

The relationship of mast cells and angiogenesis with prognosis in renal cell carcinoma

Muhammet Emin Guldur,¹ Sezen Kocarslan,² Hasan Ilyas Ozardali,³ Halil Ciftci,⁴ Dilnur Dincoglu,⁵ Kemal Gumus⁶

Abstract

Objective: To evaluate the effects of mast cell count and angiogenesis on the prognosis of renal cell carcinoma.

Methods: The retrospective study was conducted at the Harran University, Sanliurfa, Turkey, and included 64 cases with diagnosis of renal cell carcinoma between 2002 and 2012. Immunohistochemical analysis was performed on paraffin sections using the standard streptavidin-biotin immunoperoxidase method. CD31 antibodies were used to identify microvessels in tumoural tissues. The microvessel density was calculated using a serological method. The mean vascular density was equivalent to the vascular surface area (in mm²) per unit tissue volume (in mm³) (MVD=mm³). Mast cells tryptase antibody was used to evaluate the mast cell count in tumoural and non-tumoural tissues. The relationship between mast cell count and microvessel density was evaluated and compared with stage, grade, tumour diameter, and age.

Results: The mast cell count in the tumoral tissue of renal cell carcinoma was significantly higher compared with non-neoplastic renal tissue ($p<0.001$). A significant relationship was found between the mast cell count in tumoral tissue and stage, grade, and tumour diameter ($p<0.001$). However, no relation was found with age ($p>0.05$). The intratumoural mast cell count in clear cell renal carcinoma was significantly higher compared with non-clear variety ($p=0.001$). No significant relationship was found between microvessel density, age, stage, diameter, or grade of the tumour and tumoral mast cell count ($p>0.05$).

Conclusion: No significant association was found between the number of mast cells in tumoral tissue and microvessel density. Further studies are needed to demonstrate the effect of mast cells on angiogenesis in renal cell carcinoma.

Keywords: Mast cells, Renal cell carcinoma, Microvessel density, Angiogenesis. (JPMA 64: 300; 2014)

Introduction

Renal cell carcinoma (RCC) is the most commonly observed tumour of the kidney and accounts for 3% of all adult cancers.^{1,2} Many factors have prognostic significance in patients with RCC, including histopathological and clinical features and biomolecular markers. However, none of the prognostic parameters reported to be effective in RCC is adequate to predict the clinical behaviour of the tumour by itself.^{3,1} Angiogenesis is defined as the development of new blood vessels. It plays an important role in embryogenesis, wound healing, and tumour growth.⁴ In addition to tumour growth in solid tumours, invasion and metastasis have been reported to be related to angiogenesis.^{2,5,6} Neoplastic cells and the microenvironment surrounding these cells interact with each other. Inflammatory cell infiltration, including mast cells (MCs), occurs in tumour tissue.⁵ An increase in the number of MCs has been detected in neoplasias

originating in many different regions of the body, such as the breast, colon, skin, soft tissue, uterus, larynx, and oral cavity. Evidence for an association between increased MCs and the development of angiogenesis in tumoral tissue was obtained in previous studies.⁶⁻¹¹ Few studies in the literature have evaluated the association of the mast cell carcinoma (MCC) and microvessel density (MVD) in RCC. In those reports, the association of the MCC and MVD were compared with the clinicopathological features of RCC.^{2,12} We also aimed to evaluate the MCC and MVD in RCC and to analyse the relationship of these factors with the clinicopathological features of the disease.

Patients and Methods

The retrospective study comprised data of 64 patients with RCC at the Department of Pathology, School of Medicine, Harran University, Sanliurfa, Turkey. Data related to a 10-year period from 2002 to 2012 was analysed. All patients had been treated with radical nephrectomy. Slides of the cases were obtained from the archives and evaluated by a single pathologist. Diagnoses were made by light microscopy. The tumour node metastasis (TNM) stage was determined according to the seventh edition of the American Joint Committee on Cancer (AJCC) staging

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^{1,2,5}Department of Pathology, ^{4,6}Department of Urology, Harran University, Sanliurfa, ³Department of Pathology, Afyon Kocatepe University, Afyonkarahisar, Turkey.

