Immunohistochemical Expression of CD200 in Renal Cell Carcinoma

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Abstract

Background and Objectives: Renal cell carcinoma (RCC) is the most common malignant renal neoplasm in adults. CD200 is a transmembrane protein and is a promising target for cancer immunotherapy. The aim of this study is to assess the CD200 expression in RCC. **Materials and Methods:** Eighty paraffin-embedded radical nephrectomy specimens, diagnosed with RCC were evaluated immunohistochemically for CD200 expression. **Results:** Out of eighty cases studied, CD200 was expressed in $n = 73$ cases (91.25%) with high intensity in 27 cases (33.75%), moderate intensity in 22 cases (27.5%), and mild intensity in 24 cases (30%). No staining was observed in the adjacent apparently normal renal tissue in all examined sections. No significant relationship was found between CD200 expression and the gender, tumor size, tumor side, histologic type, nuclear grade, T stage, and tumor necrosis. **Conclusion:** CD200 expression in most of the studied cases of RCC may refer to the potential therapeutic of anti-CD200 antibody for this cancer.

Keywords: CD200, expression, immunohistochemistry, renal cell carcinoma

INTRODUCTION

Renal cell carcinoma (RCC) is the most common malignant renal neoplasm in adults. It is a common tumor of the genitourinary system and accounts for about 3% of all types of cancer.[1] Until now, the main treatment of RCC is partial or complete surgical resection of the kidney.

RCC includes a heterogeneous group of tumors with different histologic and molecular variations.[2] With this heterogenicity of RCC, individualized immunotherapy using a useful biomarker is still lacking.[3]

Multivariable analysis of 4260 RCC patients showed that nephrectomy type, pathological T stages, and nuclear grade were common significant risk factors for patients' survival.[4] T stage in RCC depends largely on the tumor size and the extension beyond the kidney while the nuclear grade depends on the presence/absence of nucleoli and the nuclear pleomorphism.

It is well known that the immune system can elicit a response against many types of cancers. However, this response is largely insufficient to eradicate the disease due to factors in the tumor microenvironment (TMI) that defeat a tumor immune response. In RCC, the clinical outcome is largely associated with the immune response, as the immune cells infiltrating the tumor form an ecosystem in the (TMI) that regulate the progression of cancer and have a potential prognostic value.[5]

CD200 is a transmembrane protein, coded on chromosome 3q12 and belongs to the immunoglobulin supergene family, with two extracellular domains, a transmembrane region, and a cytoplasmic tail with no known signaling motif.[6] It is normally expressed by human thymocytes, neurons, activated T-cells, B-cells, dendritic cells, and endothelial cells.[7]

Apart from normal tissue, expression of CD200 is well studied in multiple types of hematologic malignancies[8] and solid tumors. Although less well studied in solid tumors than hematologic malignancies, its expression was examined in melanoma,[9] ovarian carcinoma,[10] basal cell carcinoma,[11]

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colorectal carcinoma,[12] small cell lung carcinoma[13] and some neuroendocrine neoplasms.[14]

The expression of the receptor for CD200 (CD200R) is strong in monocyte/macrophage lineage, neutrophils, mast cells, and certain populations of T-cells. CD200R through an immunoregulatory signal imparts a suppression of T-cell-mediated immune responses. This indicates that CD200–CD200R interactions are primarily involved in limiting the cellular functions of myeloid lineages.[7]

In knockout mice of the CD200 gene and using blocking antibodies and recombinant Fc fusion proteins containing the CD200 or CD200R extracellular domains have shown that CD200 is a potent immunosuppressant in the setting of autoimmune diseases and organ transplantation.[15]

CD200 has also been suggested as a stem cell marker in many tumor types and it has been proposed that cancer stem cells may evade the immune system by inducing a tolerogenic response through CD200/CD200R interaction. In addition, it is considered that CD200 expression by cancer cells has a protumor effect and plays an important role in tumor progression of various types of cancers.[16,17]

Recently, a potential role of CD200 has emerged as a prognostic factor and as a possible target of new anticancer engineered drugs. Since RCC represents one of the most immunogenic cancers, it is believed that the emergence of several immunotherapeutic strategies in its management will offer hope for patients with RCC.

