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Original Article

Expression of CD44 and P53 in Renal Cell Carcinoma: Association with Tumor Subtypes

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ABSTRACT. Renal cell carcinoma (RCC) is a common malignancy of the kidney and accurate prediction of prognosis is valuable for the design of adjuvant therapy and counseling and effective scheduling of follow-up visits. Molecular genetic investigations of CD44 and P53 in RCC may be helpful in this regard. We studied the CD44 and P53 expressions semi-quantitatively on paraffin-embedded specimens of 64 RCC patients (37 male/27 female) who underwent surgery from 2003 to 2008 by immunohistochemistry and analyzed the correlation of P53 and CD44 expression in RCC and outcome. Thirteen of 64 (20.3%) specimens were P53 positive, 30/64 (46.9%) were CD44 positive and five tumors with positive P53 expressed CD44 protein (P = 0.5). A statistically significant correlation was not found between CD44 and P53 expression (P = 0.5) and age (P = 0.07), sex (P= 0.3), tumor size (P = 0.7), grade (P = 0.23), vascular invasion (P = 1.00) and ureteral invasion (P = 1.00). Furthermore, a significant correlation was not found between P53 expression with age (P = 0.3), sex (P = 0.7), tumor size (P = 0.7), grade (P = 0.1), vascular invasion (P = 1.00) and ureteral invasion (P = 1.00). According to our findings, only P53 expression is generally accompanied by non-conventional subtype tumor.

Introduction

Renal cell carcinoma (RCC) is a common cancer, and the incidence of it has been rising steadily in the recent years. RCC constitutes approximately 2% of all adult cancers.[1] Because more than two-thirds of RCC patients will die of their disease, the accuracy of pathology specimen assessment is very important for patient follow-up.[1] Molecular genetic investigations have recognized two distinctive subtypes of RCC: Conventional (clear cells) and non-conventional (papillary, chromophobe, collecting and unclassified).[2] It is accepted that prognosis differs according to the histological type, nuclear grade and tumor stage.[3] However, in many cases of conventional RCC, staging and grading are not sufficient to predict the clinical outcome of these tumors.[4] Several reports have focused on the evaluation of new markers and, accordingly, the prognos-

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tic value of P53 mutation and CD44 has been recently investigated.[5]

P53 tumor suppressor gene is located on chromosome 17p13, which encodes wild-type P53 protein.[6] This protein has a role in cell-cycle arrest after apoptosis, DNA damage and mitotic checkpoint regulation. In other words, this gene plays an important role in the control of cell proliferation. Therefore, P53 is one of the most important cancer-related genes and its mutation has been reported in a variety of cancers, such as lung, breast and prostate cancers.[7] In RCC, the role of P53 remains undetermined and the results obtained regarding the prognostic significance of its mutation have been presented.[7,8]

Cell adhesion molecules seem to have a role in tumor cell invasion and tumor dissemination by mediating interactions between tumor cells and their environments.[9] Altered expression of cell adhesion molecules such as CD44 on tumor cells suggests a pathogenetic mechanism for tumor metastasis and may provide prognostic information for particular tumors. CD44 is a surface transmembrane glycoprotein that was initially identified on lymphocytes. The extracellular domain of CD44 is the principal receptor of an extracellular matrix molecule, hyaluronic acid. Overexpression of hyaluronic acid has been correlated with the metastatic potential of certain human tumors, and these cell–matrix interactions of CD44 play a role in tumor cell invasion and metastasis.[10,11]

We aimed in this study to determine the correlation of P53 protein and CD44 expression in RCC subtypes. The immuno-histochemical findings were subsequently correlated with multiple clinico-pathological parameters like nuclear grade and tumor size as prognostic markers in RCC.

Materials and Methods

A total of 64 renal tissue biopsies (formalin-fixed, paraffin-embedded renal tissue) from patients with RCC pathologically confirmed were collected from 2003 to 2008. Characteristic data for sex, age and tumor size of patients were obtained from patients' medical records. The study protocol complied with the ethical guidelines of the Medical Ethics Committee, Ministry of Health, Iran.

