCC and are decidedly an ominous feature of these tumors [4]. The most common characteristics of RCC are hypervascularity, tendency to metastasize widely before giving rise to any symptoms or signs, and poor prognosis [5]. It is generally believed that one key element of the metastatic process is enhanced proteolysis of both basement membrane and stromal extracellular matrix [6]. One family of enzymes that has been shown over the years to play a role in tumor progression is the matrix metalloproteinase family [7].

MMPs are zinc-dependent endopeptidases known for their ability to cleave one or more extracellular matrix (ECM) constituents [8]. They now include more than 20 related enzymes. Ample evidence exists on the role of MMPs in normal and pathological processes, including embryogenesis, parturition, wound healing, inflammation, and cancer [9]. A positive correlation between tumor progression and expression of multiple MMP family members in tumor tissues has been demonstrated in numerous animal and human tumors [10]. For instance,

expression of MMP-2 immunoreactive protein has been associated strongly with shortened survival, independent of the major prognostic indicators in patients with primary breast carcinoma [11]. Moreover, MMP-2,-9,-13 are involved in the neoangiogenetic and focal clonal selection and expansion phenomena associated with in situ tumor progression, invasion of the microvasculature and metastasis in cases of lung carcinoma [12]. It has been suggested that MMPs expression is correlated not only with the aggressiveness of renal cell carcinoma, but also with the histologic tumor type [5]. This association of MMPs with cancer progression and metastasis has raised considerable interest because they represent an attractive target for development of novel antimetastatic drugs aimed at inhibiting MMP activity [13].

Therefore, this work was planned to investigate the expression of MMP-2 immunoreactive protein in cases of RCC, and its correlation with the histopathological findings and other possible clinical data, using the immunohistochemical staining technique.

## MATERIAL AND METHODS

This study included 50 archival cases of primary RCC, obtained from the files of the department of pathology, Faculty of Medicine, Menoufyia University. They were randomly selected, based on the availability of paraffin-embedded blocks for serial cutting and examination. Clinicopathological data were obtained from patients' medical records and included age, sex, tumor size, tumor side (right or left), lymph node status and presence of metastasis. From each representative paraffin block, three contiguous 4- $\mu$ m-thick sections were cut and mounted on glass slides, one slide for routine hematoxylin and eosin (H & E) staining, and two on poly-L-lysine-precoated slides for immunostaining.

### I- Histopathologic Examination:

Histopathological examination of H&E-stained sections was performed to confirm the diagnosis. Tumors were classified as conventional, papillary, chromophobe, and RCC unclassified (sarcomatoid) [4], graded according to Fuhrman's grading system [14], and staged according to modified TNM criteria for RCC

[15,16]. Also, mitotic and apoptotic figures were counted for each case and the mitotic (MI) and apoptotic (AI) indices were calculated [17,18].

### II- Immunohistochemistry:

Two paraffin sections from each case were stained by the immunohistochemical method (one test slide and one negative control slide for each case). The improved streptavidin-biotin amplified system was used in this work. The primary antibody was a purified mouse monoclonal antibody (NeoMarkers' cat.# Ap9003, U.S.A.) raised against MMP-2/72 KDa (collagenase IV/ Gelatinase-A, Ab-4) antigen. This antibody recognizes a protein of MW72 KDa which is identified as pro (latent) and active forms of matrix metalloproteinase-2. It is received as a "ready to use" solution in a 7ml. vial. NeoMarkers gelatinase-A shows no cross-reaction with either latent or active forms of other MMPs.

### Steps of Staining:

The sections were deparaffinized in xylene and re-hydrated by immersion in descending grades of ethanol (100%, 90%, 70%) and finally, in distilled water. To reduce non-specific background staining due to endogenous peroxidase, the sections were incubated in hydrogen peroxide (3% H<sub>2</sub>O<sub>2</sub> in absolute methyl alcohol). The heat-induced epitope retrieval (HIER) procedure was used. Ultra V Block was applied and slides were incubated for 5 minutes in a humidity chamber. Monoclonal mouse MMP-2 antibody was applied on the tissue sections in a humidity chamber over night, at room temperature. Negative control slides were prepared, by omitting the primary antibody from the staining procedure. The substrate-chromogen (Diaminobenzidine, DAB) was applied. Then, counterstaining was done using Mayer's hematoxylin (Bio Genex, cat. No. 94583, U.S.A.). Dehydration of the tissues was done in ascending grades of ethanol (70%, 90%, 100%) and finally cleared in xylene. The sections were mounted with Canada Balsam and covered by a cover slip.