To the best of our knowledge, CD200 has not been well characterized by immunohistochemistry in RCC, so the aim of this study is to assess its expression in this cancer.

MATERIALS AND METHODS

A retrospective study of 80 paraffin-embedded radical nephrectomy specimens, diagnosed as RCC. Gender, age, tumor site, and tumor size for each patient were obtained from the data on the histopathology request sheet form. Tumor stage was identified based on the data found on the sheets according to the AJCC Cancer Staging Handbook from the American Joint Committee on Cancer.[18] The specimens were collected from the tissue block archive of the Pathology Department, Faculty of Medicine, Cairo University, during the period from 2016 to 2019. The study was approved by the Institutional Medical Ethical Committee.

A paraffin block for each primary tumor was re-cut at 4-μm thickness and stained with hematoxylin and eosin (H and E) for routine histopathological examination. Deparaffinization in xylene and rehydration in descending grades of ethanol was done. Then, sections were stained with hematoxylin for 10 min, followed by washing and staining in eosin and washing again. Dehydration was done in ascending grades of ethanol, and finally, sections were cleared in xylene then mounted on slides and covered.

The following histopathological features were evaluated, and the diagnosis was confirmed; histologic classification was done according to the World Health Organization 2016,[19] nuclear grade (according to International Society of Urological Pathology "ISUP" grading system)[20] and the presence or absence of tumor necrosis.

Immunohistochemistry assay
The 4-μm thick formalin-fixed and paraffin-embedded tissue sections were cut and mounted on Silane-coated slides. For specimens examined as conventional sections, slides were de-waxed followed by re-hydration. Immunohistochemical staining was performed in an autostainer (Dako autostainer link 48).

Antigen retrieval was carried out in hot 10 mm sodium citrate buffer at pH 6.0 gradually from 50°C to 100°C in a microwave oven for 40 min. A rabbit polyclonal antibody to CD200 was used for immunostaining (Catalogue number # YPA 1760, antibody lot number Y15/20180, RRID number; Gene ID: 4345 and Uniport ID: p41217). CD200 is a specific antibody; till now, it is used to detect CD number 200 only. It was manufactured by: Chongqing Biospes Co Ltd, Chongqing, China. It was used at 1:200 dilution, incubated for 45 min, followed by Vectastain Universal Elite ABC immunohistochemistry kit (with 1:100 dilution of secondary antibody) and DAB was used as a chromogen. Pancreatic normal tissue was used as a positive normal control for the marker.

Evaluation of CD200 expression
Sections were examined microscopically by the three researchers, and the intensity of immunohistochemical expression was graded on a scale of 0–3 as follows: 0 = no staining; 1 = mild intensity, 2 = moderate intensity, and 3 = high intensity. Positive immunohistochemical expression was defined as positive membranous staining of at least 20% of the neoplastic cells.[21]

Statistical analysis
Microsoft Excel (office 2013) was used for data entry, and the Statistical Package for Social Science (SPSS) version 21 (SPSS, Armonk, New York: International Business Machines Corporation) was used for data analysis. Simple descriptive statistics (arithmetic mean and standard deviation) were used for the summary of normal quantitative data, and the frequencies for qualitative data. The bivariate relationship was displayed in cross-tabulations, and comparison of proportions was performed using the Chi-square and Fisher's exact tests where appropriate. Independent t-test, one-way ANOVA, and post-hook tests were used to compare normally distributed quantitative data. The level of significance was set at a probability $P < 0.05$.

RESULTS
Clinicopathological features of the studied cases
The ages of the patients ranged from 30 to 76, with a median of 55 years. The tumor size ranged from 3 to 20 cm in greatest

dimensions with 7 cm median. The other clinicopathological features of the studied 80 RCC cases are also presented in Table 1.

CD200 expression and its relationship to the clinicopathological features

Positive CD200 expression was found in 73 cases (91.25%) of the 80 studied RCC cases with different intensities; 27 cases (33.75%) had high intensity, 22 cases (27.5%) had the moderate intensity, and 24 cases (30%) had mild intensity. No CD200 expression was observed in the adjacent apparently normal renal tissue in all examined sections [Figure 1].

