Significance of tumor-associated macrophage density and CXCR4 expression in renal cell carcinoma

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Objective **Renal cancers are considered immunogenic tumors that are frequently infiltrated by immune cells. Tumor-associated macrophages (TAMs) represent a major leukocyte population, infiltrating tumors, and are associated with poor prognosis. Chemokines and their receptors play an important role in the tumorigenesis of many malignancies. The purpose of this investigation was to assess the immunohistochemical expression of TAM-related marker CD68 and chemokine receptor CXCR4 and study their correlation with other clinicopathological factors to detect the usefulness of these markers in the diagnosis and prognosis of renal cell carcinoma (RCC).**

Materials and methods **Immunohistochemical expression of CD68 + ve TAM density and CXCR4 was analyzed in 57 cases of RCC and 10 cases of normal renal tissue.**

Results **The higher expression of CD68 + ve TAMs was 57.8% and CXCR4 in 59.6% in RCC, which was statistically significantly different from the control group, which showed lower expression ($P < 0.01$ and <0.05, respectively). The expression of CD68 + ve tumor-associated macrophages was significantly related to high grade ($P < 0.01$), advanced stage ($P < 0.05$), and vascular invasion ($P < 0.01$). There was a significant correlation between CXCR4 overexpression in tumor cells and poor differentiation ($P < 0.01$), higher stage ($P < 0.01$), lymph node metastasis ($P < 0.05$), and vascular invasion ($P < 0.01$). A significant positive correlation between CD68 and CXCR4 in RCC was detected ($r = 0.018$, $P < 0.05$).**

Conclusion **Higher expression of CD68 + ve TAM density and CXCR4 may participate in the carcinogenesis, progression, and aggressiveness of RCC. Therefore, combined detection of these biomarkers could provide a prognostic role as well as novel therapeutic strategies, being new targets in the treatment of RCC.** ***Egypt J Pathol*** **38:344–350 © 2018 Egyptian Journal of Pathology.**

Egyptian Journal of Pathology 2018, **38:**344–350

Keywords: CXCR4, RCC, TAMs

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Received 12 October 2018 **accepted** 5 November 2018

Introduction

Cancer of the kidney and renal pelvis is the commonest urological malignancy, representing 3% of all human malignancies. In Egypt, renal cell carcinoma (RCC) accounts for approximately 1% of all cancers. The incidence was increased during the last years, and the rate of death is higher than other tumors of the genitourinary tract (Ramana, 2012; El Bolkainy *et al*., 2016).

Many patients are likely to develop metastasis. Metastatic RCC is resistant to both conventional chemotherapy and radiotherapy. So, the majority of these patients need alternative therapy, but treatment options are of limited value (Ljungberg *et al*., 2015; Belldegrun, 2007), as the stage and the grade of RCC have limited value in predicting the clinical outcome of certain patients. Moreover, there are many histological subtypes with distinct genetic and biologic features that determine clinical course and outcome (Steffens *et al*., 2011). Therefore, further studies of the mechanism of initiation, progression, and identification of diagnostic and prognostic markers are still needed.

Clear cell renal cell carcinoma (ccRCC) is the most common type, which accounts for 75–80% of cases, whereas papillary RCC and chromophobe RCC are the least common types (Faragalla *et al*., 2012).

The malignant behavior of RCC is intimately related to the immune system. Previous studies have suggested that tumor cells may interact with their surrounding stromal microenvironment, which has a vital role in the malignant aggressiveness of many types of solid tumors, including RCC (Nakanishi *et al*., 2018).

The tumor microenvironment consists of proliferating tumor cells, tumor stroma, blood vessels, infiltrating inflammatory cells, and diverse types of associated cells including the immune, inflammatory, and hypoxic microenvironment (Szebeni *et al*., 2017).