### MMP-2 Immunostaining Interpretation:

Both the intensity of staining and the percentage of MMP-2 positive tumor cells were considered in a semi-quantitative assessment. Positive staining was identified when the cytoplasm showed brown staining, whereas, nega-

tivity was considered when no cytoplasmic staining was noticed. The distribution of positive staining in tumor tissue was graded as focal (<10%), regional (11-50%), or diffuse (>50%). The staining intensity was subjectively scored as weak, moderate, or intense [5].

### III- Statistical Analysis:

The data were collected, tabulated and statistically analyzed, using a personal computer with "Statistical Package for the Social Sciences (SPSS), version 11" software program with the level of significance at 0.05 [19].

## RESULTS

This study comprised 50 cases of primary renal cell carcinoma. The patients were 31 (62%) males, and 19 (38%) females, with a male to female ratio of 1.6:1. Their ages ranged from 37 to 79 years, with a mean of 52.12±10.3 years. Gross pathological data of the specimens revealed a single renal mass in all cases. The clinicopathologic data of the studied RCC cases are presented in Table (1). We put sarcomatoid changes as a separate entity because the cases studied showed extensive sarcomatoid changes without recognizable epithelial elements.

Table (1): Clinicopathologic data of the studied RCC cases.

Variables	Histologic tumor type				Test of significance	p value
	Conventional (28)	Papillary (9)	Chromophobe (7)	Sarcomatoid (28)		
Age of patients in years (Mean ± S.D.)	52.29±8.38	59±12.53	48.86±11.71	44.83±9.11	2.86*	0.047
<b>Tumor side:</b>						
Right No. (%)	13 (46.4%)	4 (44.4%)	2 (28.6%)	4 (66.7%)	1.898**	0.549
Left No. (%)	15 (53.6%)	5 (55.6%)	5 (71.4%)	2 (33.3%)		
<b>Tumor size:</b>						
≤ 7 cm. No. (%)	6 (21.4%)	4 (44.4%)	2 (28.6%)	1 (16.7%)	2.191**	0.53
> 7 cm. No. (%)	22 (78.6%)	5 (55.6%)	5 (71.4%)	5 (83.3%)		
<b>Tumor grade:</b>						
I & II. No. (%)	17 (60.7%)	6 (66.7%)	7 (100%)	0 (0%)	13.84**	0.003 <sup>a</sup>
III & IV. No. (%)	11 (39.3%)	3 (33.3%)	0 (0%)	6 (100%)		
<b>Pathologic stage:</b>						
T1: No. (%)	7 (25.0%)	3 (33.3%)	2 (28.6%)	1 (16.7%)	2.97**	0.40
T2: No. (%)	18 (64.3%)	4 (44.4%)	3 (42.9%)	5 (83.3%)		
T3: No. (%)	3 (10.7%)	2 (22.2%)	2 (28.6%)	0 (0%)		
<b>Nodal status:</b>						
Negative: No (%)	27 (96.4%)	8 (88.9%)	7 (100%)	4 (66.7%)	6.906**	0.05 <sup>a</sup>
Positive: No.(%)	1 (3.6%)	1 (11.1%)	0 (0.0%)	2 (33.0%)		
<b>Microvascular invasion:</b>						
Negative: No.(%)	22 (78.6%)	7 (77.8%)	4 (57.1%)	2 (33.3%)	5.631**	0.702
Positive: No. (%)	6 (21.4%)	2 (22.2%)	3 (42.9%)	4 (66.7%)		
<b>Capsular invasion:</b>						
Negative: No.(%)	16 (57.1%)	5 (55.6%)	3 (42.9%)	2 (33.3)	0.747**	0.862
Positive: No. (%)	12 (42.9%)	4 (44.4%)	4 (57.1%)	4 (66.7%)		
<b>Mitotic index (MI):</b> (mean ± S.D.)	[1] 0.12±0.09	[2] 0.07±0.08	[3] 0.04±0.05	[4] 0.36±0.08	19.81***	0.001 <sup>a</sup>
<b>Apoptotic index AI:</b> (mean ± S.D.)	[1] 0.40±0.18	[2] 0.58±0.23	[3] 0.24±0.06	[4] 0.16±0.12	8.14***	0.001 <sup>a</sup>