Correspondence: Muhammet Emin Guldur. Email: eminsevim01@hotmail.com

system. The Fuhrman nuclear grade was used to identify the tumour grades. In every case, two paraffin blocks were used for immunohistochemical staining. One of these two blocks were selected from tumour tissue and the other block was from the non-neoplastic kidney tissue. Sections of 4µm thickness obtained from paraffin blocks were stained with a standard streptavidin-biotin immunoperoxidase method using CD31 antibody (Clone: 1A10, Catalogue No: PA0250, Leica). Cells with cytoplasmic staining were evaluated as endothelial cells. The mean vascular density was equivalent to the vascular surface area (in square millimeters) per unit tissue volume (in cubic millimeters) ($MVD = mm^3$).¹³ MC tryptase staining (clone: 10D11, product code: NCL-MCTYP-428, Leica) was used to identify MCs in both tumoural and non-tumoural tissues. The number of MCs was counted at 400x in 10 fields. The average number was used as the final score.

The data obtained was statistically evaluated using SPSS 11.5. The data was compared using Student's t-test and the Mann-Whitney U-test, and Pearson's correlation and Spearman's correlation tests were performed to determine correlations.

A post-hoc sample size calculation was done according to the results of the intratumoural MCC of the RCC cases that revealed; effective size as 0.48 and study power as 87%.

Results

Of the total, there were 36 (56.25%) men. The overall age ranged from 30 to 80 years with a mean of 54.3 ± 10.5 years. The mean tumour diameter was 7.7 ± 3.92 cm (range: 2-18). Clear, chromophobe, papillary, and sarcomatoid RCCs were observed in 42 (65.7%), 8 (12.5%), 7 (10.9%) and 7 (10.9%) cases. Eleven (17.2%) of the tumours were Fuhrman grade I, 26 (40.6%) were grade II, 18 (28.1%) were grade III, and 9 (14.1%) were grade IV. Distant metastasis (M1) was present in 48 (75%) cases, and no distant metastasis (M0) was detected in 18 (25%). The MCC was significantly higher in tumoral tissue compared to the non-tumoral tissue ($p < 0.001$). The values in tumoral tissue were between 0 and 13, and the mean MCC in each microscopic field was 3.86 ± 2.72 (median: 3.2) (Figure-1). The MC values

Table: Comparative analyses.

	Clear RCC(mean±SD)	Non-clear RCC(mean±SD)	p-value
Intratumoral MCC	4,68±3,02	2,28±0,65	0,001
M0 (median ±iqr)		M1 (median ±iqr)	p-value
MVD	5,42±1,02	5,31±0,36	0,587
Intratumoral MCC	3,05±1,92	5,1±7,95	0,002

RCC: Renal Cell Carcinoma, SD: Std. Deviation, MCC: Mast Cell Count, iqr: Interquartile Range, M0: Non-metastatic RCC, M1: Metastatic RCC, MVD: Microvessel Density.

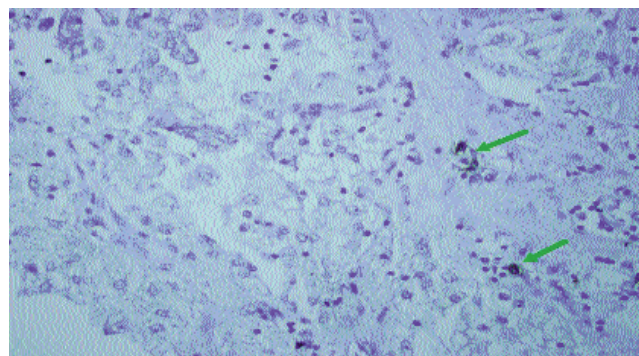


Figure-1: Mast cells in tumoral tissue that stained positively with mast cell tryptase (X400).

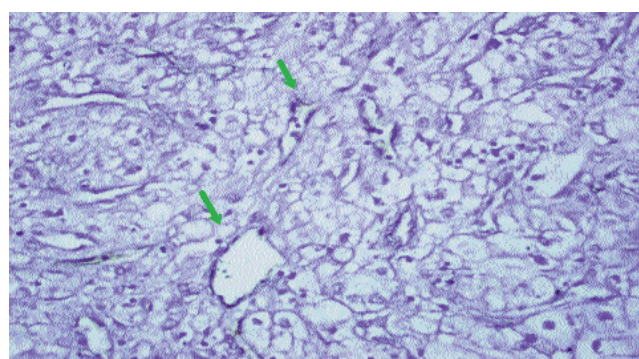


Figure-2: Vascular structures detected by CD31 observed in tumoral tissue (X400).

