Morfometrična analiza tkivnega označevalca p53 v adenokarcinomu prostate v povezavi z oceno po Gleasonu in vrednostjo PSA

Morphometrical analysis of the p53 tissue marker in prostatic adenocarcinoma and its relationship to Gleason score and PSA level

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Ključne besede:
rak prostate, tkivni označevalci, p53, imunohistokemija

Key words:
prostate cancer, tissue markers, p53, immunohistochemistry

Članek prispel / Received
26.07.2012

Članek sprejet / Accepted
13.03.2013

Naslov za dopisovanje / Correspondence
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Izvleček

Namen: *Rak prostate je v svetu zelo pogosta maligna bolezen. K natančnejši oceni bolezni bi lahko pripomogli tkivni označevalci, kot je na primer tudi p53. V vzorcih adenokarcinoma prostate slovenske populacje smo morfometrično analizirali izražanje p53 in njegovo povezavo z oceno po Gleasonu in vrednostjo PSA ter starostjo bolnikov.*

Metode: *V retrospektivno pilotno študijo smo vključili 25 bolnikov z opravljeno radikalno prostatektomijo. Tkivni označevalec p53 smo določili imunohistokemično in ga izrazili kot indeks p53. Statistično smo poiskali povezave indeksa p53 z oceno po Gleasonu pred in po operaciji, vrednostjo PSA in starostjo bolnikov.*

Abstract

Purpose: *Prostate cancer is one of the most common malignancies worldwide. The possible prognostic value of tissue markers, such as p53, may give a better understanding of this disease, improve staging accuracy, and help in choosing optimal treatment. In this study, we examined p53 expression and its correlation with Gleason score, prostate–specific antigen (PSA) levels, and patient age in a Slovenian population.*

Methods: *This retrospective pilot study included 25 radical prostatectomy patients. The immunohistochemical expression of p53 was determined and expressed as a p53 index. In addition, correlations be-*

Rezultati: *Tkivni označevalec p53 je v raku prostate neenakomerno porazdeljen neodvisno od lokalnega Gleason vzorca, vendar korelira z oceno po Gleasonu po operaciji in mejno korelira z vrednostjo PSA. Statistično značilno so med seboj povezani tudi ocena po Gleasonu pred in po operaciji, vrednost PSA in starost bolnika.*

Zaključek: *Potrdili smo, da ima p53 lahko napovedno vrednost kljub manjšemu številu primerov, vključenih v to raziskavo. Rezultati pilotne študije kažejo, da je p53 obetajoč tkivni označevalec in bi lahko bil uporaben kot dodatni diagnostični parameter.*

tween p53 index, Gleason score before and after prostatectomy, PSA level, and patient age were statistically evaluated.

Results: *The p53 tissue marker was unevenly distributed in prostate cancer independently of local Gleason pattern; however, its expression correlated with Gleason score after prostatectomy and showed borderline correlation with PSA. There were also statistically significant correlations between Gleason score before and after prostatectomy, PSA level, and patient age.*

Conclusions: *Despite the low number of cases presented in this study, our results demonstrate that p53 may have predictive value in prostate cancer. Thus, p53 is a promising tissue marker that can be used as an additional diagnostic parameter.*

INTRODUCTION

Prostate cancer is one of the most common malignancies worldwide (1), particularly in developed countries (2), as well as in Slovenia (3). There are many diagnostic and prognostic predictors for prostate cancer, among which Gleason histologic scoring (4) and prostate–specific antigen (PSA) levels are the most clinically relevant. However, these criteria and other clinical diagnostic data often do not sufficiently satisfy the clinical requirements for predicting the course of disease development or for deciding optimal therapy (5–8). Some tissue markers, such as bcl–2, p53, Ki–67, and caveolin–1, play an important role in the detection and identification of prostate cancer (5, 9). The possible predictive value of tissue markers may lead to a better understanding of this disease, improve staging accuracy, and help in choosing optimal treatment.

Tumor growth occurs due to the loss of balance between cell growth (proliferation) and/or cell death (apoptosis), mostly because of increased cell proliferation (10). Proliferation and apoptosis could be determined by tissue markers. Induction of the gene that encodes p53, a tumor–inhibitory protein, controls the cell cycle of damaged cells, and if required, directs the cell to undergo apoptosis, thereby preventing irreparable cells from proliferating (11, 12). In tumor cells, the p53 gene is mainly mutated causing these cells to divide uncontrollably (13). It has been reported that in prostate cancer, p53 expression correlates with clinical parameters, and as such, can predict biochemical recurrence following radical prostatectomy (14–20).

The purpose of this preliminary study was to determine the possible significance of the p53 tissue marker as a prognostic indicator in patients undergoing radical prostatectomy. To this end, we morphometrically evaluated p53 expression levels in prostate cancer specimens, and determined its relationship to Gleason score, PSA levels, and patient age in a Slovenian population.

