p53 Protein Expression and Its Relation to the Apoptotic Index in Prostate Adenocarcinoma

Nasser Gholijani[1], Ahmad Monabati[2], Zahra Amirghofran[1*]

Departments of [1]Immunology and [2]Pathology, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran

ABSTRACT

Background: Prostate cancer is one of the most commonly diagnosed cancers in males. Tumor suppressor gene p53 plays an important role in causing cell cycle arrest and allowing apoptosis to proceed. **Objective:** To investigate the expression of p53 protein and its relation to apoptosis and prostate cancer traditional prognostic indicators. **Methods:** In this study expression of p53 was examined in paraffin-embedded tissues from 50 cases of prostate carcinoma by immunohistochemistry and evaluated using an index of staining. Correlation between p53 expression and apoptosis was detected by TUNEL method. Pathological grade, Gleason score and stage of carcinoma were also determined. **Results:** P53 expression was observed in 48 of 50 cases (26-100% of tumor cells) with mean staining index of 141±65. A significant association between p53 expression and pathologic grade (r=0.37, p=0.004) and Gleason score (r= 0.4, p=0.009) of patients was observed. Apoptosis was detected in only 6 patients. p53 expression showed no correlation with apoptotic index. No correlation between p53 expression and stage or apoptosis and clinicopathological characteristics of patients was found. **Conclusion:** p53 expression showed a significant correlation with differentiation status of the prostate carcinoma and can be helpful as a prognostic marker. Decreased level of apoptosis observed in our cases was not correlated with p53 expression indicating the possible role of other regulatory molecules involved in the apoptosis.

Keywords: Prostate carcinoma, p53, Apoptosis

*Corresponding author: Dr. Zahra Amirghofran, Immunology Department, Medical School, Shiraz University of Medical Science, P.O. Box: 71345-1798, Shiraz, Iran. Fax: +98-711-2304069, 2359847, e-mail: amirghz@sums.ac.ir

INTRODUCTION

The most commonly altered gene in a wide variety of tumors is p53 tumor suppressor gene (1-2). p53 gene, located on short arm of chromosome 17 encodes a 53 KD phosphoprotein involved in the cell cycle that allows cellular DNA repair and/or apoptosis to occur by controlling cellular progression from the G1 to S phase (3). In western population considerable data has shown that lesions of p53 are significantly associated with a poor prognosis in prostate cancer, both with respect to over-expression of the protein and mutation of the gene (4-6). Point mutations in the p53 gene result in an increased stability of the mutant protein, which can be detected by immunochemistry, whereas the wild-type p53 protein is undetectable due to its short half-life (7). Although in several studies the prognostic value of p53 in prostate cancer has been examined (8-10), the relationship between apoptosis and p53 has not been fully investigated. The aim of the present study is to examine this issue. Moreover, the genetic background and ethnicity are reported to be involved in carcinogenesis of prostate cancer (11), therefore, the prognostic value of p53 protein expression in our patients was also examined.

MATERIALS AND METHODS

Samples. 50 untreated patients proven to have prostate adenocarcinoma, with adequate clinical and paraclinical information were selected from pathology archives of Shiraz University affiliated hospitals. The average patient age was 72 ± 7 years (range 60-90 years). Information on patient stage based on TNM classification was obtained from the hospital clinical records. The best paraffin-embedded block of neoplastic tissue was processed for further studies. The neoplastic tissues were either from tumor biopsies, transurethral resection of prostate or prostectomy speciemens.5-µm sections were cut for histological evaluation by routine hematoxylin and eosin staining as well as immunohistochemical staining. Samples were graded according to Gleason score.

Immunohistochemical analysis. Monoclonal anti-p53 antibody DO1 was kindly provided by Dr. Nouri AME, The Royal London Hospital, London. Streptavidin-biotin immunoperoxidase staining was performed on all cases. Briefly, after tissue sections were deparaffinized and rehydrated, they were incubated with 3% H_2O_2 to inactivate endogenous peroxidase activity. Slides were heated in 10 mM citrate buffer to enhance antigen retrieval. Following a 20 minute blocking step, the primary antibody was applied

and incubated for overnight. After treating with biotinylated anti-mouse IgG and avidin-peroxidase for 30 minutes each, diaminobenzidine 1mg/ml in PBS containing 0.03% hydrogen peroxide was applied as the chromogen. Sections were counterstained with hematoxylin for 15 seconds. Negative control study was performed in which PBS was used instead of primary antibody. Paraffin sections from human bladder cancer with known immunoreactivity to p53 were used as positive control. All slides were analyzed and scored by two researchers. To determine the expression of markers at least 1000-tumor cells were assessed and the proportion of cells showing reactivity was determined. Immunostaining intensity was also rated as follows: 0 none, 0.5 very week, 1 weak, 2 moderate and 3 strong. An index of staining was determined by multiplying the percentage and intensity of positive tumor cells.

