

ORIGINAL PAPER

Immunohistochemical detection of p53 protein as a prognostic indicator in prostate carcinoma

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Abstract

The aim of our study was to evaluate the prognostic significance of p53 protein immunoreactivity for prostate cancer and to determine whether p53 immunoreactivity correlates with the Gleason tumor grade in primary adenocarcinoma. Prostate fragments were fixed in 10% formalin, paraffin-embedded, sectioned and standard Hematoxylin–Eosin stained, then examined using histological grade (Gleason system). P53 expression was studied using immunohistochemistry with monoclonal antibody anti-p53, 1 : 100 (BIOX) on tissue samples obtained during transurethral electroresection, adenectomy or needle biopsy in 30 patients with prostate carcinoma: group 1 (n = 7) Gleason score 5, group 2 (n = 10) Gleason score 6, group 3 (n = 11) Gleason score 7, group 4 (n = 2) Gleason score 8. Also, we noted the cases with high grade prostatic intraepithelial neoplasia (high grade PIN). All specimens prior to initiation of any treatment were submitted for this study. Staining was defined as positive for p53 whenever any specific nuclear staining was detected. We considered tumors to overexpress p53 protein only when strong nuclear staining was present. Cases exhibiting weak or equivocal nuclear staining were classified as negative, as were cases with extremely rare isolated positive nuclei. A semiquantitative scoring system was employed to assess the level of p53 reactivity. Six of 17 (35.2%) moderately differentiated tumors (Gleason score 5–6) and five of 13 (38.4%) moderate to poorly differentiated (Gleason score 7 and above) revealed strong nuclear positivity for p53. In addition, we noted occasional p53 reactivity in high-grade PIN. *Conclusions.* We interpret these data to demonstrate a positive association between p53 reactivity and higher Gleason grade tumors; p53 might be an independent prognostic indicator among metastatic risk cases

Keywords: immunohistochemistry, prostate carcinoma, p53.

Introduction

The currently most established prognostic factors in prostate carcinoma are histological grade (Gleason system) and tumor stage [1].

There is a large discrepancy between the rate of clinical diagnosis of prostate cancer and the much higher incidence of latent cancer diagnosed at autopsy in men who died of other causes [2].

Cellular proliferation and programmed cell death are associated with tumor growth in general and prostate cancer growth in particular. Protein expression of the tumor suppressor gene p53 has been proved as useful prognostic indicators in prostate cancer progression [3, 4].

The p53 tumor suppressor gene encodes a phosphoprotein involved in the regulation of cell cycle, causing a G1 block in cell cycle progression and in certain cell types precipitating apoptosis. Mutation of the p53 tumor suppressor gene is the most common genetic alteration in malignant human tumors. Functional inactivation may result from genetic aberration within the p53 gene, most frequently missense mutations or inactivation by interacting with viral and cellular oncoproteins.

Loss of wild-type p53 function leads to deregulation of the cell cycle check point and DNA replication, defective or inefficient DNA repair, selective growth advantage and, as a result, tumor formation and progression [5].

The abnormal p53 protein produced by the mutant gene is more stable than the wild type protein, tends to accumulate in the cell, and thus can be detected by immunohistochemistry using monoclonal antibody [6].

The wild type p53 protein has a very short half-life (20 min. vs. 44 min.) and consequently its concentration in the nucleus is believed to be below the limits of detection by immunohistochemical staining [7].

The importance of p53 in the pathogenesis of prostatic adenocarcinoma was first postulated by Rubin SJ *et al.* [8] and Isaacs WB *et al.* [9] who demonstrated mutations of p53 gene in prostate cell lines and in a primary human prostatic adenocarcinoma.

Since then, a number of additional investigators have also demonstrated p53 over expression in human prostatic adenocarcinomas [10–13].

Unfortunately, the immunohistochemical studies of p53 in prostate cancer have presented conflicting conclusions on several important issues including:

the frequency of p53 overexpression in the cancers, whether p53 immunoreactivity can be observed in benign glands and whether p53 positivity correlates with tumor grade.

Chen YQ *et al.* indicate that p53 abnormalities occur at a high rate during prostate cancer development and the frequency of p53 alternations appears to correlate with tumor grade/stage [14].

Visakorpi T *et al.* found a high level p53 immunoreactivity which was limited to 6% of the cancers, was associated with high histological grade, DNA aneuploidy and high cell proliferation rate and defined a small subset of aggressive prostate carcinoma [15].