MATERIAL AND METHODS

At the beginning of this study, the p53 (NCL–L–p53–DO7, Leica Biosystems, Melbourne, Australia) antibody was tested to determine optimal staining

conditions. Specifically, different dilutions (1:25, 1:30, 1:50, 1:60, 1:75, 1:100) were tested with the immunohistochemical protocol to determine the best dilution to use. We also examined p53 expression in various tissues of different tumor types to determine the best tissue to use as a positive control in our experiments. We performed a preliminary retrospective study on 25 patients with prostatic adenocarcinoma. Gleason score before (needle biopsy) and after operation (radical prostatectomy), PSA levels before needle biopsy, and patient age were included in the data pool. Patient data were anonymously obtained from the Department of Urology at the University Medical Centre Maribor. The tissue blocks were collected from the Department of Pathology, where the tissue samples were fixed in 10% buffered formalin for 1–3 days after radical prostatectomy, processed in a tissue processor (TP 1020, Leica), and embedded in paraffin (Paraplast, McCormick Scientific, St. Louis, MO 63134, USA). Paraffin–embedded tissue samples were cut in 4–µm–thick sections and treated according to the p53 immunohistochemical staining protocol (clone DO7, Leica/Novocastra,). Briefly, deparaffinization, rehydration in 100%, 96%, and 70% alcohol, heat–induced antigen retrieval in citrate buffer (pH 6.03), and blocking of endogenous peroxidase activity were performed. This was followed by blocking of nonspecific binding, incubation with anti–p53 (1:50 dilution) for 90 min, incubation with polymeric peroxidase–linked secondary antibody for 40 min, and treated with diaminobenzidine (DAB, 1:20 dilution) for 5 min. In the last step, the nuclei were counterstained with Mayer's aqueous hematoxylin for 1 min, after which slides were covered with a cover glass. Breast cancer tissue was used as a positive control for p53 expression, and prostatic adenocarcinoma with no primary antibody applied was used as the negative control. Analysis of p53 tissue marker expression was performed under a microscope (DM 2500, Leica). For each specimen, three fields of view with the highest density of p53 nuclear staining ("hot spots") (Fig. 1) were examined under a 400X magnification and chosen for quantitative analysis. Cell nuclei (1000) of prostatic glands were counted in the three fields of view on each slide, and those stained with p53 were counted separately. The nuclear staining of p53 was expressed as a p53 index (number of cells with positive p53 staining per 1000 counted cells). The p53 index was correlated with Gleason scores and PSA levels using Spearman's rank correlation coefficient. Statistical analysis was carried out using the IBM SPSS Statistics version 19 (SPSS Inc., Chicago, IL, USA). P–values less than 0.05 (two tailed) were considered statistically significant.

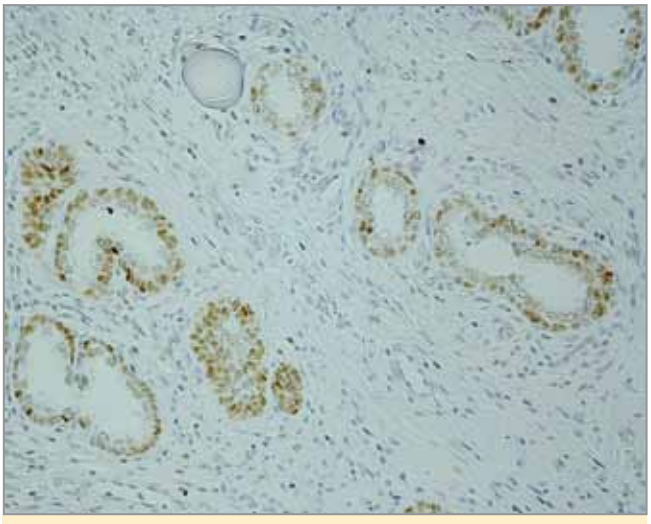

Figure 1. *Hotspot of intensive p53 staining at Gleason score of 6; 100X magnification.*