Tumor-associated macrophages (TAMs) are a major leukocyte population that infiltrate the tumor stroma. High frequency of TAMs is associated with poor prognosis (Zhang *et al*., 2012). The main functional phenotypes for macrophages are the classic M1 and M2, but recently multiple variations of the M1/M2 phenotype exist (De Palma and Lewis, 2013). The main function of M1 cells is the ability to phagocytize pathogens and produce proinflammatory cytokines, such as tumor necrosis factor-α, interleukin (IL)-12, IL-23, and IL-6 (Movahedi *et al*., 2010). However, M2 phenotype stimulates higher production of anti-inflammatory cytokines such as IL-10 and upregulates molecules that inhibit the adaptive immune responses, which promotes wound healing and reduces inflammation (Biswas and Mantovani, 2010).

CD68 represents a macrophage marker that is expressed on all macrophages. Many studies indicate that TAMs act as tumor promoter by stimulating angiogenesis, tumor-

cell proliferation, and metastasis, as well as change the adaptive immune responses (Lewis and Pollard, 2006).

Chemokines and their receptors, which represent important components in the tumor inflammatory microenvironment, have an essential role in chemotaxis and spread of malignant tumors. They have the ability to regulate cell trafficking, survival, and growth. Chemokines are small, secreted G2 proteins (8.1 kD) that attract leukocytes into inflammatory sites and to secondary lymphoid organs. Moreover, they are critical in tumor progression, in addition to their role in the immune system (Baggiolini *et al.*, 1997; Yusen *et al.*, 2018).

C-X-C chemokine receptor 4 (CXCR4) is considered one of the most predominant cancer stem cell markers and is the major chemokine receptor in many solid tumors, and it binds selectively to the chemokine stromal cell-derived factor 1, also known as CXCL12. Stromal cell-derived factor-1/CXCR4 is considered as a biological axis in tumor invasion and metastasis (Matsusue *et al.*, 2009; Gassenmaier *et al.*, 2013).

The interaction between TAMs and CXCR4 and mechanism of infiltrating RCC microenvironment is not well understood. This investigation aimed at assessment the immunohistochemical expression of TAM-related marker CD68 and chemokine receptor CXCR4 and study their correlation with other clinicopathological factors to detect the usefulness of these markers in the diagnosis and prognosis of RCC.

Materials and methods

Patients and clinical data

This is a retrospective study performed at the Departments of Pathology and Surgery, Benha University Hospital, Egypt. The study included 57 cases of RCC that were randomly obtained from the archives of pathology departments between November 2011 and August 2014 obtained from surgically resected specimens (radical nephrectomy). The control group consisted of 10 normal kidney tissues (renal biopsy from donors of kidney transplant). All tissue samples were formalin fixed and paraffin embedded. All collected blocks were cut at 4 µm and stained with ordinary hematoxylin and eosin to confirm the diagnosis.

We collected the clinical and pathological information from the medical records of the patients including age, sex, stage, lymph node metastasis, and distant metastasis. No patients had received preoperative radiotherapy or chemotherapy. Tumor were staged according to the AJCC (2017). Histological grading followed the Fuhrman grading system. Tumor size was classified into three groups: less than or equal to 4, 4–7, and more than 7 cm (Fuhrman *et al.*, 1982; Turun *et al.*, 2012). The study was conducted with full local ethics approval.

Immunohistochemistry

Immunohistochemical analysis was applied using the standard streptavidin–biotin technique following the manufacturer's instructions. The details of used primary antibodies are shown in Table 1. For the secondary developing reagents, a labeled streptavidin–biotin kit (Neomarker; Labvision, Waltham, USA) was used. The sections were stained with 0.02% diaminobenzidine solution used as chromogen, and negative controls for both markers were obtained by omitting the primary antibody.

Evaluation of immunohistochemical staining

CD68 antibody gives strong and distinct cytoplasmic granular staining of macrophages. In each slide at ×40 power field, three areas of high TAM density (hot spots) were identified and counted (Tsutsui *et al.*, 2005). The final score was categorized into low and high according to the mean count of TAM density, which was 22.56.