Most recently, several important studies have suggested a significant association between p53 immunoreactivity and aggressive biological behavior in prostatic adenocarcinoma [16, 17].

Although several studies have suggested that molecular analyses can provide useful prognostic information if largely biopsy samples or entire prostate are examined, there is a little information on the clinical significance of these molecular examination in core needle biopsies.

The purpose of the present study was to evaluate the prognostic significance of p53 protein immunoreactivity for prostate cancer and to determine whether p53 reactivity correlates with the Gleason tumor grade.

☞ Material and methods

The tissue samples obtained during transurethral electro resection, enucleation or needle biopsies in 30 patients with prostate carcinoma were retrospectively identified from the files and archives of the Pathology Department of "Prof. dr. Theodor Burghele" Hospital: group 1 (n = 7) Gleason score 5, group 2 (n = 10) Gleason score 6, group 3 (n = 11) Gleason score 7, group 4 (n = 2) Gleason score 8. Also, we noted the cases with high grade prostatic intraepithelial neoplasia (high grade PIN). Gleason histological grading system was determined by adding the numbers for the two most predominant patterns.

All specimens prior to initiation of any treatment were submitted for this study.

Prostate fragments were fixed in 10% formalin, paraffin-embedded, sectioned and standard Hematoxylin–Eosine and van Gieson stained, then examined by light microscopy (Nikon Eclipse E 600) using histological grade (Gleason system). The Gleason grading system takes into account the heterogeneity of prostate cancer Representative photomicrographs were taking using Nikon Plan $\times 20$ and $\times 40$.

A single representative block was selected for immunohistochemical staining.

Immunohistochemistry was performed on 3 μ m thick sections from 10% formalin-fixed paraffin-embedded specimens, according to the Avidin Biotin Complex method of the tissue [18], modified by Bussolatti G and Gugliotta P [19] and Miller K [20].

Briefly, the procedure was: deparaffinization in xylene and alcohol series rehydration, washing in

phosphate saline buffer (PBS), incubation with normal serum for 20 min. incubation with primary antibody overnight, standard labeled streptavidin–antibody biotin (LSAB) kit (DAKO), washing in carbonate buffer and development in 3,3'-DAB hydrochloride/H₂O₂.

Tumor fragments were tested by p53 monoclonal antibody BIOX, 1 : 100 [21]. All specimens were counterstained with Mayer's hematoxylin, examined and photographed on a Nikon Eclipse 600 microscope. A known p53 positive specimen was used as a positive control. Immunohistochemical stains were interpreted without knowledge of clinical data.

Staining was defined as positive for p53 whenever any specific nuclear staining was detected. Three staining patterns were recognized: diffuse nuclear staining, regional nuclear staining and focal nuclear staining.

A semiquantitative scoring system was employed to assess the level of p53 reactivity: 0 – was assigned when no staining was observed, 1 – when less than 10% of tumor cell nuclei were reactive, 2 – when more than 10%, but less than 33% of the nuclei stained, and 3 – if more than 33% of nuclei were positive.

☞ Results

Positive staining with anti-p53 was seen in 11 of the 30 primary prostate cancers (36.6%) examined.

We considered tumors to over express p53 protein only when strong nuclear staining was present (Figure 1).

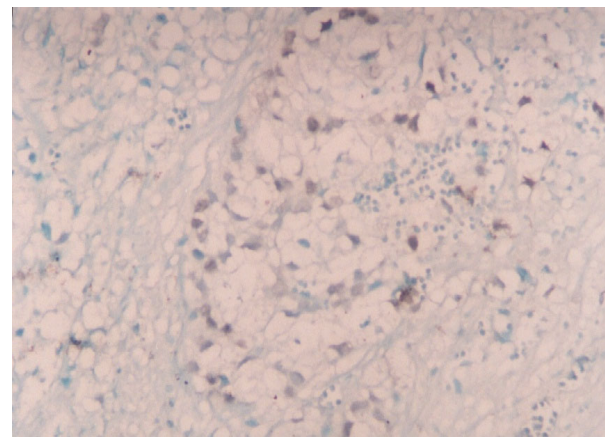


Figure 1 – Moderate staining of p53 protein in prostate carcinoma ($\times 200$)

Cases exhibiting weak or equivocal nuclear staining were classified as negative, as were cases with extremely rare isolated positive nuclei.