No significant relationship was found between CD200 expression, and all studied clinicopathologic parameters [Table 2]. For statistical purposes, according to CD200 expression, cases were grouped as no/low intensity and moderate/high intensity.

Table 1: Clinicopathological features in 80 renal cell carcinoma cases

Clinicopathological features	Cases, *n* (%)
Gender	
Male	52 (65)
Female	28 (35)
Tumor size (cm)	
≤7	44 (55)
>7	36 (45)
Tumor side	
Right	38 (47)
Left	42 (52)
Bilateral	0
Histopathological subtype for RCC	
Clear cell	59 (73.75)
Papillary	12 (15)
Chromophobe	4 (5)
Tubulocystic	3 (3.75)
Collecting duct	2 (2.5)
Nuclear grade	
G1	17 (21.25)
G2	45 (56.25)
G3	15 (18.75)
G4	3 (3.75)
Nuclear grade (grouped)	
G1-G2	62 (77.5)
G3-G4	18 (22.5)
T stage	
T1	42 (52.5)
T2	14 (17.5)
T3	23 (28.75)
T4	1 (1.25)
Tumor necrosis	
Present	27 (33.75)
Absent	53 (66.25)

n: number, RCC: Renal cell carcinoma

DISCUSSION

RCC is considered to be the most fatal urologic cancer. Its incidence rate is increasing worldwide as it is ranked as the 9th most common cancer in males and 13th for females.[22] RCC is categorized into numerous subtypes based on the pathological classification with different molecular and histopathological features, with clear-cell RCC subtype being the most common.[19,23]

Despite advances in screening, diagnosis, surgical treatment, and drug therapy, the clinical outcome of RCC remains unsatisfactory.[24]

The marker CD200 is a membrane-bound glycoprotein, and its expression may promote tumor formation and metastasis by helping malignant cells evade the immune system.[25] Several studies have shown that tumor cells overexpressing CD200 can better escape the host immune system.[26] The expression of CD200 is an independent prognostic factor for multiple myeloma and acute myeloid leukemia. In addition, it can predict the reduced overall survival of these patients.[8]

In a recent study by Love *et al.* on CD200 immunohistochemical expression in different types of neoplasms, only five cases of RCC (72%) out of the only seven studied cases showed positive staining for CD200.[14] This percentage may be inaccurate because of the small number of examined cases. The present study examined the expression of CD200 in a relatively larger number of cases, and the percent of CD200 positive cases was much higher (91.25%) and this may reflect more accurate results in a larger number of studied cases and/or the different expression in the examined histologic variants.

Several studies have shown that overexpression of CD200 in tumor cells has been correlated with aggressive tumor progression, greater metastatic potential, and reduced patient survival.[27] However, in this study, these important data and other important factors that largely affect the prognosis were lacking and no significant relationship was found between CD200 expression and the available histopathologic features of the cases. Although in RCC, pathological T stage and nuclear grade are significant risk factors for survival,[4] the relatively small number of examined cases may result in nonsignificant relations. A study using larger sample size and more prognostic factors may be better predicting the relation, if any, between CD200 expression and tumor prognosis.

Although many cancers approved systemic therapies have improved the clinical outcomes for many patients with advanced RCC, most patients still do not derive optimal benefit from anyone specific therapy, and metastatic RCC remains a lethal diagnosis.[28] It is suggested that CD200 is a promising target for cancer immunotherapy not only for cases of lymphoma but also in other tumor types with increased CD200 expression.[29] A subject that deserves further studying.