CXCR4 expression was regarded positive if there was any brown membranous, cytoplasmic or nuclear staining of tumor cells. Using the average percentage of positive tumor cells as a cutoff, CXCR4 expression was classified into high-expression group if the percentage of positively stained cells more than or equal to 30% and low-expression group if the percentage of positively stained cells less than 30% or no staining (Wang *et al.*, 2012).

Statistical analysis

Values are expressed as mean ± SD. Unpaired Student's *t* test was used to determine statistical significance between groups (SPSS Inc., Chicago, Illinois, USA). Spearman's correlation was done for correlation analysis between CD68 + ve TAMs and CXCR4. The statistical analyses were done using SPSS software (version 19.0; SPSS, Chicago, Illinois, USA), *P* value less than 0.05 was considered significant and less than 0.01 was highly significant.

Results

Clinicopathological results

The mean age of the studied 57 cases of RCC cases was 63.2 ± 11 years (range, 22–71years). The characteristics of the patients and tumors are listed in Table 2.

Immunohistochemical expression of CD68 + ve tumor-associated macrophage density

The TAM density varied from 5.6 to 41.5, with a mean value of 22.56 per field (×400). CD68 immunoreactivity was overexpressed (high TAMs) in 57.8% (33/57) of the studied RCC cases with significant difference from the control cases, which showed lower expression ($P < 0.01$), as shown in Table 3.

A total of 57 patients with RCC were analyzed for the correlation between clinicopathological characteristics, and the CD68 expression was listed in Table 4. CD68 + ve TAM

Table 1 **Study markers**

Markers	Vendor	Clone	Host/isotope	State	Dilution	Incubation	Positive control	Antigen retrieval
CD68	Thermo Fisher scientific	Monoclonal	Rabbit/IgG2	Concentrated	1 : 100	2 h at RT	Spleen	Citrate buffer (pH 6.0)
CXCR4		Polyclonal	Rabbit/IgG2	Concentrated	1 : 50	Overnight at RT	Cervical carcinoma	Citrate buffer (pH 6.0)

Rt, room temperature.

expression in RCC was significantly lower in patients aged less than 60 years ($P<0.01$). CD68 expression was significantly higher in T3 stage RCC ($P<0.05$) and was more strongly expressed in higher grade ($P<0.01$), as shown in Fig. 1. Moreover, CD68 expression in RCC with vascular invasion was significantly higher than without vascular invasion ($P<0.01$). There was no significant correlation with histological type, tumor size, lymph node status, distant metastasis, or clinical TNM stage ($P>0.05$) (Table 4).

Immunohistochemical expression of CXCR4

In this study, all cases showed CXCR4 positive immunoreactivity, and it was highly expressed in 59.6% of studied RCC cases, whereas control group showed low expression with statistically significant relationship ($P<0.05$) as shown in Table 3.

Analysis of CXCR4 immunoexpression with the clinicopathological criteria of the studied 57 RCC revealed that CXCR4 was significantly upregulated in high-grade carcinomas ($P<0.01$), as shown in Fig. 2, advanced T stage ($P<0.01$) and lymph node metastasis positive group ($P<0.05$) and was highly expressed in cases with vascular invasion ($P<0.01$). There was no significant correlation with age, tumor size, histological type, distant metastasis, and clinical stage ($P>0.05$) (Table 5).

The correlation analysis between CD68 and CXCR4 expressions among the studied renal cell carcinoma cases

Based on a correlation analysis between the results of immunohistochemical expression of the two markers among the studied RCC cases, a significant positive correlation was found between the expression of CD68 and CXCR4 proteins [Spearman's correlation (r) = 0.018, $P<0.01$) (Table 6).

