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EVALUATION OF THE VALUE OF p53 PROTEIN EXPRESSION IN THE EXTRA-CAPSULAR EXTENSION OF PROSTATE CANCER

Saidi S[1], Georgiev V[1], Stavridis S[1], Penev M[1], Stankov O[1], Dohcev S[1], Banev S[2], Danilovski D[3], Ivanovski O[1], Popov Z[1]

[1]University Urology Clinic, Medical Faculty,
Ss Cyril and Methodius University, Skopje, R. Macedonia
[2]Institute of Pathology, Medical Faculty,
Ss Cyril and Methodius University, Skopje, R. Macedonia
[3]Institute of Epidemiology and Statistics, Medical Faculty,
Ss Cyril and Methodius University, Skopje, R. Macedonia

A b s t r a c t: *Introduction & objectives:* The objective of this study is to identify the nuclear expression of the p53 protein in prostate cancer and to determine its relationship with clinico-pathological variables.

Material & methods: The research included 83 patients, 43 of whom are patients with prostate cancer who underwent radical prostatectomy and a control group of 40 patients with benign hyperplasia of the prostate in whom a transurethral resection or a transvesical prostatectomy was undertaken. In all cases the nuclear expression of p53 protein was evaluated. A hystopatological evaluation of the tumour characteristics and the data of the local progression of the cancer were undertaken in the research group.

Results: The results show that the expression of the p53 protein does not have an important correlation with the preoperative PSA, but that it is in direct correlation with the malign potential of the cancer (Gleason score, Gleason sum, primary tumour) and with the features of the disease (metastatic lymph nodes, stage of the disease).

Conclusion: p53 protein could be used as a valid biomarker in determining the malignant potential of the tumour and the prognosis of the disease. There is no practical use in predicting the extraprostatic extension.

Key words: Prostate carcinoma, p53 protein, extra-capsular extension.

Introduction

In the United States in 232,000 men were found in 2005 with newly diagnosed prostate cancer [1]. Since the intruduction of prostatic specific antigen (PSA) as a screening method prostate cancer incidence has increased over the last two decades. The incidence was 6, 18 and 14% in the periods 1985–89, 1989–92 and 1992–95 respectively [2]. During the last ten years mortality has decreased significantly from approximately 40,000 dying in 1990 to 29,900 in 2004 [3]. According to the World Health Organistaion (WHO) 679,023 new cases were diagnosed in 2002, while 221,002 died from CaP.

According to the Cancer Register in the Republic of Macedonia, in 1994 the incidence of this disease was 6.55 cases in 100,000 inhabitans. The incidence increased with age. In 60-year-old males, it was 6; in 70-year-olds, 14; and in 75-year-olds, 55 (Registar za rak na RM). In countries with an absence of organized screening of CaP only 55% [4] of CaPs were organ confined cancers at the time of diagnosis, whereas 30–45% showed pathological staging of the extracapsular extenion [5].

For these reasons it is important to identify new markers that could predict the progression of the disease in terms of extracapsular extension. The aim of this study was to determine the nuclear expression of p53 protein in prostate cancer as well as the correlation with extracapsular extension, such as semen vesical involvement, capsule penetration, and metastatic involvement of local lymph nodes. The second aim was to determine the correlation of nuclear expression of p53 protein with histopathological findings and the Gleason score, Gleason grade, Gleason sum and primary tumour.

Material and methods

The study was realised at the University Urology Clinic and the Pathology Institute in Skopje from May 2006 to June 2010. A total of 83 patients were included in the study. The study group consisted of 43 patients who underwent radical prostaectomy for treatment of clinically localized cancer whereas the control group consisted of 40 patients who underwent prostatectomy for nonmalignant disease of the prostate. The histopathological result was the gold standard. The inclusion factors for the study group were: PSA levels before or 28 days after the prostate biopsy, and patients who had not been treated with radiation, hormonal or another therapy for prostate or other cancer. All cases that did not comply with the survey were excluded.

