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Immunohistochemical study of C-kit expression in subtypes of renal cell carcinoma

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ABSTRACT

Objective: Renal cell carcinomas (RCCs) include about 2% of adult neoplasms and 90-95% of all renal tumors. Mostly, it is possible to distinguish RCC subtypes using hematoxylin-eosin staining. However, overlapping morphologic features cause some difficulties in making a precise diagnosis. In order to render an accurate diagnosis, additional methods such as immunohistochemical staining for c-kit have been recommended. In this study, we aimed to investigate c-kit gene expression in various subtypes of RCC.

Material and methods: We reviewed 65 diagnosed RCC cases. Formalin- fixed, paraffin- embedded specimens were available for the cases. The expression of c-kit was evaluated using immunohistochemistry. The correlation between c-kit expression and clinicopathological parameters including patients' age and gender in addition to grade, stage, and size of the tumor were investigated.

Results: Six cases of 39 clear cell types (15.4%), 8 of 13 papillary types (61.5%), 11 of 12 chromophobe types (91.7%), and no sarcomatoid type were positive for c-kit expression. Based on chi-square test results, there was a significant relationship between RCC subtypes and c-kit expression (p=0.001). There was no significant correlation between age, sex, grade, stage, and size of the tumor and c-kit expression.

Conclusion: The expression of c-kit in RCC may have diagnostic significance in subtypes of RCC especially papillary and chromophobe subtypes of RCC.

Keywords: C-kit; H&E staining; KIT; renal cell carcinoma.

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Introduction

Renal cell carcinoma (RCC) encompasses about 2% of adult malignancies[1] and its incidence has been continuously rising by the annual rate of 2-4% in recent years.[2] The main etiology of RCC is not obvious in most of the cases but there are some known risk factors for its incidence including obesity, smoking, hypertension, and/or drugs used in order to control hypertension.[3] World Health Organization (WHO) classifies RCC into three main subtypes including clear cell, papillary, and chromophobe RCC.[1] Comprising about 70% of all RCCs, clear cell RCC is the most common subtype with overall 5-year survival rate of 55-60%.[4] The second most common subtype of RCC is papillary RCC. It approximately accounts for 15-20% of RCCs and its 5-year survival rate is 80-90%.[5] Finally, 6-11% of RCC cases belong to chromophobe RCC. This subtype has the best prognosis among all RCC subtypes with an approximate 5-year survival rate of 90%.[6] In addition to these subtypes, sarcomatoid RCC is also a scarce variant of RCC which accounts for 0.7-13.2% of all renal parenchymal carcinomas.[7] The prognosis of this aggressive carcinoma is relatively poor and it is very likely to cause metastasis.[8] Since the prognosis of RCC is fairly poor and higher than 60% of the patients die, accurate study of pathology specimens is so vital for the proper management of the dis-

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ease.[9] It is usually possible to differentiate the subtypes using routine hematoxylin and eosin (H&E) staining but because of several common morphologic characteristics, some specimens are very difficult to diagnose even by conversant pathologists.[10]

Protoencogen c-kit encodes growth factor receptor protein KIT or CD117, a membrane receptor type III tyrosine kinase whose expression has been observed in several types of neoplasms.[11] The intracellular portion of KIT has the enzymatic kinase domain and the extracellular part bands with a ligand called stem cell factor (SCF).[12] SCF-kit system starts a cascade of signals acting within the cytoplasm and by the means of these signals, several types of cells such as hematopoietic stem cells (HSCs), melanocytes, germ cells, and interstitial cells of Cajal develop. Consequently, KIT expression can be used in order to detect the tumors derived from these cells.[12-16] Also, studies show that KIT is expressed in some subtypes of RCCs such as chromophobe RCC.[17] So we intended to carry out this study in order to explore the expression of c-kit gene in various subtypes of RCC in addition to investigating the correlation between c-kit expression and clinicopathological parameters such as age and gender of the patients and also grade, stage, and size of the tumor.