CONCLUSION

As many previous studies examined the role of tumor CD200 expression in tumor formation and metastasis, the identification

Table 2: Relationship between CD200 expression and clinicopathological features of 80 renal cell carcinoma cases

Clinicopathological features	CD200 expression		P
	Cases (*n*)		
	No/mild intensity	Moderate/high intensity	
Gender			
Male	18	34	0.301
Female	13	15	
Tumor size (cm)			
≤7	19	25	0.368
>7	12	24	
Tumor side			
Right	17	21	0.296
Left	14	28	
Histopathological subtypes for RCC			
Clear cell	23	36	0.927
Papillary	5	7	
Chromophobe	2	2	
Tubulocystic	1	2	
Collecting duct	0	2	
Nuclear grade			
G1	9	8	0.282
G2	14	31	
G3	6	9	
G4	2	1	
T stage			
T1	14	28	0.592
T2	6	8	
T3	11	12	
T4	0	1	
Tumor necrosis			
Present	20	33	0.794
Absent	11	16	

The level of significance was set at probability *P*<0.05. *n*: Number of cases, RCC: Renal cell carcinoma
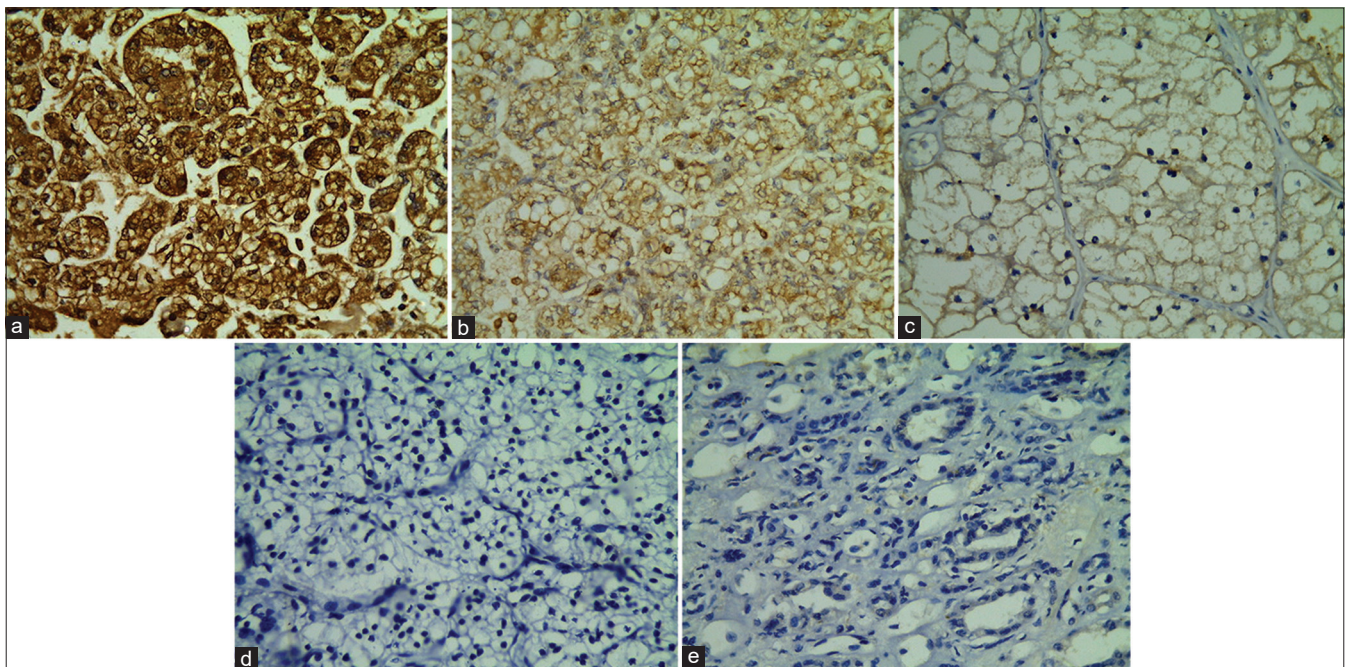
Figure 1: Immunohistochemical expression of CD200 in renal cell carcinoma. Clear-cell carcinoma showing strong membranous staining for CD200 (a). Clear-cell carcinoma showing moderate membranous staining for CD200 (b). Clear-cell carcinoma showing weak membranous staining for CD200 (c). Clear-cell carcinoma showing negative membranous staining (d). Negative staining in nearby apparently normal renal tissue (tubules) (e)

of CD200 expression in most of the studied cases of RCC and the absence of its expression in nearby nontumor part of renal tissue represents an exciting point for other larger studies to determine the therapeutic potential of anti-CD200 antibody.

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Conflicts of interest

There are no conflicts of interest.

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