Original Article

Expression of P53 and Ki67 Proteins in Renal Cell Carcinoma and Its Relationship with Nuclear Grade

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ABSTRACT

Background and Objective: **Evaluation of tumor proliferative activity may provide a predicting parameter to estimate biologic aggression and a subsequent prognosis that has been evaluated in many malignancies. We have selected renal cell carcinoma (RCC) in this study. To determine tumor proliferative activity, KI67 antibody was applied and results were compared with apoptosis, applying P53 antibody and using immunohistochemical staining.**

Patients and Methods: **Specimens of 30 patients who underwent radical nephrectomy for RCC were selected for histopathology and immunohistochemical study. Two different grading systems (S&H, Fuhrman) were used to calibrate average nucleoli diameter and tumor grading on all specimens. After processing of paraffin-embedded samples, they were immunohistochemically stained applying (MIB-1) KI67 monoclonal and P53 antibodies. Then, statistical analysis was done.**

Results: **Tumor grading correlated with the average nucleus diameter. Positive reaction to KI67 and P53 antibodies in tumors increased as compared to control group. No significant relationship between age, sex and tumor grade was obtained.**

Conclusion: **These two antibodies are as easy and reliable markers that could be applied on formalin-fixed tissues for better assessment of the biologic behavior of RCC and probably prediction of patients' outcome.**

Key words: **P53 protein, Ki-67 Antigen, Renal cell carcinoma, Immunohistochemistry**

Introduction

Renal cell carcinoma (RCC) is the most frequent renal neoplasia (90-95%) which includes 3% of malignancies among adults. Tumor etiology and histogenesis has not been completely known (1-3). Tumor is subdivided into different subgroups which clear cell type is the most frequent one. Tumor prognosis is dependent on different factors including early weight and dimensions, stage and tumor cell morphology (4,5). Different grading systems are used for RCC and specialists have considerably different grading viewpoints. Tumor reactivity rate to prognosis has been measured by assaying patients' survival. Increasing severity and reactivity rate to these markers have been always followed by poor prognosis. These markers were applied to predict metastasis possibility.

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Cellular proliferation rate and apoptosis may provide another predictive variable for biologic aggression of RCC and therefore prognosis. Cellular proliferation rate could be evaluated by studying rates of KI67 antigen and PcNA (proliferate cell nuclear antigen). Detecting mutant P53 antigen demonstrates apoptosis degree in tumor. Since in different studies, KI67 and P53 have been considered as a good predictive marker for RCC aggression and survival outcome of patients (6), therefore, the purpose of this study was to assess nuclear grading of RCC on nucleolar morphometric basis and its correspondence with apoptosis and proliferative marker.

Patients and Methods

This study was carried out on archival revision of the pathologic findings of patients who underwent radical nephrectomy at Imam Reza hospital from 1995 to 2006. Histopathologic revision evaluated the nephrectomy speciemens for the type of primary tumor. Tumor grading was classified according to Syrjanen and Hjelt (SH grading) and Fuhrman (F grading) (Table 1). To carry out Fuhrman grading system, oculometer was applied to calibrate nucleus diameter. The mean of 5 fields in each case was calculated and compared to different grading system.

Then, grade, diameter, shape and uniformity of nucleus regarding the presence or absence of nucleoli were evaluated using Fuhrman system. Thereafter, a suitable block from each tumor was selected for immunohistochemical staining and 5μm tissue sections were prepared on adhesive slides. Then, sections were deparaffinized and rehydrated. After rinsing in deionized water, sections were immersed in phosphate buffer saline (PBS; pH 7.2). The labeled streptovidin-biotin-peroxidase technique was undertaken with the monoclonal antibody MIB-1 directed against KI67 antigen and anti-P53 against P53 antigen. Negative controls prepared by substitution of non-immune serum on sections and known positive controls were run in parallel to give reliable results. Immune reaction was proved by development of brown nucleus after applying DAB chromogen. Normal renal tissues in 5 specimens were used as control. Immunoreactivity was identified by nuclear brown color. Two pathologists observed and reported tumors with their positive reaction and percentage of stained nucleoli in each tumor.

