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Original Article

Prognostic value of CD44 in renal cell carcinoma

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Abstract

BACKGROUND: CD44 is a transmembrane glycoprotein involved in cell-cell and cell-matrix interactions. De novo expression of CD44 and its variant isoforms has been associated with aggressive behavior in various tumors. Since little data is available on the role of CD44 expression in renal cell carcinoma, we evaluated CD44 expression to determine its prognostic value.

METHODS: Forty-six patients with renal cell carcinoma were studied. CD44 expression was evaluated semiquantitively on paraffin-embedded tumor tissue by immunohistochemistry. The prognostic value of CD44 was tested using Kaplan Meier plots by the log rank test and Cox regression analysis.

RESULTS: Fifteen out of 46 specimens (32.6%) were CD44-positive. According to bivariate analysis, tumor stage, tumor size, nuclear grade and CD44 expression were significant prognostic factors.

CONCLUSIONS: CD44 expression can be considered as a useful prognostic parameter in renal cell carcinoma.

KEY WORDS: CD44 expression, renal cell carcinoma, immunohistochemistry.

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Renal cell carcinoma (RCC) is a common cancer and its increasing incidence is partly related to improvement in diagnostic tests. Recent advances in molecular genetic analysis have led to the recognition of 5 distinct types of RCC, namely conventional, papillary, chromophobe, collecting and unclassified [1]. It is accepted that prognosis differs according to the histological type, tumor stage and nuclear grade [2,3].

However, in many cases of conventional RCC, staging and grading are not sufficient to predict the clinical behavior of these tumors. Therefore, several studies have focused on the evaluation of new markers. Indeed, the prognostic value of P53 mutation and ki67 and VEGF expression have been recently investigated [4,5]. Results from these studies are discordant, and up to now, none of these parameters appear to be better predictive prognostic factor than the usual staging and grading.

CD44 is an adhesion molecule involved in cell-matrix interaction and its expression has been linked to tumor metastasis in several cancers [6]. To date, only a few studies have evaluated CD44 expression in RCC in vivo [5]. The aim of this study was to investigate the role of CD44 as a prognostic marker in RCC.

Methods

Case selection

This is a historical cohort study that was performed on paraffin-embedded specimens from patients with primary RCC who underwent

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surgery between 1994 and 2004 at the Urology Department. Out of a total of 125 cases, we excluded cases that did not come for follow up. We only included cases that had been operated on using the radical nephrectomy technique. More cases were excluded owing to incomplete clinical data and inadequate archival material. Finally, a total of 46 cases were included in the survival analysis. All specimens were reevaluated for pathological stage, grade and histological subtypes by two pathologists and compared with previous pathological reports. Clinical data were obtained from patient medical records at the archives of Al-Zahra and Seyed-al-Shohada Hospitals, as well as the author's archive. The pathological stage was adjusted according to the 2002 TNM staging system [7]. The nuclear grade was determined according to the Fuhrman classification [8]. The histological subtype was assessed according to the consensus classification of RCC [9]. The routine follow-up regimens were history, yearly physical examinations and liver function tests for T1 tumors, history, yearly physical examinations, liver function tests, CXR and abdominal ultrasonography for T2 tumors, and history, 6-month physical examinations, liver function tests and CXR for 3 years and then yearly with abdominal ultrasonography in the first year and then yearly for T3 tumors.

Equivocal ultrasonographic findings were further evaluated by abdominal CT scanning. No informed consent was required for such studies dealing with archived material.

Immunohistochemistry (IHC)

Five-micron sections were mounted on slides for IHC analysis. Sections were deparaffinized with xylene, treated with hydrogen peroxide and washed after 5 minutes. Biotin was added and washed after 10 minutes; then streptavidin was added and washed after 10 minutes. Finally, monoclonal antisera to CD44 (DAKO) were used and assessed by pathologist. Pathologists were unaware of the clinicopathological data, especially the pathological stage and patient outcome. Tumor cells with less than 5% immunoreactivity were considered negative and those with more than 5% immunoreactivity were considered positive (figure 1). CD44 was reported as positive or negative with no grading. Positive and negative controls were included.

Statistical analysis

According to pathologic stage, grade, histological subtype and sex, the subgroups were compared for possible differences in CD44 immunoreactivity. Survival of patients with and without CD44 immunoreactivity was evaluated by the Kaplan-Meier method and compared by the log-rank test and then, multivariate analysis was performed with Cox regression.

Results

Forty-six patients met the inclusion criteria. The mean age was 52.64 years (range: 26-77 years) and male-to-female ratio was 1.48 (59.7% male; 40.3% female). Pathological stage was I in 18 cases (39.1%), II in 10 (21.7%) and III or IV in 18 (39.1%). Sixteen lesions were grade I (34.7%), 21 were grade II (45.65%), and 9 were grades III and IV (19.56%) (table 1). Total 10-year survival of patients was 69.44% (figure 2). Mean tumor size was 7.5 cm (range: 2-20 cm). The CD44-positive frequency was 32.6% (15 of 46). Eight patients died of cancer and 38 are alive without any evidence of dis-

Table1. Patients characteristics

Number of patients	46
Mean age (range)	52.64 (26-77)
Men/women (%)	59.7/40.3
Pathological stage (%):	
I	18 (39.1%)
II	10 (21.7%)
III + IV	18 (39.1%)
Histopathological grade (%)	
1	16 (34.7%)
2	21 (45.65%)
3 + 4	9 (19.56%)
Histological subtypes	
Conventional	35 (76.08%)
Papillary	6 (13.04%)
Sarcomatoid	2 (4.34%)
Collecting duct	1 (2.1%)
Papillary and clear	1 (2.1%)
Chromophobe	1 (2.1%)

ease. The mean follow-up period was 31.3 months (range: 1-120 months). Mean survival time was 70.16 months (range: 33.57-106.75 months) for CD44-positive patients, and 84.42 months (range: 73.94-94.89 months) for CD44-negative patients. Patients with CD44-positive RCC had shorter survival than those with CD44-negative tumors (log rank test: P = 0.0269) (figure 3). Cox regression analysis showed CD44 expression to be an independent prognostic factor (Regression coefficient = B = -2.412, P value = 0.039, Odds Ratio = 0.09).