\* : F test. \*\* : Chi-Square test. \*\*\* : Kruskal-Wallis test.

p Value for AI between different groups:

1&2 = 0.01 1&3 = 0.04 1&4 = 0.006

a p-values ≤ 0.05 are considered significant.

p Value for MI between different groups:

2&3 = 0.42 2&4 = 0.001

2&3 = 0.001 2&4 = 0.001

**Immunohistochemistry:**

Regarding MMP-2 immunostaining in this work, the non-tumorous renal tissue (parenchymal and stromal) in all sections showed no MMP-2 immunoreactivity, hence considered as negative control. In the MMP-2 positive sections, immunostaining was observed in tumor cells, stromal fibroblasts, as well as in vascular endothelial cells. However, the tumor cells displayed moderate - to - intense positivity as opposed to relatively weaker expression in the stromal fibroblasts. MMP-2 positivity was noticed to be higher at the tumor/stromal interface than other tumor zones. Positive MMP-2 immunostaining was observed in 43/50 (86%) of the studied RCC cases. Regarding intensity of staining, 20/43 cases (46.5%) were weakly stained, 5/43 cases (11.7%) were moderately stained, and 18/43 cases (41.8%) were intensely stained. For the distribution of MMP-2 immunostaining, 15/43 cases (34.9%) were focally stained, 8/43 cases (18.6%) were regionally stained, and 20/43 cases (46.5%) were diffusely stained, (Table 2, Figures 1-5).

Regarding MMP-2 expression, a significant correlation was observed with tumor size, histologic types, microvascular and capsular inva-

sion (Table 3). Moreover, a significant positive correlation was observed between intensity of MMP-2 immunostaining and histologic tumor type and stage of RCC cases studied (Table 4). Also, a significant correlation was noticed between the distribution of MMP-2 immunostaining and the histologic tumor types as well as tumor stage of RCC studied cases (Table 5). MMP-2 positive cases displayed higher mean MI than MMP-2 negative ones and the difference was statistically significant. However, there was no significant difference between the two groups regarding the mean AI (Table 6).

Table (2): Results of MMP-2 immunostaining in the studied RCC cases.

Variable	Number (50)	%
<i>MMP-2 expression:</i>		
Positive	43	86
Negative	7	14
<i>Intensity of MMP-2 staining in positive cases:</i>		
Weak	20	46.5
Moderate	5	11.6
Intense	18	41.8
<i>Distribution of MMP-2 staining in positive cases:</i>		
Focal	15	34.9
Regional	8	18.6
Diffuse	20	46.5

Table (3): Correlation between MMP-2 expression and clinicopathologic variables in RCC cases studied.

Variable	Expression		Test of significance (Chi-Square)	p value
	Negative (N=7)	Positive (N=43)		
<i>Tumor Size:</i>				
≤ 7 cm. No. (%)	4 (30.7%)	9 (69.3%)	0.928	0.05*
> 7 cm. No. (%)	3 (8.1%)	34 (91.9%)		
<i>Histologic type:</i>				
Conventional No. (%)	3 (10.7%)	25 (89.3%)	6.249 <sup>⊕</sup>	0.053*
Papillary No. (%)	0 (0.0%)	9 (100%)		
Chromophobe No. (%)	1 (13.3%)	6 (85.7%)		
Sarcomatoid No. (%)	3 (50.0%)	3 (50.0%)		
<i>Nodal Status:</i>				
Negative (%)	7 (15.2%)	39 (84.8%)	0.708	0.4
Positive (%)	0 (0%)	4 (100%)		
<i>Microvascular invasion:</i>				
Negative (%)	7 (20%)	28 (80%)	3.488	0.05*
Positive (%)	0 (0%)	15 (100%)		
<i>Capsular invasion:</i>				
Negative (%)	7 (26.9%)	19 (73.1%)	7.51	0.006*
Positive (%)	0 (0%)	24 (100%)		
<i>Grade:</i>				
I & II: No. (%)	4 (13.3%)	26 (86.7%)	0.03	0.87
III & IV: No. (%)	3 (15%)	17 (85%)		
<i>Stage:</i>				
T1: No. (%)	2 (15.4%)	11 (84.6%)	1.34	0.51
T2: No. (%)	5 (16.7%)	25 (83.3%)		
T3: No. (%)	0 (0%)	7 (100%)		

<sup>⊕</sup> Fisher exact test

\*p-values ≤ 0.05 are considered significant.