Apoptosis detection. Apoptotic carcinoma cells were identified using the Cell Death Kit (Boehringer Mannheim GmbH, Germany) as recommended by the manufacturer. Briefly, sections were deparaffinized and rehydrated, and the endogenous peroxidase activity was blocked. The sections were incubated in proteinase K then incubated with 50µl of TUNEL reaction mixture. Following rinsing in PBS, slides were incubated with 50µl converter-peroxidase solution. The reaction was visualized with diamineobenzidine/H_2O_2. Slides were subsequently washed and counterstained. Negative control sections included the above process except the enzyme solution and positive control were prepared by treating sections with 1µg/ml DNase 10 minutes before the above protocol. Apoptotic cells were identified using light microscope, and a total of 1000 carcinoma cells were evaluated, whereby the apoptotic index was determined

Statistics. SPSS version 10 and Spearman correlation coefficient was used for Statistical analysis.

RESULTS

Paraffin-embedded specimens of prostatic cancer were analyzed for the pattern of expression of p53 as well as apoptosis. The immunohistochemistry results were evaluated in terms of the percentage of immunopositive tumor cells and relative immunointensity. An immunostaining index was calculated for each marker. As it is shown in table 1, of 50 cases 32% were well differentiated (Gleason score 2-4), 54% moderately (5-7) and 14% were poorly differentiated (8-9). Staging was available on 62% of cases in that 29% were stage B, 38% stage C and 33% were clinically recorded to be

stage D.
P53 was over expressed in 96% of cases ranging from 26 to 100% of tumor cells. Nuclear staining index of p53 varied from 13 to 300 with an average of 102 ± 68 (Figure 1). A significant association between p53 expression and pathologic grade (r=0.37, p=0.004) and Gleason score (r= 0.4, p=0.009)

Table1. Gleason score and stage in patients with prostate carcinoma.

Gleason score	Number of patients(%)
2-4	16(32)
5-7	27(54)
8-9	7(14)
Stage	**Number of patients(%)**
A	0(0)
B	9(29)
C	12(38)
D	10(33)

of patients was observed (Figure 2). p53 expression in poorly differentiated tumor cells was 172 ± 100 compared to moderately (95 ± 57) and well differentiated tumors (63 ± 40) showing the higher expression of p53 in less differentiated tumor cells. Correlation between p53 expression and stage was not significant.
Apoptosis was detected in 12% of prostate carcinoma samples. The mean apoptotic index was 31 ± 28. Study of the relationship between p53 expression and apoptosis showed no significant correlation.

DISCUSSION

In order to find the relevance of p53 expression and apoptosis in prostate

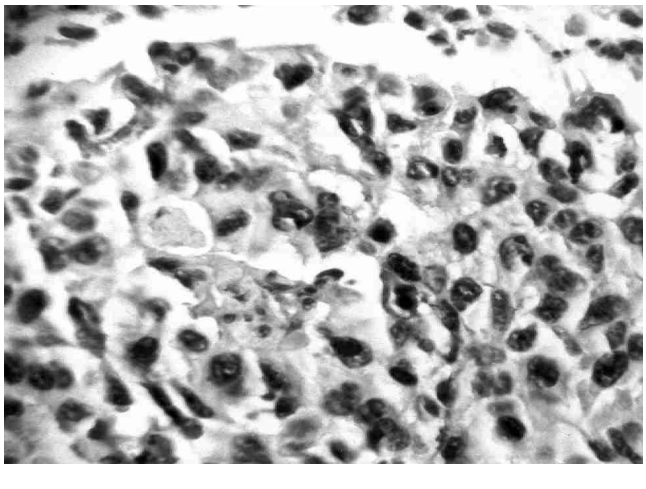

Figure 1. Nuclear staining of p53 in prostate adenocarcinoma. X400.

cancer we retrospectively investigated a series of 50 patients using embedded materials by immunohistochmistry and evaluated the relationship of this expression to apoptotic index and clinopathologic characteristics of patients. Results obtained showed the expression of p53 in nearly all cases of prostatic carcinoma with a mean staining index of 102 ± 68. p53 was more expressed in poorly differentiated tumor cells than moderately and well differentiated ones indicating the prognostic value of this oncogene in prostate carcinoma. In a previous study, Matsushima et al. reported the p53 positivity in 27% of prostate carcinoma (12). In his study the frequency of p53 positive cases

