Original paper

Augmented immunoexpression of survivin correlates with parameters of aggressiveness in prostate cancer

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The clinical significance of the immunoexpression of survivin in prostate cancer and its correlation with the biological aggressiveness of prostate cancer remains unclear. Therefore, the present study was undertaken to compare the immunoexpression of survivin in prostate cancer (PCa) and benign prostatic hyperplasia (BPH) as well as to determine whether this immunoexpression could correlate with Gleason score, proliferation activity and prostate specific antigen (PSA) levels. The prostate needle biopsies from 28 patients with elevated serum PSA levels were studied. As a control, 12 needle biopsies of prostate diagnosed as BPH were used. The immunoexpression of survivin was evaluated semiquantitatively, whereas the Ki-67 index was assessed quantitatively. The immunoexpression of survivin and Ki-67 in epithelial cells in the prostate cancer group was significantly increased as compared to BPH cases. In the prostate cancer group there were positive significant correlations between the immunoexpression of survivin and Gleason score as well as Ki-67 antigen. The correlation between the immunoexpression of survivin and PSA levels was also positive, but it did not reach statistical significance. In conclusion, we can confirm that in prostate cancer the immunoexpression of survivin is augmented as compared to BPH and positively correlated with parameters of tumor aggressiveness.

Key words: prostate cancer, survivin, Ki-67, PSA.

Introduction

Worldwide, prostate cancer (PCa) is the second most frequently diagnosed cancer in males [1-4]. There are over 540 000 new cases diagnosed each year worldwide [5]. It occupies second place in mortality in Poland and first place in the USA [6, 7].

The classic, widely used prognostic factors for prostate cancer are prostate specific antigen (PSA) serum levels and histological grading – Gleason score and staging represented by the TNM system. Prostate specific antigen serum level is widely used for PCa screening and has resulted in an overall decrease in PCa metastasis and death. However, this screening has also led to over-detection and overtreatment as elevations in PSA levels can also be present as a result of infection, chronic inflammation or benign prostatic hyperplasia (BPH) [1]. Thus, searching for new methods of therapy and prognosis becomes a key task for many researchers around the world. Recently, the prognostic role of angiogenesis was investigated [8].

Prostate cancer is slow-growing malignancy that is characterized by an imbalance in the rates of cell division and cell death which mainly depends on apoptosis [1, 9]. Survivin is a new and the smallest member of the inhibitor of apoptosis family and is expressed predominantly in fetal tissue but is also found in many human malignancies. It is a 142-amino acid, 16.5-kDa protein coded by a single-copy gene on the human 17q25 chromosome [10-12]. Although the

immunoexpression of survivin in prostate cancer has been reported, the clinical significance of its expression and correlation with the biological aggressiveness of prostate cancer remains unclear [13, 14]. Especially the correlation of expression of survivin and Gleason score is controversial [1, 13, 15, 16].

Therefore, the present study was undertaken to compare the immunoexpression of survivin in PCa and BPH as well as to determine whether this immunoexpression could correlate with Gleason score, proliferation activity and PSA levels.

Material and methods

Patients

The investigation was confined to study prostate needle biopsies from 28 patients diagnosed in the Department of Pathology, Medical University of Lodz (years 2007-2012). In all cases indication for the biopsy was elevated serum PSA level. Mean age of the patients was 68.85 ±5.91, and PSA level varied from 4.02 ng/ml to 103 ng/ml, mean 34.62 ±35.2. All patients of this group had prostate cancer diagnosed using light microscopy and standard hematoxylin-eosin stain. As a control, 12 needle biopsies of prostate diagnosed as BPH were used. In all these patients the serum level of PSA was within normal limits.