Six of 17 (35.2%) moderately differentiated tumors (Gleason score 5–6) and five of 13 (38.4%) moderate to poorly differentiated (Gleason score 7 and above) revealed strong nuclear positivity for p53 (Table 1) (Figure 2).

Table 1 – P53 positivities compared to Gleason score

Gleason score	No. of cases	P53 positivity	
		No.	%
2–4	0	0	0
5–6	17	6	35.2
7 and above	13	5	38.4

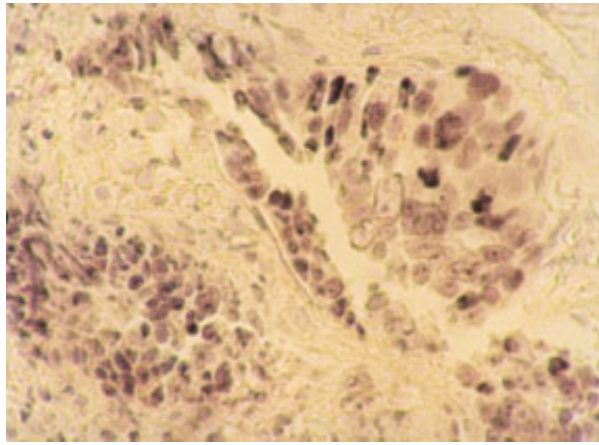


Figure 2 – Strong staining of p53 protein in prostate carcinoma ($\times 400$)

In addition, we noted occasional p53 reactivity in high grade PIN. There was some variation in staining intensity and proportion of positive nuclei among tumors. Among p53 reactive cancers 2 received a score of 1, four a score of 2 and 5 a score of 3. No staining was observed in normal or hyperplastic benign prostate tissue.

☐ Discussions

While pathological stage, grade, positive surgical margins and tumor volume are perhaps the most commonly accepted prognostic factors, they can not be used preoperatively.

p53 over expression has been investigated independently in a large number of different malignancies for their potential value as a prognostic marker.

Mutation of the p53 tumor suppressor gene is a common genetic alteration in malignant human tumors and can be immunohistochemical detected [6].

The role of p53 in human prostate adenocarcinoma is still unclear and remains controversial.

While a number of groups demonstrated a high p53 mutation and/or protein accumulation rate in prostate cancer (PCa) [10] others reported rare mutations [12].

Such frequency differences of the p53 in PCa among various groups could partially be due to the geographic or demographic factors as well as methods used for detecting p53 abnormalities.

Bookstein R *et al.* [22] reported that 23% of stage III or IV tumors and 4% of stage 0–II tumors had abnormal nuclear p53 accumulation and that 20–25% of advanced cancers, but none of early PCa had mutations of the p53 gene. However, two studies suggested that p53 abnormalities may be an early event in PCa progression [23].

Kubota Y *et al.* [24] screened PCa specimens for p53 gene mutations in axons 1–11 and found that 9% of well and moderately differentiated and 30% of poorly differentiated PCa had p53 mutations. This result also supports that p53 mutation is a late event in the development of PCa.

Another interesting observation in our study was the focal intermediate intensity p53 immunoreactivity of

high grade prostate intraepithelial neoplasia cells adjacent to areas harboring tumor. This finding has been consistently reported by previous investigators [12].

The presence of p53 over expression in PIN tissues raises the question as to whether the occurrence of p53 mutations in PCa is an early event. With the passage of time some of these basal cells might sustain further somatic mutations that allow progression to malignancy.

We reported a significant association between p53 protein over expression and Gleason score.

These results strongly imply that p53 mutations play a role in the pathogenesis of a subset of Pca. We also reported a focally positive p53 staining of high grade PIN cells adjacent to areas harboring tumor. Our results demonstrate that the pattern of p53 expression is complex and suggest that over expression of mutant p53 may play a particularly important role in mediating the early cellular changes that lead to metastasis.

The precise molecular role played by the over expressed p53 protein in mediating oncogenesis in prostate epithelium remains to be determined.

☐ Conclusions

We interpret these data to demonstrate a positive association between p53 reactivity and higher Gleason grade tumors. It may be possible to prospectively predict the tumor behavior and prognosis on the basis of needle biopsies. It is likely that p53-positive tumors detected at biopsy display aggressive biologic features; p53 might be an independent prognostic indicator among metastatic risk cases

Further prospective clinical studies including long term follow up patients with p53-positive primary PCa, need to be undertaken to understand the biology of the p53 protein and to assess its prognostic significance in patients with PCa.

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