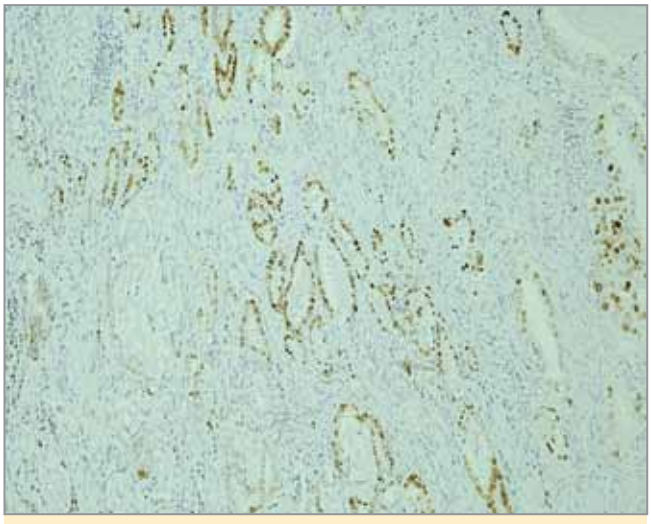

Figure 2. *Uneven p53 staining at Gleason score of 6; 100X magnification.*

RESULTS

Gleason score before and after operation, PSA levels before needle biopsy, patient age, and tissue blocks were available for 25 patients with prostate adenocarcinoma. The mean patient age at the time of diagnosis was 63±5 years (range 53–71). The results of our statistical analyses are in Table 1. The nuclear staining of p53 was unevenly distributed, regardless of local Gleason pattern (Fig. 2). The p53 index ranged between 0.050 and 0.206 (mean 0.135±0.42). It significantly and positively correlated with Gleason score after operation (r=0.483; P=0.023) (Table 1), and showed a borderline statistically significant correlation (r=0.413; P=0.056) with PSA level. Gleason scores before (r=0.439; P=0.028) and after operation (r=0.414; P=0.04) correlated with PSA level. In addition, Gleason scores after operation also correlated with patient age (r=0.598; P=0.002). There was also a significant correlation (r=0.414; P=0.013) between Gleason score before and after operation. PSA levels ranged between 1.81 and 34.4 ng/ml (mean 8.02 ng/ml ±7.02). PSA level before needle biopsy significantly (r=0.478; P=0.016) correlated with patient age. There were no significant correlations between p53 index and patient age or between p53 index and Gleason score before operation.

CONCLUSIONS

The prognosis after prostatectomy is usually based upon clinical findings, such as the preoperative PSA level, and pathological findings, such as Gleason score and pathological stage. These conventional variables have proved useful in predicting biochemical failure. However, cancers with similar characteristics can exhibit different chemical behaviors and biological properties, thereby yielding different clinical outcomes. On the other hand, currently available conventional clinical variables may not be sufficient to identify indolent disease for which an operation may not be necessary. Other variables that may improve outcome prediction in prostate cancer include tissue markers. Knowledge of the staining characteristics of these tissue markers may explain the tumor dynamics and aggressiveness, which could consequently lead to a better prediction of disease outcome.

The goal of our study was to determine the correlation between p53 expression with Gleason score, PSA level, and patient age, and to identify the possible predictive value of p53 in Slovenian patients following radical prostatectomy. p53 staining in slow progressing prostatic adenocarcinoma is weakly expressed and is irregularly disseminated in the tissue, while in some other cancer types it is distributed more evenly, and is thus easier to evaluate. We used a specific three field counting approach on classic microscopy image processing, trying to reach more indicative p53 values. In the present pilot study, we found out that p53 expression strongly correlates with Gleason score after operation, and exhibits a borderline statistically significant correlation with PSA level. These data indicate that p53 expression could be an indicator of malignancy. In addition, as

Table 1: *Correlations between p53 index, Gleason score, PSA level, and patient age.*
(ng – nanogram; mL – milliliter; NS – not significant; RP – radical prostatectomy; r – correlation coefficient)

	p53 index (0.135 ± 0.42)	PSA level (8.02 ng/mL ± 7.02)	Gleason score after RP
age (63 years ± 5)	NS	r=0.478 P=0.016	r=0.598 P=0.002
Gleason score after RP	r=0.483 P=0.023	r=0.414 P=0.040	—
Gleason score on needle biopsy	NS	r=0.439 P=0.028	r=0.492 P=0.013
PSA level (8.02 ng/mL ± 7.02)	r=0.413 P=0.056	—	r=0.414 P=0.040

expected, patient age and Gleason score after operation positively correlate as well.

Despite the low number of cases, our results show that PSA serum levels have a significant dual correlation with Gleason score before operation (on needle biopsy), and with Gleason score after operation. A study by Bauer and coworkers (16) demonstrated that p53 expression in prostatectomy specimens significantly predicts disease recurrence. It has also been reported that pre–operative patients who are p53–negative have a better prognosis for a longer period of time following radical prostatectomy (9). Thus, our data are in accordance with other studies, which have shown that p53 is a useful prognostic factor of patients with radical prostatectomy (21, 22), and also of patients after radiation therapy failure (23).

Here, we confirm that p53 may have predictive value according to its significant correlation with Gleason score and PSA level after operation, despite the low number of cases that were included in our study. Future studies will include a larger sample size, and will also investigate other tissue markers, as we believe that examining a combination of different tissue markers might improve their prognostic value.

These preliminary results were presented at the International Symposium of Clinical and Applied Anatomy, Maribor, Slovenia, July 2011.

ACKNOWLEDGEMENTS

We would like to thank Dr. Boris Pospihalj, Head of the Department of Pathology, Hospital in Slovenj Gradec, Slovenia, for his help and support.

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