Above-mentioned data including age, sex, nucleus diameter and grading, and tumor positive reaction percentage in different grades were assayed by ANOVA and Spearman statistical analysis. A p-value less than 0.05 considered significant.

Table 1. Practical grading systems for RCC in this study

A: Syrjanen and Hjelt system (1978)
SH I- good differentiation, uniformed round nucleus, slight mitosis
SH II- moderate differentiation, some anisocaryosis and hyperchromasia
SH III- Poorly differentiated, anisocaryosis, frequent mitosis
B: Fuhrman system (1982)
F I- uniform round nucleus, nucleus diameter 15 μm
F II- slightly irregular nucleus, non-prominent nucleolus, nucleus diameter 15 μm
F III- highly irregular nucleus, prominent nucleus, nucleus diameter 20 μm
F IV- large nucleolus, lobulated nucleolus with dominating nucleolus and ellipsoid cells, nucleus diameter over 20 μm

Results

Thirty suitable samples among all dispatched nephrectomies during 10 years were selected to be studied. Studying microscopic slides, 28 cases diagnosed as having RCC with clear cells and 2 cases as having sarcomatoid. In descriptive statistical analyses, 17 males and 13 females with an average age of 53 years and an age range of 26-75 years were evaluated. Malignancy rate in males was 1.3% more than females. Other descriptive statistics considering nucleus diameter and immunohistochemical studies including KI67 and P53 can be found in Figure 1 with regard to criteria of Table 1.

Most tumors were grouped as grade II according to Fuhrman grading system, 43% of tumors (13 cases) were as grade II, 9 as grade I (30%), 5 as grade III (16.9%) and 3 as grade IV (10%). However, 50% of them according to S&H system were grouped as grade II, 9 as grade I (30%) and 6 as grade III (20%).

Table 2. Descriptive statistics regarding quantitative variables in this study

	Average	Minimum	Maximum	Criterion deviation
Nucleus diameter (mm)	14.5	10	26	5.2
%KI67	22	0	100	24
%P53	24	0	80	25

The mean nuclear diameter significantly increased with grade. The expression of the proliferative marker KI67 by the nuclear reactivity was 20 and for P53 it was 13 out of 30 tumors (66.6% and 43.3% respectively) (Figure 1). According to S&H system for KI67, 9 cases with positive reaction were as grade II (60%), 6 as grade III (100%) and 5 were grouped as grade I (55%). Positive reaction to KI67 using Fuhrman system covers 9 tumors as grade II (30%), 5 as grade I (16.9%), 3 as grade III (10%) and 3 (100%) as grade IV (Table 2).

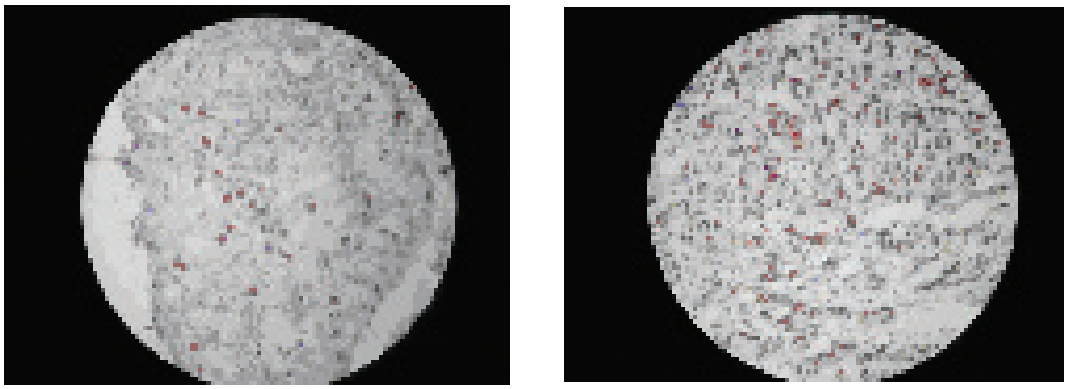

Figure 1. Positive reaction after IHC staining with markers of KI67 (A) and P53 (B) (brown nuclei in tumors cells) (x400)

Similar positive reaction against P53 marker was observed in 4 out of 8 tumors in grade I of Fuhrman system and in all cases of grades III and IV. High grade tumors also showed high apoptotic marker reactivity rate in S&H system. Reactivity to KI67 and P53 in both systems increased in parallel with nucleus grade and there was considerable increase of marker expression in nucleus grades 3 and 4 in both systems. Comparison between grades of the tumor for KI67 and P53 markers is demonstrated in Table 3.