Table 2 Clinicopathological characteristics of studied cases of renal cell carcinoma

Clinicopathological variables	*n* (%)
Histologic type	
Clear cell RCC	45 (79)
Papillary RCC	6 (10.5)
Chromophobe RCC	6 (10.5)
Sex	
Males	35 (61.4)
Females	22 (38.6)
Age (years)	
< 60	26 (45.7)
> 60	31 (54.3)
Tumor size	
≤ 4	11 (19.2)
4–7	30 (52.6)
> 7	16 (28.2)
Fuhrman grade	
1	17 (29.8)
2	26 (45.6)
3	14 (24.6)
Stage	
T1 and T2	33 (57.8)
T3	24 (42.2)
N stage	
N0	37 (46.9)
N1	20 (35.1)
M stage	
M0	41 (71.9)
M1	16 (28.1)
TNM stage	
Stage I + II	32 (56.1)
Stage III + IV	25 (43.9)
Vascular invasion	
Negative	33 (57.9)
Positive	24 (42.1)
Total	57

RCC, renal cell carcinoma.

Table 4 CD68 expression in relation to different variables in the studied cases

		CD68 [*n* (%)]		
	Total	Low	High	*P* value
Histologic type				
Clear cell RCC	45	20 (44.4)	25 (55.6)	0.5
Papillary RCC	6	2 (33.3)	4 (66.7)	
Chromophobe RCC	6	2 (33.3)	4 (66.7)	
Sex				
Males	35	17 (48.6)	18 (51.4)	0.1
Females	22	7 (31.8)	15 (68.2)	
Age (years)				
< 60	26	17 (65.4)	9 (34.6)	< 0.01**
> 60	31	7 (22.6)	24 (77.4)	
Tumor size				
≤ 4	11	4 (36.3)	77(63.7)	0.563
4–7	30	16 (53.3)	14 (46.7)	
> 7	16	4 (25)	12 (75)	
Fuhrman grade				
1	17	12 (70.6)	5 (29.4)	< 0.01**
2	26	12 (46.2)	14 (53.8)	
3	14	0	14 (100)	
Stage				
T1 and T2	33	18 (54.5)	15 (45.5)	< 0.05*
T3	24	6 (25)	18 (75)	
N stage				
N0	37	19 (51.3)	18 (48.7)	0.056
N1	20	5 (25)	15 (75)	
M stage				
M0	41	18 (43.9)	23 (56.1)	0.667
M1	16	6 (37.5)	10 (62.5)	
TNM stage				
Stage I + II	32	16 (50)	16 (50)	0.178
Stage III + IV	25	8 (32)	7 (68)	
Vascular invasion				
Negative	33	21 (63.6)	12 (36.4)	< 0.01**
Positive	24	3 (12.5)	21 (87.5)	
Total	57	24 (42)	33 (58)	

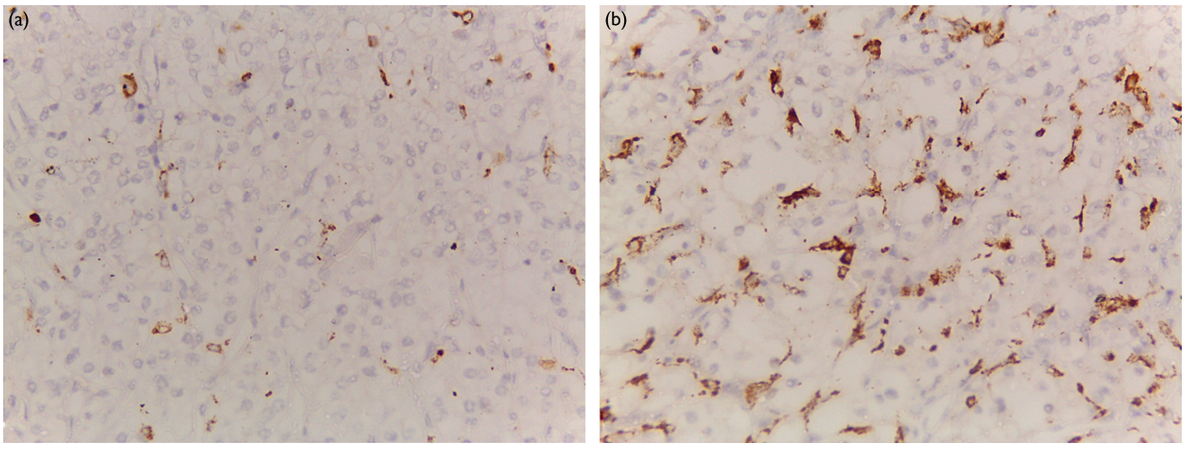
RCC, renal cell carcinoma.
**Correlation is significant at the 0.01 level (two tailed).
*Correlation is significant at the 0.05 level (two tailed).