Table (4): Intensity of MMP-2 immunostaining in different histologic tumor types, grades and stages.

Histologic tumor type	No.	Intensity				Test of significance (Chi-Square)	p value
		Negative (7)	Weak (20)	Moderate (5)	Intense (18)		
<i>Histologic tumor type:</i>							
Conventional No. (%)	28 (100%)	3 (10.7%)	8 (28.6%)	3 (10.7%)	14 (50.0%)	17.0	0.04*
Papillary No. (%)	9 (100%)	0 (0.0%)	4 (44.4%)	1 (11.1%)	4 (44.4%)		
Chromophobe No. (%)	7 (100%)	1 (14.3%)	5 (71.4%)	1 (14.3%)	0 (0.0%)		
Sarcomatoid No. (%)	6 (100%)	3 (50.0%)	3 (50.0%)	0 (0.0%)	0 (0.0%)		
<i>Grade:</i>							
I & II. No. (%)	26 (100%)	—	12 (46.2%)	5 (19.2%)	9 (34.6%)	4.1	0.02*
III & IV. No. (%)	17 (100%)	—	8 (47.1%)	0 (0%)	9 (52.9%)		
<i>Stage:</i>							
T1: No. (%)	11 (100%)	—	7 (63.6%)	3 (27.3%)	1 (9.1%)	8.3	0.04*
T2: No. (%)	25 (100%)	—	9 (36%)	2 (8%)	14 (56%)		
T3: No. (%)	7 (100%)	—	4 (57.1%)	0 (0%)	3 (42.9%)		

\*p-values ≤ 0.05 are considered significant.

Table (5): Distribution of MMP-2 immunostaining in different histologic tumor types, grades and stages.

Histologic tumor type	No. (%)	Distribution				Test of significance (Chi-Square)	p value
		Negative (7)	Focal (15)	Regional (8)	Diffuse (20)		
<i>Histologic tumor type:</i>							
Conventional No. (%)	28 (100)	3 (10.7%)	6 (21.4%)	5 (17.9%)	14 (50.0%)	15.5 <sup>⊕</sup>	0.029*
Papillary No. (%)	9 (100)	0 (0.0%)	2 (22.2%)	3 (33.3%)	4 (44.4%)		
Chromophobe No. (%)	7 (100)	1 (14.3%)	4 (57.1%)	0 (0.0%)	2 (28.6%)		
Sarcomatoid No. (%)	6 (100)	3 (50.0%)	3 (50.0%)	0 (0.0%)	0 (0.0%)		
<i>Grade:</i>							
I & II. No. (%)	26 (100)	—	8 (30.7%)	7 (26.9%)	11 (42.3%)	3.0	0.22*
III & IV. No. (%)	17 (100)	—	7 (41.1%)	1 (5.9%)	9 (52.9%)		
<i>Stage:</i>							
T1: No. (%)	11 (100)	—	5 (45.5%)	4 (36.4%)	2 (18.2%)	8.3	0.05*
T2: No. (%)	25 (100)	—	6 (24.5%)	4 (16.0%)	15 (60%)		
T3: No. (%)	7 (100)	—	4 (57.1%)	0 (0.0%)	3 (42.9%)		

⊕ Fisher exact test \*p-values ≤ 0.05 are considered significant.

Table (6): Correlation between MMP-2 expression and mean mitotic and apoptotic indices.

Variable	MMP-Expression		Test of significance t-test	p value
	Positive	Negative		
<i>Mitotic index:</i>				
MI±SD	0.24 ± 0.15	0.114 ± 0.11	2.27	0.009*
<i>Apoptotic index:</i>				
AI±SD	0.27 ± 0.42	0.39 ± 0.22	1.29	0.2

\*p-values ≤ 0.05 are considered significant.