Table 3. P-Value averages of quantitative data in different levels of two grading systems using one way analysis of variance (ANOVA)

Degree / variables / Quantitative variables	Fuhrman grading	S&H grading
Nucleus diameter	0	0
KI67	0.028	0.002
P53	0.01	0.046

Evaluating correlation coefficient of quantitative data by Spearman statistical method, the highest coefficient (r = 0.835) was found between KI67 and P53 (Table 4), which proves the strong relationship between P53 antigen expression and tumoral cells proliferation.

Table 4. Correlation coefficient between KI67, P53 and nuclear diameter

Pearson coefficient	The two considered variables
0.456	Nucleor diameter and KI67
0.835	KI67, P53
0.63	Nuclear diameter and P53

Using Tau-kendall statistical method for correlation coefficient for Fuhrman and S&H grading systems, values of 0.762 and 0.802 was obtained for the two systems respectively, which shows a considerable relationship between the two separate grading systems.

Discussion

Renal cell carcinoma includes almost %3 of malignancies in adults and 90-95% of renal neoplasm. In similar studies it has been reported that this tumor is more frequent in males than females and mostly occurs at an age range of 50-70 years (3). In the present study, 43-50% of the patients were as grade II according to Fuhrman and S&H systems which is similar to the studies around the world (45%) (2;4;6).

Studying the average age of patients at different levels of grading factors using ANOVA statistical method showed no significant relationship between age and tumor grading (Fuhrman and S&H systems)($p = 0.926$ and $p = 0.576$ respectively). In other words, increasing age has no significant effect on tumor grade increase which is possibly due to the lack of factual differences or insufficient sample size. This warrants further studies in the future. No meaningful relationship between patient gender and other variables was also observed as it was expected.

There have been many reports on KI67 cell proliferative marker and P53 administrating apoptosis. Tumor reactivity rate to prognosis has been measured by assaying patients' survival. Increasing severity and reactivity rate to these markers have always been followed by poor prognosis. These markers were applied to predict metastasis possibility (7). This unfortunately was not possible for our patients and we could not follow up the consequences; therefore we decided to measure the relationship between them and the grade of tumor by assigning two different systems in order to detect the relationship of these markers, their presence or absence with renal carcinoma aggressiveness. Indirectly, S&H grading system was administered since it has the lowest viewpoint discrepancy among different observers. Fuhrman system is one of the criteria to determine nucleus diameter which shows its valuable power. Positive rate (degree) of P53 and KI67 (MIB-1) in this study was in parallel with similar studies like Helmy et al (6), Yang (8), Pinto (9), Kashyap (3), Shvart (10), Bui (11), Kankuri (12), Uzunlar (13), Ljungberg (14), Girgin (15), Olumi (16), and Rioux (17). In these reports and our study, tumor grade increase corresponds with reaction severity increase. Antibodies against KI67 and P53 are easily available markers and valuable. Facts concerning tumor aggressiveness and estimating metastasis possibility could be achieved. Further studies are required to evaluate these proliferative and apoptosis markers to decreases discrepancy among observing pathologists in determining the grade of tumors.

Conclusion

Using immunohistochemical staining by KI67 and P53 markers, the results were consistent regarding mean nuclear diameter and grading in both systems. These two antibodies are easy and reliable markers that could be applied on formalin fixed tissue for better assessment of the biologic behavior of RCC and probably predicting patients' outcome. In addition, the assessment of these markers will help to reduce inter-observer variability which is a frequent issue.

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