Table 3 Expression of CD68 and CXCR4 proteins in control group and renal cell carcinoma tissues

		CD68			CXCR4		
	Total	Low [*n* (%)]	High [*n* (%)]	*P* value	Low [*n* (%)]	High [*n* (%)]	*P* value
Control group	10	9 (90)	1 (10)	< 0.01**	8 (80)	2 (20)	< 0.05*
RCC	57	24 (42)	33 (58)		23 (40.3)	34 (59.7)	

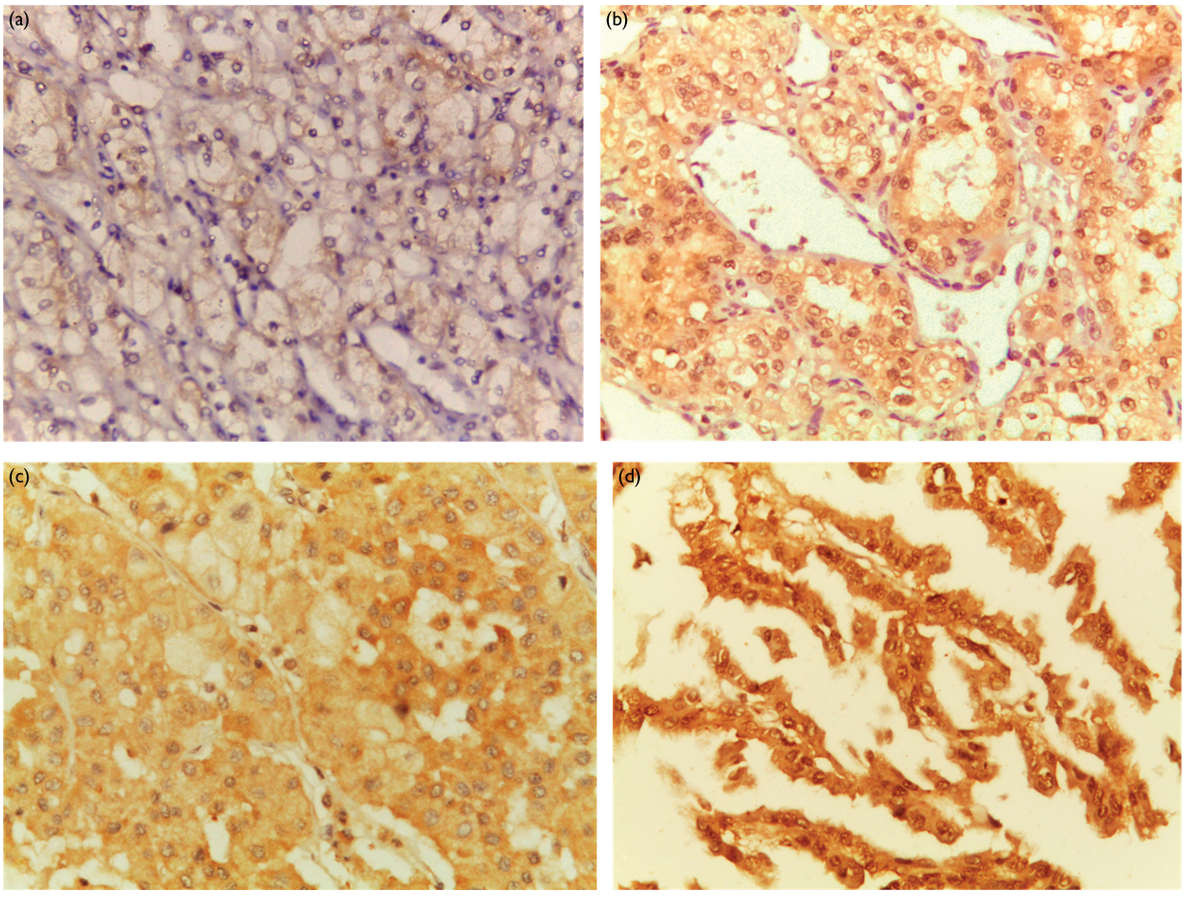
RCC, renal cell carcinoma.
**Correlation is significant at the 0.01 level (two tailed).
*Correlation is significant at the 0.05 level (two tailed).