were between 0 and 4 in non-neoplastic tissue, and the mean MCC in each microscopic field was 0.83 ± 0.70 (median: 0.75). MCs in non-neoplastic renal parenchyma were distributed along the interstitium and subcapsular field. The MCCs in tumoral tissue in clear cell renal cancers were significantly higher compared with the non-clear RCC subtypes ($p = 0.001$). The number of MCs was correlated with stage ($\rho = 0.616$; $p < 0.001$), grade ($\rho = 0.436$; $p < 0.001$), and tumour diameter ($\rho = 0.624$; $p < 0.001$). However, no correlation was found between the number of MCs and age ($p > 0.05$). The number of MCs was significantly higher in RCC with metastasis compared with cases without metastasis ($p < 0.002$). The MVD varied between 1.44 and 8.86, and the mean value was 5.41 ± 1.57 (median: 5.4) (Table, Figure-2). No significant associations between MVD and age, stage, tumour diameter, grade, or number of intratumoral MCs were identified ($p > 0.05$). There was no significant difference in MVD between metastatic RCC and non-metastatic RCC ($p = 0.587$).

Discussion

Tumour angiogenesis is a necessary process for the

development of solid tumours. Newly formed blood vessels provide a source of nutrition and oxygen. Angiogenesis develops through many steps. Tumour cells, endothelial cells, and extracellular matrix interact during angiogenesis.¹³⁻¹⁶ Angiogenic molecules, such as vascular endothelial growth factor, fibroblast growth factor-2, and IL-8, cause tumour angiogenesis. Mediators released from lymphocytes, macrophages, neutrophils, and MCs surrounding tumour cells and receptors of these mediators support the development of angiogenesis.^{5,17} Some studies have evaluated the prognostic significance of angiogenesis in RCC, with conflicting results.^{2,12,15,18-20} Yoshino et al.²⁰ demonstrated that survival was affected by the MVD in T1 and T2 tumours. However, this finding was not confirmed in other studies.^{2,12,15,18,19} In our study, we also could not find a significant association between angiogenesis and age, stage, tumour diameter, or grade. The role of MCs in angiogenesis has been evaluated in many different types of neoplasias. For example, Anak Lamaroon et al.⁸ demonstrated that the number of MCs and angiogenesis in oral squamous cell carcinoma were higher compared to normal oral mucosa and hyperkeratosis. In contrast, Motohiro Sawatsubashi et al.¹⁰ suggested that cancer cells and MCs controlled angiogenesis in laryngeal squamous cell carcinomas. Similar results were detected in malignant lesions of the uterine cervix, lung adenocarcinomas, and gastric cancers.^{6,9,11} MCs exist in the interstitium of the renal parenchyma in the kidney. These cells are found around the renal tubules or around blood vessels in the renal cortex. MCs are thought to be effective in the development of interstitial fibrosis in the renal parenchyma. These cells can be detected with histochemical and immunochemical methods.²¹ Few studies have evaluated MCs in RCCs.^{2,12,22} Mohseni MG et al.² and Tuna B et al.¹² evaluated associations between the number of MCs and microvessel intensity and some clinicopathological features in RCCs. MCs were more concentrated in tumoral tissue and peritumoral inflammatory spaces compared with non-tumoral renal tissues. MCs were rarely observed between renal tubules or around blood vessels in the renal cortical interstitium in non-tumoral spaces in both studies. No associations were found between the number of MCs, age, stage, grade, tumour diameter, or survival in previous studies. The number of MCs was significantly higher in clear cell renal carcinoma compared to non-clear RCC in both studies. Tuna B et al.¹² found a significant relation between the number of MCs and MVD, while Mohseni MG et al.² found no statistical significance. We also obtained similar findings

as these two studies. We identified that MCs were more concentrated in tumoral tissue and peritumoral inflammatory renal tissue compared to the non-tumoral renal parenchyma. MCs were distributed in the interstitium and subcapsular space in non-tumoral renal parenchyma. The number of MCs in clear cell renal cancer was significantly higher compared to the non-clear RCC. In contrast, we did not find a significant relation between the number of MCs and MVD. In contrast to the two studies mentioned above, we found a significant relation between the number of MCs and the tumoral tissue and stage, grade, and tumour diameter. However, we did not find a significant association between the number of MCs and age. In addition, we found a statistically significantly higher tumoral MCC in RCC cases with metastasis compared to cases without metastasis.

Conclusion

The findings of the study were in line with literature. No statistically significant relation was found between the number of MCs and MVD in the tumoral tissue. A marked increase in the number of MCs in tumoral tissue compared to non-tumoral renal parenchyma suggests that MCs might have an effect on angiogenesis. Because few studies have evaluated the association between the number of MCs and angiogenesis in RCCs, we suggest that further studies be conducted on this subject.

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