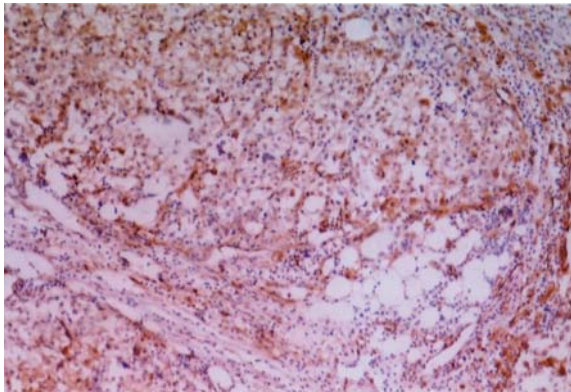


Fig. (1): Renal cell carcinoma, conventional type, grade I, MMP-2 immunostaining: The tumor cells show intense cytoplasmic staining with diffuse pattern of distribution, immunoperoxidase with Mayer's haematoxylin. (x 100).

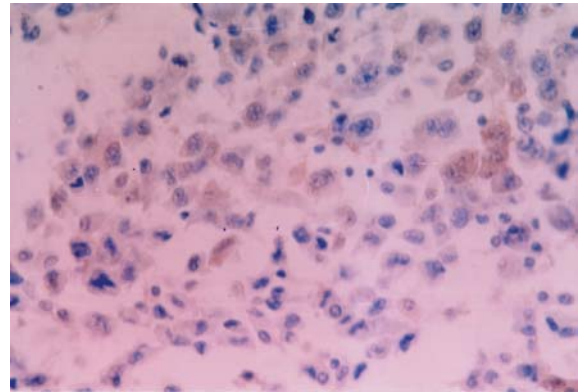


Fig. (4): Renal cell carcinoma, sarcomatoid type, grade III, MMP-2 immunostaining: The tumor cells' cytoplasm show weak positivity with focal pattern of distribution, immunoperoxidase with Mayer's haematoxylin (x 400).

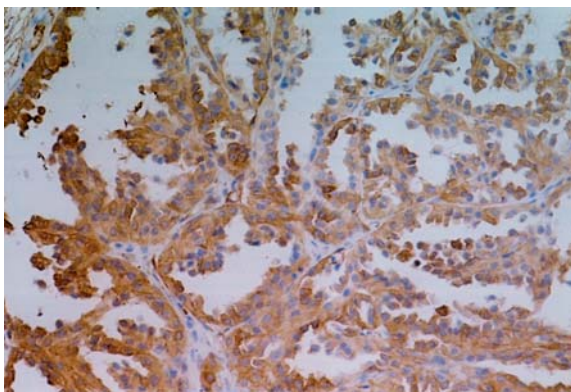


Fig. (2): Renal Cell Carcinoma, Papillary type, Grade II, MMP-2 Immunostaining: The staining is confined to the cell cytoplasm with no nuclear staining, immunoperoxidase with Mayer's haematoxylin (x 200).

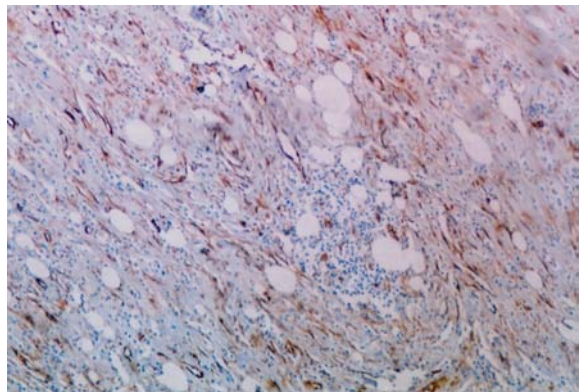


Fig. (5): Tumor stromal cells showing weak positive MMP-2 immuno-staining, immunoperoxidase with Mayer's haematoxylin (x 100).