Fig. 1



Immunohistochemical expression of CD68 + ve TAMs in RCC. (a) Clear cell RCC, grade 1 showing low cytoplasmic expression for CD68 in macrophages. (b) Clear cell RCC, grade 2, shows high cytoplasmic expression for CD68 (immunohistochemical, ×400). RCC, renal cell carcinoma; TAM, tumor-associated macrophage.

Fig. 2



Immunohistochemical expression of CXCR4 in RCC. (a) Clear cell RCC, grade 2 showing low weak membranous staining of CXCR4 in tumor cells. (b) Clear cell RCC, grade 3 showing diffuse strong cytoplasmic, nuclear, and membranous staining of CXCR4. (c) Chromophobe RCC, grade 2, showing diffuse strong membranous, cytoplasmic, expression of CXCR4. (d) Papillary RCC, grade 2 showing diffuse moderate cytoplasmic, and nuclear expression of CXCR4 (immunohistochemical, ×400). RCC, renal cell carcinoma.

Discussion

Tumorigenesis is a multifactorial process. Almost all cancers are caused by chronic inflammation mediated by inflammatory factors and cells (Almatroodi *et al.*, 2016). Macrophages are considered an important component of the immune system. TAMs have the potential role in promoting cancer progression and metastasis (Qian and Pollard, 2010).

Tumor cells could secrete specific chemokines and their receptors, and through their interaction, they can promote cancer cell proliferation, tumor growth, and apoptosis prevention (Yusen *et al.*, 2018). CXCR4 was overexpressed in various types of carcinomas and was correlated with poor prognosis. In RCC, the CXCR4/CXCL12 system is most important because CXCR4 is regulated by von Hippel-Lindau/hypoxia-inducible factor (HIF) (Staller *et al.*, 2003; Balkwill, 2004; Chawla *et al.*, 2008).

Table 5 CXCR4 expression in relation to different variables in the studied cases

		CXCR4 [*n* (%)]		
	Total	Low	High	*P* value
Histologic type				
Clear RCC	45	19 (42.2)	26 (57.8)	0.6
Papillary RCC	6	2 (33.3)	4 (66.7)	
Chromophobe RCC	6	2 (33.3)	4 (66.7)	
Sex				
Males	35	6 (17.1)	29 (82.9)	< 0.01**
Females	22	17 (77.3)	5 (22.7)	
Age (years)				
< 60	26	10 (38.5)	16 (61.5)	0.8
> 60	31	13 (41.9)	18 (85.1)	
Tumor size				
≤ 4	11	4 (36.3)	7 (63.7)	0.995
4–7	30	13 (43.3)	17 (56.7)	
> 7	16	6 (37.5)	10 (62.5)	
Fuhrman grade				
1	17	13 (76.5)	4 (23.5)	< 0.01**
2	26	8 (30.8)	18 (69.2)	
3	14	2 (14.3)	12 (85.7)	
T stage				
T1 and T2	33	20 (60.6)	13 (39.4)	< 0.01**
T3	24	3 (12.5)	21 (87.5)	
N stage				
N0	37	19 (51.3)	18 (48.7)	< 0.05*
N1	20	4 (20)	16 (80)	
M stage				
M0	41	16 (39)	25 (61)	0.749
M1	16	7 (43.7)	9 (56.3)	
TNM stage				
Stage I + II	32	15 (46.9)	17 (53.1)	0.264
Stage III + IV	25	8 (32)	17 (68)	
Vascular invasion				
Negative	33	19 (57.6)	14 (42.4)	< 0.01**
Positive	24	4 (16.7)	20 (83.3)	
Total	57	23 (40.3)	34 (59.7)	

RCC, renal cell carcinoma.
**Correlation is significant at the 0.01 level (two tailed).
*Correlation is significant at the 0.05 level (two tailed).