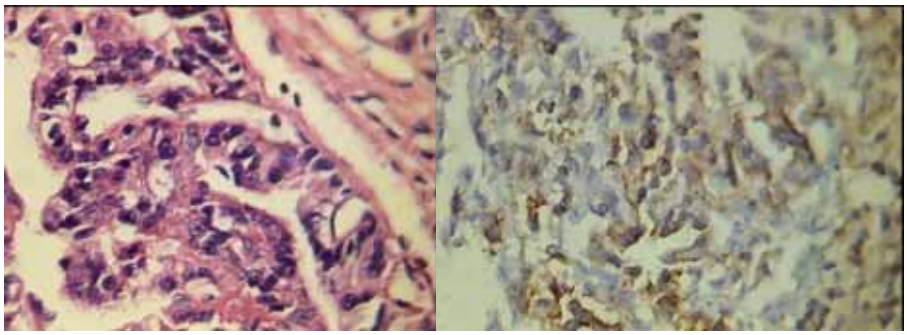

Figure 1. Renal cell carcinoma, H&E and CD44 immunostaining.

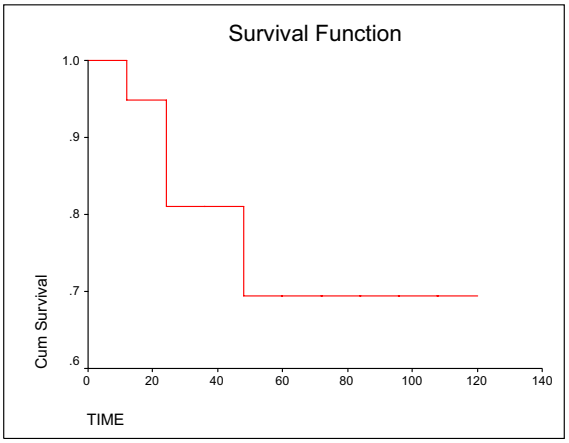

Figure 2. Overall survival rate of patients with RCC.

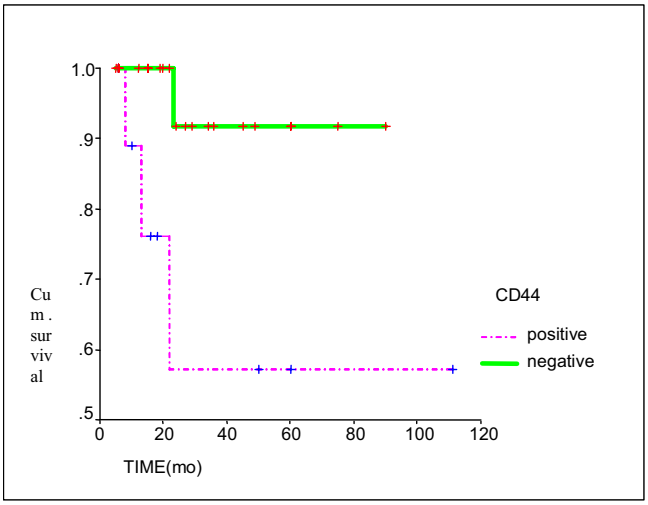

Figure 3. Survival rates of patients with RCC in relation to the expression of CD44.

Discussion

RCC is well-recognized as a malignancy with unpredictable course [10]. Tumor stage and nuclear grade are usually considered the main pathological prognostic factors [11], but improved prediction is needed and attempts to find better prognostic criteria remain under investigation [4-6,12]. Cell adhesion molecules are thought to participate in tumor metastasis by mediating interactions between tumor cells and their environment. CD44 is a transmembrane glycoprotein known as hyaluronan.

CD44 has been associated with diverse physiological functions such as cell-cell and cell-matrix interactions, as well as lymphocyte homing [13]. The increased expression of CD44 may be associated with unfavorable clinical behavior in non-small-cell lung carcinoma and gastric carcinoma [14,15]. Terpe et al [16] showed a strong correlation between the expression of CD44 and grade in RCC. They did not correlate CD44 expression with clinical data. Paradis et al [12] showed that CD44 expression correlated with the nuclear grade, size and stage of the tumor. They only evaluated locally-confined conventional renal cell carcinomas. Gilcrease et al [17] found an association between CD44 expression and progression and recurrence in RCC and suggested that CD44 may be an important prognostic factor in RCC. Li et al [18] showed that the expression of CD44 correlated significantly with grade, stage and survival in patients with clear cell RCC. Lucin et al [19] showed that CD44 expression may play a role in the progression of conventional RCC.

In the present study, the prognostic value of CD44 was evaluated in 46 patients with long-term follow up. CD44 was a significant marker of prognosis in the univariate and multivariate analyses. It is important to note that based on statistical calculations, about 35 deaths would be expected in this study in each group (35 in the CD44-positive and 35 in the CD44-negative group). However, the incidence and mortality of RCC is not high enough to produce such a situation and as seen in similar studies, there are an insufficient number of cases. Help can be obtained from meta-analysis.

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