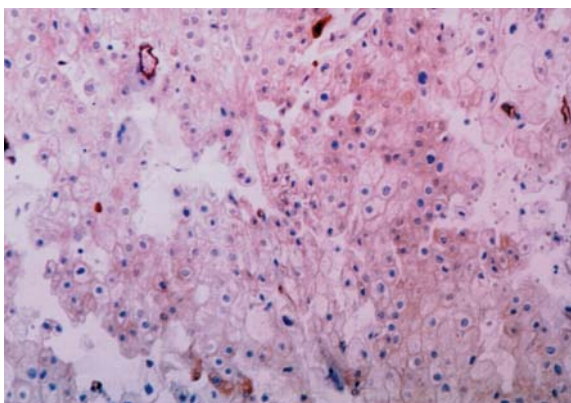


Fig. (3): Renal cell carcinoma, chromophobe type, grade II: The malignant cells show weak MMP-2 positivity with diffuse pattern of distribution, immunoperoxidase with Mayer's haematoxylin (x 400).

## DISCUSSION

MMP-2 (gelatinase-A, collagenase IV) is an MMP family member that is capable of degrading collagens type I, IV and V, besides fibrillar collagens and gelatins [20]. A unique structural feature of MMP-2 is the presence in its catalytic domain of "fibronectin-like domain", that is responsible for collagen binding and subsequent collagen degradation [21].

Regarding MMP-2 immunostaining in this work, the non-tumorous renal tissues both parenchymal and stromal, showed no MMP-2 immunoreactivity. This finding is consistent with the fact that MMPs are produced in response to a variety of cytokines and growth factors [22]. Gohji and associates [23] stated that

MMP-2 production in human RCC is regulated by basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and transforming growth factor beta-1 (TGF-beta-1). In the MMP-2 positive sections, the tumor cells, the stromal cells, and the vascular endothelial cells all showed positive cytoplasmic expression. The tumor cells displayed moderate - to - intense positivity as opposed to relatively weaker expression in the stromal fibroblasts. These findings are in accordance with previous studies [5,24].

It was reported that MMPs in tumor tissue are produced by the reactive stromal fibroblasts surrounding the tumor cells and not by the tumor cells themselves [25]. An explanation for this came from the discovery of Extracellular Matrix MetalloProteinase INducer, (EM-MPRIN), by Biswas et al. [26]. EMMPRIN is an intrinsic plasma membrane glycoprotein produced in high amounts in cancer cells stimulating stromal fibroblasts to synthesize MMPs.

Despite its stromal production, MMP-2 expression was more in RCC tumor cells. This goes in line with the study conducted by Kallakury et al. [5]. This finding was explained by Zucker et al. [27], who postulated that the tumor cells probably possess docking sites which bind stromal cell-secreted MMP-2 thus functioning as receptacles for the secreted MMP-2. This reinforces Brooks' findings [28] who previously stated that MMP-2 binds to malignant cell  $\alpha v \beta 3$  integrin surface receptors. Another explanation for such epithelial cell localization of MMP-2 is the activation of stromal-cell secreted pro-MMP-2 by the membrane type-1 MMP (MT-MMP-2) bound to the epithelial cell membrane [29].

In this work, the MMP-2 positivity was noticed to be higher at the tumor-stromal interface than other tumor zones. This supports the fact that at the invading edge of the tumor the balance between proteases and antiproteases is tilted in favor of proteases [2]. An explanation to why both tumor and adjacent stromal cells express MMP-2 enzyme may be that both cellular components contribute to the metastatic cascade. The tumor MMP-2 may contribute to the invasive and expansive tumor growth, while stromal MMP-2 contributes to the remodeling process and desmoplastic reaction in the tissues adjacent to the tumor [24].

As RCC, particularly the conventional type, is one of the most vascular of all solid tumors, the markers of pathological neovascularization have been intensely investigated [30]. Many studies have shown that the endothelial cells are capable of differentially expressing and activating MMPs, including MMP-2 [24]. The proteolytic activity of MMPs is required during the capillary bud formation in order for the endothelial cells to migrate out through the precapillary membrane and through the ECM. In addition, capillary elongation, lumen formation, and ECM remodeling all require proteolytic activity [31]. In one study, RCC tumor microvessels expressed MMP-2 and MMP-9 with incomplete pericyte covering in comparison to the tumor free tissue indicating immature active angiogenesis [32]. The VEGF, a major angiogenic factor, was said not only to induce MMP-2 expression but also to activate its latent form [33]. The bFGF plays a similar role [34,35].