Table 6 The correlation analysis between CD68 and CXCR4 expressions among the studied renal cell carcinoma cases

		CD68 [*n* (%)]		
CXCR4	Total	Low	High	*P* value
Low	23 (40.3)	14 (60.9)	9 (39.1)	< 0.05*
High	34 (59.7)	10 (29.4)	24 (70.6)	
Total		24 (42)	33 (58)	

Spearman's rho = 0.018, *P* value less than 0.05.
*Correlation is significant at the 0.05 level (two tailed).

In this study, we examined CD68 + ve TAM density and CXCR4 expression in a group of Egyptian patients with renal cell cancer. The study included 57 cases of RCC and 10 normal renal tissues.

Our analysis of CD68 immunoreactivity revealed that high TAM expression was detected in 57.8% of the studied RCC, and the expression was highly significantly different from the normal renal tissue ($P < 0.01$). The same results were reported by Santoni *et al.* (2013) in RCC and Tsutsui *et al.* (2005) in breast cancer, suggesting that TAMs are important mediators in the tumor inflammatory microenvironment and could play a vital role in early carcinogenesis and diagnosis of RCC.

This study reported that density of CD68 + ve TAMs was significantly higher in T3 stage RCC than in T1 and T2 stage ($P < 0.05$) and increased with higher grade of RCC ($P < 0.01$). Therefore, high TAM density is highly correlated with the malignant degree of RCC.

In agreement with our finding, the study performed by Nakanishi *et al.* (2018) declared that CD68 + ve cells were significantly correlated with higher grade and pT stage in RCC. Moreover, Wyler *et al.* (2014) in RCC, Guo *et al.* (2017) in gastric carcinoma, and Morita *et al.* (2017) in breast cancer concluded the same results. Therefore, the overexpression of CD68 + ve macrophages may be considered a poor prognostic marker in various cancers.

This may be attributed to the results studied by Stefanie *et al.* (2013) in ccRCC, which suggested that accumulation of TAMs is promoted by malignant cells. Furthermore, TAMs induce the conversion of CD4 + T cells toward a more immunosuppressive type with decreased secretion of cytokines, increased production of IL-10, and enhanced expression of programmed death 1 and T-cell immunoglobulin mucin 3, leading to the growth and spread of tumor. Moreover, high TAM infiltration had a putative role in the progression of breast (Qian *et al.*, 2011) and gastric cancer (Hwang *et al.*, 2012).

Moreover, this work showed that density of CD68 + ve TAMs was significantly higher in RCC cases with vascular invasion ($P < 0.01$). These results were compatible with Hanada *et al.* (2000). In urinary bladder cancer, this could be explained through the ability of TAM to facilitate angiogenesis and extracellular matrix breakdown, promoting tumor-cell motility, and to facilitate the entry of tumor cells into blood vessels in response to chemoattractants such as endothelial growth factor. The signals between the macrophage and the tumor cell result in the formation of invadopodia in tumor cells promoting vascular invasion by affecting the activity of actin regulators in the surrounding stroma (Condeelis and Pollard, 2006).

There was no significant correlation with tumor size, histological type, lymph node status, distant metastasis, or clinical TNM stage ($P > 0.05$), which is in agreement with Bolat *et al.* (2006) who stated that in the breast

cancer group, negative expression of CD68 + ve TAMs was found in advanced TNM stage and positive lymph node metastasis.