Material and methods

We analyzed 65 cases of RCC from the pathological documents which had gone under surgery from March 2011 to March 2015 in Urmia Imam Khomeini Hospital, Urmia, Iran. The primary diagnosis was made according to imaging studies and clinical presentation of the patients. All procedures involving human participants were in accordance with the ethical standards of the Ethics Committee, Urmia University of Medical Sciences and the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All of the patients in the study had undergone radical nephrectomy at the Urology Department of Imam Khomeini Hospital.

Formalin-fixed and paraffin-embedded 3-mm tissue sections of each patient's tumor were stained using H&E technique. These specimens were extracted from the archives of pathology department. The slides were analyzed by a pathologist. RCC cases were classified according to WHO classification.[1] Then, grading of RCC was performed according to the criteria proposed by Fuhrman et al.[18]. According to this grading system, tumors with inconspicuous and basophilic nucleoli at 400× magnification were categorized as grade I, while tumors with conspicuous and eosinophilic nucleoli at 400× which were visible but not prominent at 100× were categorized as grade II. Also, grade III tumors had conspicuous and eosinophilic nucleoli at 100×, and finally grade IV tumors were categorized as nuclear with an extreme pleomorphism, and multinuclear giant cells.

Also, according to the criteria of the American Joint Committee on Cancer[19], stage I and II tumors were categorized based on their size (< or >7 cm) or extent of their spread as only limited to the kidney with no invasion of lymph nodes or distant organs. Stage III tumors were categorized as tumors growing into a major vein (for example renal vein or vena cava) with no invasion of the adrenal gland or beyond Gerota's fascia. In this stage, invasion of lymph nodes and distant organs was not detected. Stage IV tumors were categorized as any size of the tumor which had grown outside the kidney. In this stage, tumor invades the distant lymph nodes and/or other organs and invasion of nearby lymph nodes may be observed.

Finally, the proper tissue block appropriate for immunohistochemical staining was selected and a new tissue section was obtained by cutting with a microtome. Immunohistochemical staining for CD117 was performed and the new slides were analyzed by another pathologist. The relation between the variables was assessed using chi-square test.

Results

Out of 65 patients undergoing nephrectomy for suspected RCC, 38 patients (58.5%) were male and 27 patients (41.5%) were female with a male to female ratio of 1.41:1. The age of the patients ranged from 25 to 86 years and the mean age was 56.47±12.57 years. No significant correlation was seen between patients' age and sex and c-kit expression. The correlation between c-kit expression and tumor characteristics including grade, stage, and size was also insignificant (Table 1).

The most common subtype of RCC was clear cell RCC comprising 60% of the cases and the least common subtype was sarcomatoid RCC encompassing 1.5% of the cases. There was a significant correlation between c-kit expression and subtypes of RCC (p=0.001). The most common subtypes expressing c-kit were chromophobe and papillary subtypes (Table 2).

Discussion

Various subtypes of RCC can be distinguished from each other by their different morphologic features. These subtypes have different prognosis and clinical approaches.[1] Studies have shown that each subtype is also genetically different from the other one.[20] Usually it is possible to differentiate the subtypes using routine H&E method but there are some common and overlapping morphological characteristics of the subtypes that cause difficulties in diagnosis. Consequently, there is a need for a supplementary method in order to distinguish RCC subtypes.[21]

CD117 or growth factor receptor protein KIT is a membrane receptor tyrosine kinase which is encoded by protoencogen

Table 1. Relationship between c-kit expressions and clinicopathological parameters

Characteristics		No. of cases	c-kit positive cases no. (%)	p
All cases		65	25 (38.5%)	0.4*
Age (years)	≤50	22	10 (45.5%)	
	>50	43	15 (34.9%)	
Sex	M	38	15 (39.5%)	0.84
	F	27	10 (37%)	
Grade (conventional)	I	14	6 (42.9%)	0.95
	II	39	14 (35.9%)	
	III	10	4 (40%)	
	IV	2	1 (50%)	
Stage	I, II	55	22 (38.6%)	0.95
	III	8	3 (37.5%)	
	IV	4	0 (0.0%)	0.10
Size (5 cm)	≤ 5 cm	47	20 (42.6%)	0.27
	> 5 cm	18	5 (27.8%)	

