Original Article

Prognostic value of p53 in renal cell carcinoma

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ABSTRACT

Background and Objectives: RCC is one of the most common genitourinary cancers. Accurate prediction of prognosis would be valuable for adjuvant trial design, counseling and effectively scheduling follow up visits.

P53 is a tumor suppressor gene that expresses a protein that involved in both cell-cycle arrests after DNA damage and apoptosis. Presence of mutated p53 protein in tumors has been related to poor prognosis in several malignancies such as lung, breast and prostate cancer. There is diverging results concerning the prognostic significance of mutated p53 in RCC. The aim of this study was to investigate the survival rate of RCC and the role of inactivated p53 protein as a prognostic marker in RCC.

Materials and Methods: Patients with nonmetastatic renal cell carcinoma were studied. Paraffin embedded specimens of patients who underwent surgery between 1994 and 2004 at our department were chosen. All specimens were reevaluated with regard to pathological stage, nuclear grade, histological subtypes and P53 expression. P53 expression was semiquantitively evaluated on paraffin-embedded tumor tissue by immunohistochemistry. The prognostic value of parameters was tested using Kaplan Meier plots by the log rank test and Cox regression analysis.

Results: This study performed on paraffin-embedded specimens of patients with nonmetastatic RCC who underwent surgery between 1994 and 2004 at our department. The mean age was 52.64yr (SD: 13.49). Mean tumor size was 7.95cm (SD: 4.00). Pathological stage was I in18 (39.1%), stage II in 10(21.7%), stage III and IV in 18(39.1%) patients. Analysis revealed that 16 lesions were grade I (34.7%), 21(45.65%) grade 2, and 9(19.56%) grades 3and 4. The 10-year total survival of patients was 69.44%.

In 28.3% of cases P53 staining was positive. In bivariate analysis tumor stage, tumor size, nuclear grade and P53 expression were not found to be significant prognostic factors.

Conclusion: P53 can not be considered as a useful prognostic parameter in renal cell carcinoma.

Key Words: P53 expression, renal cell carcinoma, immunohistochemistry

Introduction

Renal cell carcinoma (RCC) is a common cancer, and its increasing incidence is partly related to improvement in diagnostic tests(1).Recent advances in molecular genetic analysis have led to the recognition of 5 distinct types of RCC: conventional (clear cells with or without granular cells), papillary, chromophobe, collecting and unclassified (2). It is accepted that prognosis differs according to the histological type, tumor stage and nuclear grade (3, 4). However, in many cases of conventional RCC, staging and grading are not sufficient to predict the clinical behavior of these tumors (5). Therefore, several studies have focused on the evaluation of new markers. Indeed the

prognostic value of P53 mutation and ki and VEGF expression has been recently investigated (6, 7). 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 and in the other hand these useful markers have never been evaluated in Iranian patients. Tumor suppressor gene p53 is located on chromosome 17p13 that encode wild-type p53 protein(8).This protein is involved in both cell-cycle arrests after DNA damage and apoptosis, but is also believed to be involved in mitotic checkpoint regulation (9). Mutation of p53 gene is the most common single mutation found in human cancer (9). The presence of mutated p53 protein in tumors has been related to poor prognosis in several cancers such as lung, breast and prostate cancer(9).in RCC ,the role of p53 remains undetermined and diverging results have been presented concerning the prognostic significance of mutated p53(9).

The aim of this study was to investigate the role of inactivated p53 protein and tumor stage and nuclear grade and tumor size as prognostic markers in RCC.