The paraffin-embedded tissue blocks were cut into 4-μm sections and mounted on slides. Sections were de-waxed in xylene and were rehydrated in descending alcohol concentrations. Then, they were boiled in a target retrieval solution for 15 min and cooled to room temperature. All the slides were washed with 0.1% Tris-buffered saline (TBS) for 5 min. Later, they were kept in 0.3% hydrogen peroxidase for 5 min for the blockage of endogenous peroxidase activity. The slides were rinsed with TBS for 5 min and then incubated with monoclonal antibody for 5 min and rinsed again with TBS for 5 min, and Envision and chromogen (Dako Co, Denmark) was added. After 30 min, the slides were rinsed under water and countered with hematoxyline, dehydrated and mounted. They were examined regarding the P53 and CD44 protein expression and evaluated regarding the vascular invasion and ureteral invasion. According to the latest tumor subtype classification,[2] tumors were classified to conventional (include clear cell type) and non-conventional (include papillary, chromophobe, collecting and unclassified types) classes. Immunostaining of tumor cells was semiquantitatively scored according to the Fuhrman's nuclear grading system[3] as follows: No positive cells (grade 1); fewer than 25% of reactive cells (grade 2); from 25% to 75% of positive cells (grade 3); and more than 75% of positive cells (grade 4).

Validation of staining scoring of all the preparations was carried out independently by a pathologist without prior knowledge of the samples. Tumors were classified as positive if >5% of the stained cells were positive for P53 and CD44. Negative controls by the respective deletion of primary antibody were also performed.

Statistical Analysis

The mean values were expressed as mean ± standard deviation (SD). The Spearman rank correlation was used to correlate the immuno-

histochemical findings for CD44 and P53 and histopathological parameters. The chi-square test was used to evaluate the qualitative parameters. These tests were processed with SPSS 17.0 statistical software program (SPSS Inc. Chicago, Illinois, USA). *P*-value <0.05 was considered as statistically significant.

Results

The 64 study patients ranged in their age from 19 to 86 years (mean ± SD: 56 ± 6 years) and the male/female ratio was 37/27. The mean tumor size was 7.6 ± 4.0 cm. Of the 64 RCC cases, positivity for P53 was observed in 13 (20.3%) specimens (Figure 1), while 30 (46.9%) sections were positive for CD44 (Table 1, Figure 2). Thirteen of 64 (20.3%) specimens were P53 positive, 30/64 (46.9%) of specimens were CD44 positive and five tumors with positive P53 expressed CD44 protein (*P* = 0.5).

There was no statistically significant difference in P53 expression in relation to the tumor size (*P* = 0.7), age (*P* = 0.3), sex (*P* = 0.7) and grade (*P* = 0.1). Also, there was no statistically significant difference in CD44 expression in relation to the tumor size (*P* = 0.7), age (*P* = 0.07), sex (*P* = 0.3) and grade (*P* = 0.23). Two of 13 (3.1%) P53-positive cases were conventional and 11 (17.2%) were non-conventional, which was statistically significant (*P* <0.05). There was no significant correlation between CD44 expression and tumor subtype: 17/30 (26.6%) were conventional and 13/30 (20.3%) were non-conventional (*P* = 0.6).

Two of thirteen (3.1%) P53-positive specimens and six of 51 (9.4%) P53-negative specimens revealed vascular invasion. Also, four of 30 (6.3%) CD44-positive cases and four of 34 (6.3%) CD44-negative specimens showed vascular invasion. On the other hand, one of 13 (1.6%) P53-positive cases and three of 51 (4.7%) P53-negative specimens had ureteral invasion. In addition, two of 30 (3.1%) CD44-positive cases and two of 34 (3.1%) CD44-negative specimens showed ureteral invasion. There were no statistically significant correlations between ureteral and vascular invasion and both P53 and CD44 expression (*P* = 1.000)