Considering the tumor size, a statistically significant difference ( $p=0.05$ ) was noticed regarding MMP-2 expression. Most of the positive cases (79%) were more than 7 cm. It was stated that MMP-2 is involved in the generalized growth and expansion of the neoplastic cell mass in carcinoma of the lung [12]. MMP-2 may play a possible similar role in RCC.

A statistically significant difference ( $p=0.04$ ) was noticed regarding MMP-2 expression in different histologic tumor types, with the highest positivity in the papillary RCC cases, followed by the conventional (clear) RCC, and lastly the chromophobe and sarcomatoid RCC. In the study conducted by Kallakury et al. [5], it was reported that the chromophilic papillary RCC showed a significantly higher predilection to express MMP-2 than the clear cell RCC. However, the highly aggressive sarcomatoid RCC cases in the present study displayed the least positivity among the studied types. To date, no particular study was conducted to evaluate MMP-2 expression in sarcomatoid RCC.

The intensity and distribution of MMP-2 staining were statistically significant ( $p=0.04$ ) among the different histologic types. The conventional type displayed the highest values regarding both MMP-2 intensity and distribution. Fifty percent of the conventional cases showed diffuse & intense MMP-2 staining. However, regarding the weak & focal immun-

ostaining, the chromophobe cases displayed the highest values. These results go in line with Hagemann et al. [36], who reported that MMP-2 was intense in the conventional RCC than the other RCC types. This could be explained not only by its aggressive behavior but also its highly vascular structure compared to the other RCC types [37]. Moreover, the results of MMP-2 immunostaining within the chromophobe RCC cases support the fact that it is the least aggressive among all RCC types [38].

In the current study, advanced pathologic stage showed more MMP-2 expression, intensity and distribution. This goes with kallakury et al. [5] and Sumi et al. [39], who reported that MMP-2 expression was found to be significantly stronger in advanced tumor stage. Thus, MMP-2 overexpression appears to play important roles in initiating metastasis [40].

As regards to the regional lymph node status in this work, though all node positive cases (4/4) showed MMP-2 immunopositivity, these results failed to achieve significance ( $p=0.5$ ). The small number of lymph node positive cases could explain such result.

Considering microscopic vascular and capsular invasion in this study, a statistically significant correlation was observed regarding MMP-2 expression in the studied RCC cases. These results support the key role of MMP-2 in local expansion and infiltration of the tumor cell mass as well as metastatic invasion [24].

Comparison of means of mitotic and apoptotic indices for MMP-2 stained cases revealed a statistically significant difference ( $p=0.009$ ) between MMP-2 positive and negative cases regarding their mitotic index. MMP-2 positive cases displayed higher mean MI than MMP-2 negative ones. This goes with the fact that tumors with high proliferative activity behave more aggressively than those with minimal proliferation [41]. Also, it was reported that the survival of patients was shorter and the chance of metastases was higher in RCCs with higher proliferative activity [42]. Although MMP-2 positive cases displayed lower mean apoptotic index than MMP-2 negative ones (0.22 and 0.39, respectively), their differences failed to achieve significance ( $p=0.2$ ). Richter and associates [43] reported that lower levels of apoptotic cell death in RCC affect not only its growth

rate but also the cellular escape from immune attack. The results of the current study indicate that RCC with increased MMP-2 expression had high levels of cellular proliferation and low levels of apoptotic cell death and these combined factors may assist in the development and progression of cancer [44]. Therefore, these factors should be considered as relevant targets for novel therapeutic strategies [30,45]. Also, matrix metalloproteinase inhibitors (MMPIs) contribute a new class of emerging drugs targeting angiogenesis and metastasis formation through MMP inhibition that could be a possible treatment for controlling the metastatic potential of many tumors including RCC [46].

Thus, it seems that MMP-2 is involved in the tumor expansion phenomena associated with tumor progression, invasion of the microvasculature, and distant metastasis of human RCC. Therefore, from the present study it could be concluded that as MMP-2 expression may contribute to the development and progression of RCC, evaluation of its expression is important in this neoplasm and this might be of help as a target for novel therapeutic strategies for this lethal cancer.

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