*Chi-square test

Table 2. Comparison between c-kit expressions and histopathological subtypes of RCC

Tumor histology	No. of cases	c-kit positive no. (%)	p
CCRCC	39	6 (15.4%)	0.001*
PRCC	13	8 (61.5%)	
ChRCC	12	11 (91.7%)	
SRCC	1	0 (0.0%)	

*Chi-square test. RCC: renal cell carcinoma; CCRCC: clear cell renal cell carcinoma; PRCC: papillary renal cell carcinoma; ChRCC: chromophobe renal cell carcinoma; SRCC: sarcomatoid renal cell carcinoma

c-kit. It is expressed in various neoplasms such as melanoma, seminoma, small cell lung cancer, gastrointestinal stromal tumor (GIST), myeloproliferative disorders, mast cell neoplasms, and pancreatic ductal carcinoma.[16,22-25] Furthermore, studies have shown that c-kit is also expressed in renal neoplasms including renal oncocytomas, renal angiomyolipomas, and some subtypes of RCC such as chromophobe RCC. Thus, it is possible to use immunohistochemical staining for c-kit in order to diagnose and differentiate the different subtypes of RCC.[26]

In the current study, among total 65 cases of clear cell RCC, indicated percentages of cases with papillary RCC (6/39; 15.4%), chromophobe RCC (8/13; 61.5%), and sarcomatoid RCC (91.7%; 11/12) expressed c-kit which demonstrates the presence of a significant relationship between the expression of c-kit and RCC subtypes.

The study carried out by Zhang et al.[27] showed that 10.9% of the clear cell RCCs expressed c-kit. The result of this study about clear cell RCC agrees with the results of our study.

In the study conducted by Lin et al.[28], c-kit was expressed by 0% of clear cell RCCs, 100% of papillary RCCs, 100% of chromophobe RCCs, and 100% of non-neoplastic kidneys. Also the study of Liu et al.[10] shows that c-kit is expressed by 0% of clear cell RCCs, 82% of chromophobe RCCs, and 100% of renal oncocytomas. These two studies show that the expression of c-kit in papillary RCC and chromophobe RCC is higher than its expression in clear cell RCC which is similar to our results. Additionally, the findings of the studies of Miliaras et al.[15] and Ziguner et al.[12] also confirm that c-kit is frequently and significantly expressed in chromophobe RCC and papillary RCC subtypes.

According to our results, various percentages of the cases with grade I (42.9%), grade II (35.9%), grade III (40%), and grade IV (50%) were positive for c-kit immunostaining. Although higher tumor grade correlated with higher rate of c-kit expression, this relationship was not statistically significant.

In the current study positive expression of c-kit was found in tumors greater, and also less than 5 centimeters, but the correlation was also not statistically significant. Furthermore, this negative correlation was found for pathological stage of the tumor. Our results were not confirmed by those of Zhang et al.[27] study as they asserted that c-kit was associated with

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advanced pathological stage, higher nuclear grade, and larger tumor size. In contrast, our findings were consistent with the results of Ahmed et al.[29]. The findings of their study did not show significant correlation between pathological stage and positive c-kit expression but they stated that c-kit might be significantly expressed in high grade clear cell RCCs including those with sarcomatoid change and cytoplasmic expression pattern. We have considered RCCs with sarcomatoid differentiation as a subtype of RCC and this might explain the differences between the two studies.

In our study, we did not find statistically significant correlation between c-kit expression and patients' age and gender. This result was consistent with the results of both studies conducted by Ahmed et al.[29] and Zhang et al.[27].

In conclusion, overlapping morphologic characteristics cause difficulties in diagnosis of RCC subtypes. This study suggests that combining c-kit immunostaining with the routine H&E technique can increase accuracy and lead to more accurate diagnosis especially among papillary and chromophobe types of RCCs.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ethics Committee, Urmia University of Medical Sciences.

Informed Consent: Written informed consent was obtained from all patients who participated in this study.
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