Materials and Methods

Case selection

This is a historical cohort study that performed on paraffin-embedded specimens from patients with primary RCC who underwent surgery between 1994 and 2004 at our department. We included only cases that had been operated by radical nephrectomy technique. This corresponded to125 cases but we excluded cases that did not participate in follow up programs. We had a synchronous TCC and RCC which excluded from study. Additional cases were excluded on the bases of incomplete clinical data and inadequate archival material so a total of 35 cases were finally included in the survival analysis .All of the surgeries had been undergone with a similar surgical team .All specimens were reevaluated with regard to pathological stage, grade and histological subtypes by two pathologists and compared with previous pathological reports .Clinical data were obtained from patients 'medical records at the archive of AL-ZAHRA university hospital and archive of the SEIED-ALSHOHADA(OMID)cancer university hospital and also archive of the author which had designed for follow up of the patients(each patient has a card that included time of surgery, stage of cancer ,type of tumor ,radiotherapist and chemotherapist notes ,sonographic and radiographic data). The pathological stage was adjusted according to the 1997 TNM staging system (10).The nuclear grade was determined according to the Fuhrman classification (10).The histological subtype was assessed according to the consensus classification of RCC (11). The routine follow-up regimens for T1 tumors were history , physical examination and liver function tests yearly ;for T2 tumors were history, physical examination ,liver function tests ,CXR and abdominal ultrasonography yearly ;for T3 tumors were history, physical examination, liver function test and CXR every 6 months for 3 years and then yearly and abdominal ultrasonography at 1year and then yearly. If there was any doubt about the sonographic findings, we had performed Abdominal CT scanning. No

informed consent was required for such studies dealing with achieved material at our institution.

Immunohistochemistry (IHC)

Sections (5 micron) from blocks were mounted on slides for IHC analysis. In brief The section were deparaffinized with xylene, treated with hydrogen peroxides and after 5 minute washed, then added Biotin and after 10 minutes washed, then added streptavidine for 10 minutes and washed, at the end monoclonal antisera to P53 (DAKO) were used and assessed by two pathologists. Our pathologists were unaware of the clinicopathological data, especially the pathological stage and outcome of the patients. Our pathologist reported tumor cell with less than 5%immunoreactivity, negative and those with more than 5% immunoreactivity, positive .We repeat borderline cases to reach a definite answer. P53 reported as: positive and negative with no grading. Positive and negative controls included.

Statistical analysis

Subgroups according to pathologic stage, grade, histological subtype and sex were compared with respect to possible differences in P53 immunoreactivity using the chi-square test. Survival of patient with and without P53 immunoreactivity was evaluated by the Kaplan-Meier method

and compared by the log-rank test and then multivariate analysis was done with Cox regression.

Values for P less than 0.05 were considered statistically significant.

Results

Of 125 patients, who underwent radical nephrectomy between 1994 and 2004, 97 patients had RCC but only 46 cases participated in follow up programs and because of damaged paraffinated blocks, 35 cases included in survival analysis. Mean age was 52.64yr (SD: 13.49) and male to female ratio was 1.48(59.7% male &40.3% female). Mean tumor size was 7.95cm

(SD: 4.00) .Of the 46 cases who participate in follow up programs, pathological stage was I in18 (39.1%), stage II in 10(21.7%), stage III and IV in 18(39.1%) patients. Analysis revealed that 12 lesions were grade I (34.2%), 16(45.7%) grade 2, and7 (20%) grades 3and 4(table1). The 10-year total survival of patients was 69.44 %(Figure1).

Table 1. Patient characteristics

Mean age (range) …......…................…..	52.64(SD:13.49)
%men/women ....….......................….	59.7/40.3
No. pathological stage (%):	
I …………………….......……………….	18(39.1%)
II………………………………………..	10(21.7%)
III +IV……………………………………..	18(39.1%)
No. histopathological grade (%)	
1 …………………….......……………….	12(34.2%)
2 …………………….......……………….	16(45.7%)
3+4………………………………….……	7(20%)
No 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%)
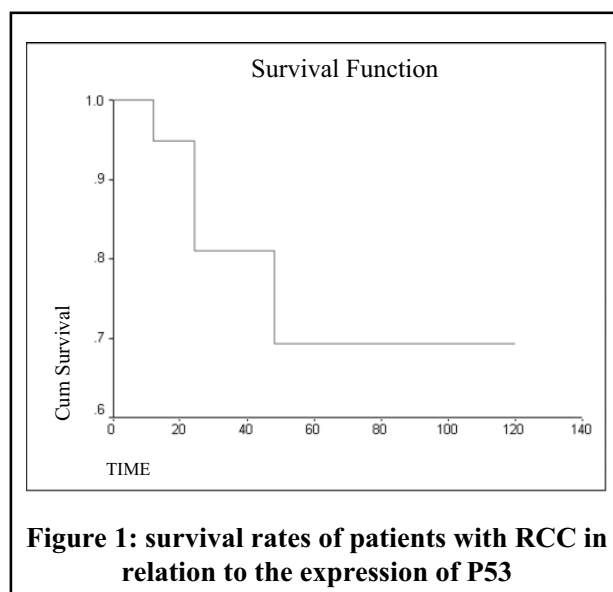
Figure 1: survival rates of patients with RCC in relation to the expression of P53