The immunostaining was estimated in tissue sections of 10% neutral formalin fixed and paraffin-embedded. In a heat antigen retrieval process the slides were placed in a citrate buffer (1 mM, pH 6.0) and heated three times for 8 minutes each time in a domestic microwave oven on high power. The slides were incubated overnight at 4°C with monoclonal antibodies to p53 (clone DO7, Dako, Glostrup, Denmark) at a dilution of 1 : 50 in phosphate-buffered saline (PBS). Biotinylated antimouse immunoglobulin G was applied at 1 : 200 dilution for 60 minutes at room temperature. The slides were rinsed with PBS for 30 minutes, incubated with peroxidase-conjugated streptavidin (streptABC Kit, Dako) at 1 : 400 dilution in PBS for 45 minutes at room temperature, and then rinsed again with PBS for 30 minutes. Colour was developed by incubating the slides in 0.06% diaminobenzidine in PBS for 15 minutes, and the slides were then rinsed in tap water, counterstained with Harris hematoxylin, dehydrated, coverslipped, and reviewed under a light microscope. For p53 any dark, brown nuclear staining was considered positive, indicating abnormal stabilization of p53. The results were observed under a light Leica type microscope. Around 500 nuclei on 10 fields at 400-magnification were evaluated. The cut-off point value for positivity was 20% of the evaluated cells. Collected data were put on an excel. Thereafter SPSS 16.0.1 Eval Version_a was used for statitical analyses. : χ^2–test and ANOVA were used for comparataive analyses.

Results

The study group consisted of 43 patients treated with radical prostatectomy for clinically localized prostate cancer. The youngest patient was 51 year old while the oldest was 75 (mean 65.3 ± 5.91). The values of preoperative serum PSA ranged from 2.14 mM to 100 mM (mean 23.6 ± 22.4). The p53 expression showed that 28% of the patients in the study group were positive whereas none were in the control group. In Table 1 we show the results from the histopathological evaluation of prostate cancer patients, the distribution of the nuclear expression of p53 and its correlations.

The results show that the expression of the p53 protein was found in 13 (28%) of cases and this is in direct correlation with the Gleason score ($p = 0.001$), the Gleason sum ($p = 0.001$), the degree of the primary tumour ($p = 0.001$), metastatic lymph nodes ($p = 0.0000$), and the stage of disease ($p = 0.026$), but that no significant correlation was found with the involvement of the seminal vesicles ($p = 0.647$), preoperative PSA ($p = 0.621$) or the age of the patient ($p = 0.341$).

Table 1

Histopathological evaluation of prostate cancer patients, the distribution of the nuclear expression of p53 as well as its correlations

Parameters		Overexpresion of p53 protein		Frequency	Chi-Square Test
		Yes	No		
Gleason score	2 + 2	0	0	0	0.001
	2 + 3	1	1	2	
	3 + 2	0	0	0	
	3 + 3	5	2	7	
	3 + 4	19	7	26	
	4 + 3	3	1	4	
	4 + 4	3	1	4	
	4 + 5	0	0	0	
	5 + 4	0	0	0	
Gleason sum	4	0	0	0	0.001
	5	1	1	2	
	6	5	2	7	
	7	22	8	30	
	8	3	1	4	
	9	0	0	0	
	10	0	0	0	
Primary tumor	T2a	3	0	3	0.001
	T2b	4	1	5	
	T2c	9	6	15	
	T3a	0	0	0	
	T3b	14	4	18	
	T4	1	1	2	
Gleason grade	1	0	0	0	0.001
	2	1	1	2	
	3	24	9	33	
	4	6	2	8	
Ekstrakapsular extension	No	16	7	23	0.647
	Yes	15	5	20	
Semen vesical involvement	No	16	7	23	0.647
	Yes	15	5	20	
Lympho node metastasis	No	28	11	39	0.000
	Yes	3	1	4	
Stage of the disease	1	0	0	0	0.026
	2	13	6	19	
	3	15	3	18	
	4	3	3	6	

Discussion

One of the goals of molecular biology is to modify diagnostic and therapeutic modalities in treating human cancer. Protein p53 is a product of the tumour suppresor gene p53 and has a role as a transcripton factor involved in the cell cycle in order to protect from damage to the genome, and by doing so to prevent malignant alterations. For these reasons in the literature this protein has been called the "guardian" of the genome. The name in fact represents the molecular mass of the protein that is 53 kDa. It is located on the chromosome 17p13.1 [6]. Its main function is to activate the proteins responsible for reparing DNA, to block the cell cycle at the point G1/S until the proteins repair the DNA. In a case where repair is impossible, the protein p53 leads the cell into apoptosis [7]. The mutated p53 protein is not able to repair efficiently the function of temporarily stopping the cell cycle, and this may lead cells to divide without control, which leads to the malignant transformation of the cell [8].

Rubin et al. [9] and Isaacs et al. [10] showed for the first time that the p53 suppressor gene is important in the pathogenesis of prostate cancer. In fact, more than 50% of all human cancer is related to the inactivacting of the tumour suppressor gene p53.