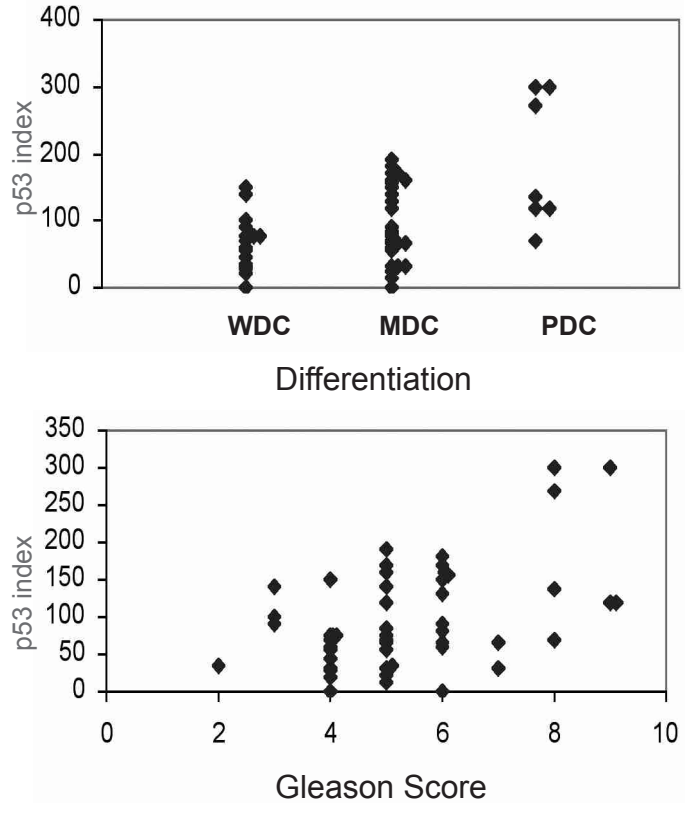

Figure 2. Relationship between p53 staining index and (a) pathological grade of differentiation and (b) Gleason score in patients with prostate carcinoma (p=0.004, p=0.009, respectively). WDC: Well differentiated; MDC: Moderately differentiated; PDC: poorly differentiated.

were found to be increased with advancing grades. In several other works p53 positive staining has been associated with high histological grades (13-15). In cohorts from Western countries, lesions of the p53 gene (such as over-expression of the protein or mutation of the gene) have been found in up to 65% of prostate tumors (16). However, less percent positivity of p53 has also been reported in other studies. As, in a study performed on an Asian population by Chia et al. (17) only 3% of cases expressed p53 indicating the role of genetic background in prostate tumorigenesis.
In our study apoptosis was detected in only 6 cases of prostate carcinoma examined. A few studies performed on the apoptosis in untreated prostate

cancer patients. The presence of apoptotic cells has been observed in all (29/29) and 61%(38/62) of samples in two studies (17-18). The rate of apoptosis in primary carcinoma has also been reported to have a prognostic significance (18-21). No correlation between apoptosis and differentiation status of carcinoma was observed in our study. This may be in part due to our low number of apoptosis positive cases.

The relation between p53 reactive cells and apoptosis in prostatic carcinoma has been considered in two previously studies. In both studies apoptosis has been shown no correlation with p53 expression (17,19). p53 protein appears to play a role as a check point control for recognizing DNA damage, resulting in either a delay in progress through the cell cycle to permit repair processes or the initiation of programmed cell death or apoptosis and eliminating the abnormal cells (22). Wild-type p53 is thought to promote apoptosis, whereas mutant p53 has a similar effect on apoptosis as bcl-2 that is inhibition of programmed cell death (23). Therefore, over-expression of p53 with a concomitant mutation may contribute to the process of tumorigenesis by reducing the rate of cell death. Although, in our study we found a high expression of p53 and a low rate of apoptosis suggesting the possible role of p53 over-expression in decreasing the rate of apoptosis, the result was not significant, indicating the possible role of other molecules involved in the regulation of cell death particularly those of the Bcl-2 family. It is noted that since not all over-expressions of p53 may be the result of altered wild-type p53, the possibility of the presence of a non-mutated p53 should also be taken into account.

In conclusion p53 immunostaining was correlated with differentiation status of prostate carcinoma showing the prognostic value of p53, and there was a statistically non-significant association between p53 and the apoptotic index.

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