In the current study, CXCR4 immunoreactivity was highly expressed in 59.6% of studied RCC cases. This finding was statistically significantly different from the finding in the normal group which showed lower expression ($P < 0.05$). This finding was in agreement with Wang *et al.*, (2012), in RCC and Yusen *et al.* (2018) in non-small cell lung cancer tissues who reported the malignant cells expressing CXCR4 higher than normal tissues. Therefore, CXCR4 may be closely associated with the tumorigenesis of RCC.

In this study, correlations of CXCR4 expression with the clinicopathological features of the studied RCC cases revealed significant association of high CXCR4 expression with high grade ($P < 0.01$), advanced stage ($P < 0.01$), and vascular invasion ($P < 0.01$) which is similar to many previous related studies such as by Wehler *et al.* (2008) and Rasti *et al.* (2017). CXCR4 was also significantly upregulated in the lymph node metastatic group ($P < 0.05$), and this is consistent with the finding of D'Alterio *et al.* (2010) and Wyler *et al.* (2014), who stated that there was a positive correlation between CXCR4 expression and higher Fuhrman grade ($P < 0.05$) in RCC. This might suggest that overexpression of CXCR4 has a role in progression and aggressiveness in patients with RCC.

Evidence suggests that HIF-1 and vascular endothelial growth factor drive the increased expression of CXCR4. This may be owing to the hypothesis that within the hypoxic regions of proliferating tumors, chemokine receptors might be overexpressed to facilitate the spread of tumor (Zagzag *et al.*, 2006). Moreover, CXCR4 may play a major role in epithelial–mesenchymal transition, which induces cancer stem cell formation in the epithelial cells of solid tumors and spread (Lichner *et al.*, 2015). Moreover, in many solid tumors, including RCC, CXCR4 is considered a very important mediator in the signaling pathways (Matak *et al.*, 2015).

However, this is different from the finding of Bo Tang *et al.* (2015) who found CXCR4 higher expression was not significantly associated with advanced stage of RCC. This discrepancy may be owing to a different methodology in quantification of marker expression and its various pattern of localization (cytoplasmic, membranous, or nuclear).

There was no significant correlation with age, tumor size, histological type, metastasis, and clinical stage ($P > 0.05$). This is consistent with Wang *et al.* (2012) who found that high expression of CXCR4 was independent of metastasis and histologic variant.

In contrast to our finding, Rasti *et al.* (2017) concluded that there was a significant difference in the expression levels of CXCR4 in the ccRCC samples compared with the ChRCC and papillary RCC samples. This difference may be attributed to different methods of immunostaining evaluation and low number of studied cases.

In this study, CXCR4 expression was positively correlated with high density of CD68 + ve TAMs among studied RCC cases [Spearman's correlation (r) = 0.018, $P < 0.05$]. This finding is consistent with Yusen *et al.* (2018).

TAMs and CXCR4 both play important roles in the tumorigenesis and progression of RCC. TAMs may create a synergistic effect between vascular endothelial growth factor and CXCR4 through the attraction of mesenchymal-derived stem cells and therefore promote the differentiation of TAMs, leading to a circuit reaction that promotes tumor immunosuppression and growth. Through proinflammatory molecules (e.g. NF-κB and HIF-1α), TAMs may interact with CXCR4, facilitating tumor immune escape through epithelial-to-mesenchymal transition, playing a vital role in carcinogenesis, invasion, and metastasis of RCC. TAMs secrete many growth factors (e.g., endothelial growth factor, PDGF, and TGF-β) that promote aggregation of TAMs and inhibit tumor immunity, exerting their effect together with CXCR4 and directly or indirectly promoting tumor growth (Yusen *et al.*, 2018).

Conclusion and recommendation

RCC is an immunogenic tumor often infiltrated by immune cells. This study suggests that higher expression of CD68 + ve TAM density and CXCR4 may participate in the carcinogenesis, progression, and aggressiveness of RCC. Therefore, combined detection of these biomarkers could provide a prognostic role as well as novel therapeutic strategies, being new targets in the treatment of RCC. Our recommendation is for additional large-scale studies to deliver more understanding of their role in the prognosis and clinical management of patients with RCC.

Conflicts of interest

There are no conflicts of interest.

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