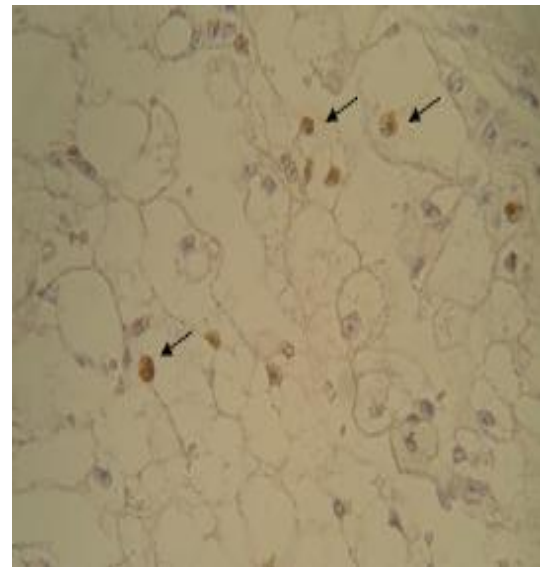

Figure 1. P53 immunoreactive tumor cells in a renal cell carcinoma (×400).

Discussion

RCC constitutes 1–3% of visceral cancers in humans and about 85% of primary malignant tumors of the kidney in adults.[12] In many cases, efforts such as grading and staging are not sufficient to define the behavior and prognosis of these tumors. Thus, several studies have focused on the evaluation of new markers. The most common mutated gene in human malignancies is P53. The mutation of this gene is reported in most of human malignancies such as astrocytoma, mesothelioma, sarcoma, leukemia and colon, bladder, lung and breast carcinomas.[13,14] The relationship between the increased expression of this protein and urogenital cancers (bladder and prostate carcinomas) has been well demonstrated;[13,15,16] while its relationship with RCC is still a matter of debate.

Studies have shown that the standard form of CD44 or one or more of its variants are overexpressed in many human malignancies, including breast, stomach, colorectal, lung, bladder, non-Hodgkin lymphoma, certain squamous cell carcinomas, melanoma and RCC.[10] The enhanced metastatic potential for tumor cells that overexpress CD44 has been well documented. CD44 overexpression in RCC has shown an

Table 1. Relationship between immunohistochemical expression of both P53 and CD44, and age, sex, tumor size, grade, invasion and tumor subtype in RCC.

Variables	P53 positive n (%)	P53 negative n (%)	*P*	CD44 positive n (%)	CD44 negative n (%)	*P*
	13 (20.3 %)	51 (79.7%)		30 (46.9%)	34 (53.1%)	
Age (years)						
≤50	6 (9.4%)	17 (26.6%)	0.3	11 (17.2%)	12 (18.7%)	1
>50	7 (10.9%)	34 (53.1%)		19 (29.7%)	22 (34.4%)	
Sex						
Male	7 (10.9%)	30 (32.8%)	0.7	16 (25%)	21 (32.8%)	0.3
Female	6 (9.4%)	21 (46.9%)		14 (21.9%)	13 (20.3%)	
Tumor size (cm)						
≤5	2 (3.1%)	14 (21.9%)	0.7	10 (15.6%)	6 (9.4%)	0.07
>5	11 (17.2%)	37 (57.8%)		20 (31.3%)	28 (43.7%)	
Tumor subtype						
Conventional	2 (3.1%)	37 (57.8%)		17 (26.6%)	22 (34.4%)	
clear cell	2 (3.1%)	37 (57.8%)		17 (26.6%)	22 (34.4%)	
Non-conventional	11 (17.2%)	14 (21.9%)	0.000	13 (20.3%)	12 (18.8%)	0.6
papillary	7 (10.9%)	6 (9.4%)		9 (14.1%)	4 (6.2%)	
chromophobe	4 (6.3%)	8 (12.5%)		4 (6.2%)	8 (12.5%)	
collecting	0 (0.00%)	0 (0.00%)		0 (0.00%)	0 (0.00%)	
unclassified	0 (0.00%)	0 (0.00%)		0 (0.00%)	0 (0.00%)	
Grade						
1	0 (0.00)	14 (21.9%)		4 (6.3%)	10 (15.6%)	
2	10 (15.6%)	28 (43.8%)	0.1	18 (28.1%)	20 (31.2%)	0.23
3	3 (4.7%)	9 (14%)		8 (12.5%)	4 (6.3%)	
4	0 (0.00%)	0 (0.00%)		0 (0.00%)	0 (0.00%)	
Invasion						
Vascular invasion	2 (3.1%)	6 (9.4%)	1	4 (6.3%)	4 (6.3%)	1
Ureteral invasion	1 (1.6%)	3 (4.7%)		2 (3.1%)	2 (3.1%)	