The P53 positive incidence was 28.26%. Four patients died of cancer and 31 patients are alive without any evidence of disease. Mean survival

time for P53 negative cases was 65.25(55.12-75.37, C=95%) and mean survival time for P53 positive cases was 76.67(21.72-131.61,C=95%). Patients with P53-positive RCC had not shorter survival than those with P53-negative tumors (log rank test: P=0.3773).

Mean survival time for male patients was 87.03 months (64.38-109.34, C=95%) and for female patients was 73.00 months (52.59-93.41, C=95%) and survival analysis for sex was not significant (Pvalue =.6082).

Analysis revealed negative correlation between age and survival (r= -0.42, p=o.oo6) and there was no correlation between size of tumor and survival (p=0.257).

In analysis p53 expression and stage revealed that 45.5 % of p53 positive patients and36.6% of p53 negative patients were in stage III or IV that were not statistically significant (Pvalue >0.05).

In analysis p53 expression and grade we found that 12.9% of p53 negative cases had grade III or IV and 27.3% of p53 positive case had grade III or IV that were not statistically significant (Pvalue >0.05).

In analysis p53 expression and type we found that 66.7% of p53 positive patients and 81.8% of p53 negative cases had conventional subtypes that were not statistically significant(Pvalue>0.05).

In analysis p53 and sex we found that 57.6% of p53 negative cases and 59.1% of p53 positive cases were male that were not significant (Pvalue>0.05).

Analysis of P53 with type of tumor, grade, sex and stage was not significant.

Discussion

In this study we gathered 10 year clinical information. Age of the patients was significantly lower in compare to other studies (1).In western countries RCC is a disease of elderly patients but in this study 33.3 % of patients were younger than 45 years. All of the tumors were sporadic. The most common type of tumor was conventional (75%) that is similar to other studies (1).

RCC is well recognized as a malignancy

with an unpredictable course (12). Therefore prognostic factors are particularly important in RCC. Tumor stage and nuclear grade are usually considered the main pathological prognostic factors (13), but improved prediction is needed and attempts to find better prognostic criteria remain under investigation (6,7). Evaluation of P53 status by IHC is a widely accepted tool in surgical pathologic evaluation; however the role of P53 overexpression in RCC is still controversial (8). The reported results regarding both the rate of immunoreactive tumors and impact of P53 overexpression on patients' prognosis is inconsistent (6, 7, and 9). ZIGEUNER et al showed that P53 over expression is prognostic marker only for conventional RCC(14).SHVARTS et al reported that P53 is a significant molecular predictor of tumor recurrence(15).LJUNGBERG et al reported that P53 is a prognostic marker for chromophobe and papillary tumors but not in conventional RCC(9).ULMAN et al found that positive P53 is associated with metastatic disease and poor survival in RCC(16).GIRGIN et al reported that P53 mutation is one of the most important prognostic factors in RCC (17). GELB et al showed that p53 expression is not an independent prognostic factor in conventional RCC(6).PAPADOPOULOS et al reported that p53 expression have no significant prognostic value in RCC(18).

Conclusion

In the present study the prognostic value of P53 was evaluated with long term follow up. P53 was not significant marker of prognosis in the bivariate analysis.

Acknowledgment

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