The rate of nuclear exression of p53 protein in prostate cancer in the literature is heterogeneous. It ranges from 6% to 34% [11–15]. It is interesting to mention the study of Yang et al. [16] who analyszed 49 patients with clinically localised prostate cancer and showed 34% of p53 expession. Moreover, after 5 years of follow-up, of 16 patients who developed local recurrence, 10 (63%) showed nuclear expression of p53. On the other hand, of 33 patients who did not develop a recurrence only 7 (21%) showed nuclear expression of p53. This study shows a significant correlation ($p < 0.01$) between nuclear expression of p53 and the recurrence of the disease.

The results of the present study show that the nuclear expression of p53 is expressed in 28% of prostate cancer specimens whereas in the control group there was no staining. This suggests a strong correlation between prostate cancer and p53.

One of the main goals of this study was to find the correlation between the clinical grade of the disease and p53. The clinical grade is defined for the purpose of this article as whether the tumour has an extracapsular extension. Analysis by χ^2-test showed this correlation to be lacking. Both groups, organ confined (T1 and T2) and organ non-confined (T3 and T4) showed the same p53 expression. This means that we cannot use this test for clinical staging of prostate cancer. The correlation with total PSA value showed only a non-significant correlation ($p = 0.621$). However, our results show a strong positive correlation between nuclear p53 expression and the histological stage of the tumour in terms of the Gleason score, Gleason sum and Gleason grade.

Similar results have been observed in other studies. Quin DI et al. shows a strong positive correlation between p53 expression and preoperative PSA value as well as the pathological grade and the Gleason score [17]. The same or similar observations have been published in the literature by various authors [15, 18, 19].

In conclusion, our results show that p53 protein could be used as a valid biomarker in determining the malignant potential of a tumour and in the prognosis of the disease. However, it has no practical use in predicting the extraprostatic extension.

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Резиме

ЕВАЛУАЦИЈА НА ВРЕДНОСТА НА ЕКСПРЕСИЈАТА НА p53 ПРОТЕИНОТ ВО ЕКСТРАКАПСУЛАРНАТА ЕКСТЕНЗИЈА НА КАРЦИНОМОТ НА ПРОСТАТА

Саиди С.[1], Георгиев В.[1], Ставридис С.[1], Пенев М.[1], Станков О.[1], Дохчев С.[1], Банев С.[2], Даниловски Д.[3], Ивановски О.[1], Попов Ж.[1]

[1]ЈЗУ Универзитетска клиника за урологија, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Р. Македонија
[2]Институт за патологија, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Р. Македонија
[3]Институт за епидемиологија и статистика, Медицински факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Р. Македонија

Протеинот p53 има улога на контролор на клеточниот циклус. Инволвиран е во контролата на обновувањето на клетката, инхибирајќи ја нејзината поделба пред деоксирибонуклеинската киселина целосно да се репарира.

Цел: Да се утврди експресијата на p53 протеинот во карциномот на простатата и можностите за негова практична примена во одредувањето на стадиумот, т.е. екстракапсуларната екстензија на болеста.

Методи: Студијата е комбинирана со ретроспективен и проспективен карактер. Во студијата се вклучени 83 пациенти од кои испитуваната група се состои од 43 пациенти со карцином на простата кај кои е направена радикална простатектомија, додека контролната група се состои од 40 пациенти со бенигна хиперплазија на простата кај кои е направена трансуретрална ресекција на простата или трансвезикална простатектомија. Кај сите примероци евалуирана е нуклеарната експресија на p53 протеинот. Кај испитуваната група се собрани податоци од хистопатолошката евалуација на туморските карактеристики, како и за локалната прогресија на болеста.

Резултати: Експресијата на p53 протеинот е во директна корелација со малигниот потенцијал на карциномот (Gleason skor-от, Gleason sum-от, степенот на примарниот тумор) и прогнозата на болеста (метастатските лимфни жлезди, стадиумот на болеста), но нема значајна корелација со предоперативниот PSA.

Заклучок: p53 протеинот може да се употреби како добар биомаркер за одредување на малигниот потенцијал на туморот и прогнозата на болеста, додека за екстрапростатичната екстензија нема употреблива вредност.

Клучни зборови: карцином на простата, p53 протеин, екстракапсуларна екстензија.

Corresponding Author:

Skender Saidi, MD,
University Clinic of Urology
Medical Faculty
Skopje, Republic of Macedonia
Phone: + 389 70 251279
Fax: + 389 2 3110 368

E-mail: skendersaidi@yahoo.com