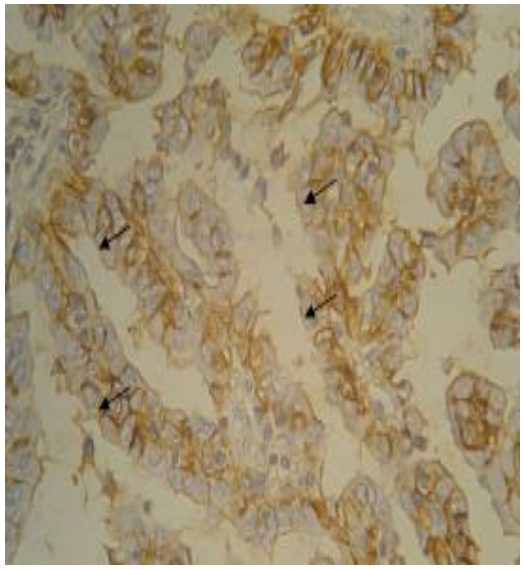

Figure 2. CD44 immunoreactive tumor cells in a renal cell carcinoma (×400).

association with tumor progression, recurrence, survival, aggressiveness and, perhaps, pathogenesis.[10,11]

It was demonstrated that positive P53 immunostaining in RCC is associated with metastatic carcinomas and poor survival in patients with early-stage disease[17] It was claimed by Mombini et al that increased expression of P53 protein is rather prevalent in RCC. This factor is associated with tumor grade and subtype. According to their findings, P53 was generally associated with non-conventional subtypes and higher tumor grades.[18]

Nuclear grade and tumor size were found to be independent predictors of survival in locally confined clear cell (conventional) RCC, as has been shown previously for locally confined RCC in general, but P53 expression did not contribute additional prognostic information.[5] In the Zigeuner et al report,[6] P53 overexpres-

sion for papillary, chromophobe and conventional RCC was 70.0%, 27.3% and 11.9%, respectively (P <0.0001). They concluded that overexpression of P53 was significantly more frequent in "nonconventional" RCC subtypes, especially papillary RCC, compared with conventional RCC in their study. Terpe et al.[19] showed that expression of variant CD44 isoforms was strongly correlated with grading and appeared to mediate a more aggressive phenotype to renal cell tumors, but they could not interpret the clinical data.

In a study on 91 patients with locally confined conventional RCCs, CD44 was found to be a useful prognostic parameter in conventional RCC.[20] In another study, the expression of CD44 was immuno-histochemically evaluated in 173 conventional RCCs, and was compared with the usual clinico-pathological parameters such as tumor size, histological grade and patho-logical stage. The study concluded that CD44 molecules may play a role in the progression of conventional RCC and may be used in the evaluation of disease outcome.[21]

In our study, we examined the immunohistochemical staining patterns of P53 and CD44 in archival tissue from patients who had undergone partial or radical nephrectomy for RCC. We found the expression of P53 protein in 20.3% of the specimens similar to previous studies (20–30%).[18] The most important finding in our study was the correlation between expression of P53 protein and non-conventional types of RCC, especially the papillary type, which was similar to that in the study of Zigeuner et al.[6]

We conclude that of the markers of RCC, only P53 was found in our study, helpful in staging of conventional tumor. A further study of other markers may be more helpful in this regard.

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