Immunohistochemistry

Paraffin-embedded tissue sections were mounted onto SuperFrost slides, deparaffinized, then treated in a microwave oven in a solution of TRS (Target Retrieval Solution, pH 6.0, Dako) for 30 minutes (2 × 6 minutes 360 W, 2 × 5 minutes 180 W, 2 × 4 minutes 90 W) and transferred to distilled water. Endogenous peroxidase activity was blocked by 0.3% hydrogen peroxide in distilled water for 30 minutes, and then sections were rinsed with Tris-buffered saline (TBS, Dako, Denmark) and incubated all night with monoclonal anti-human survivin antibody (Abcam; dilution 1 : 300) and 30 minutes with mouse monoclonal anti-human Ki 67 antibody (Dako; dilution 1 : 100). Immunoreactive proteins were visualized using the EnVision-HRP kit (Dako, Carpinteria, CA, USA) according to the instructions of the manufacturer. Visualization was performed by incubating the sections in a solution of 3,3'-diaminobenzidine (DakoCytomation, Denmark). After washing, the sections were counter-stained with hematoxylin and coverslipped. For each antibody and for each sample a positive and negative control were processed. As a positive control, according to the instructions of the manufacturer, adenocarcinoma of the colon was used (Fig. 1). Negative controls were carried out by incubation in the absence of the primary antibody and always yielded negative results (Fig. 2).

In each specimen staining intensity of survivin in epithelial cells was recorded semiquantitatively by two independent observers in 7-10 adjacent high power fields and graded 0 (staining not detectable), 1 (weak immunostaining), 2 (moderate immunostaining intensity) or 3 (strong staining). The mean grade was calculated by averaging grades assigned by the two authors and approximating the arithmetic mean to the nearest unity.

Morphometry

Ki-67 positive cells were evaluated using a computer image analysis system consisting of a PC computer equipped with a Pentagram graphic tablet, Indeo Fast card (frame grabber, true-color, real-time), produced by Indeo (Taiwan), and color TV camera Panasonic (Japan) coupled with a Carl Zeiss microscope (Germany). This system was programmed (MultiScan 8.08 software, produced by Computer

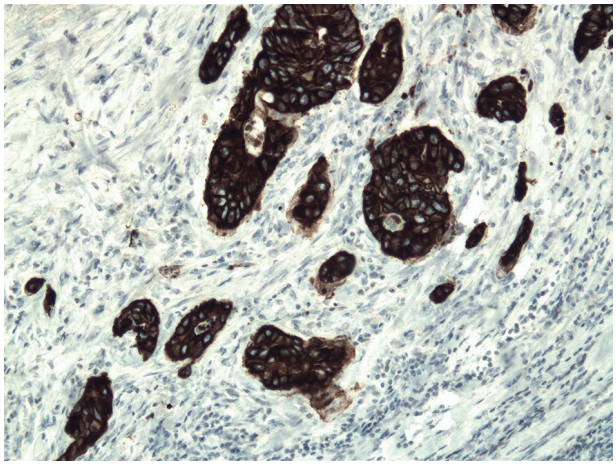

Fig. 1. Positive control for survivin (adenocarcinoma of the colon). Magnification 100×

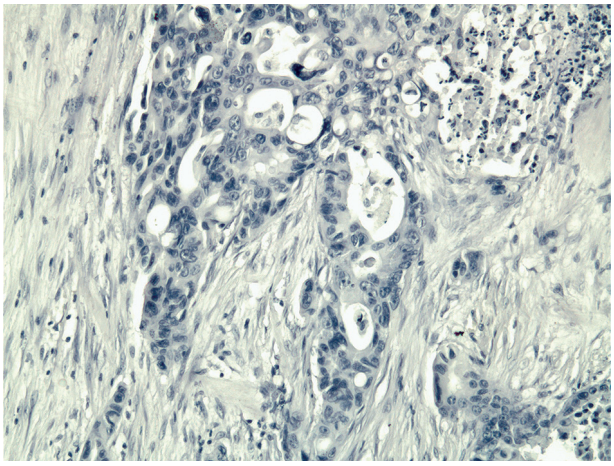

Fig. 2. Negative control for survivin (adenocarcinoma of the colon). Magnification 100×

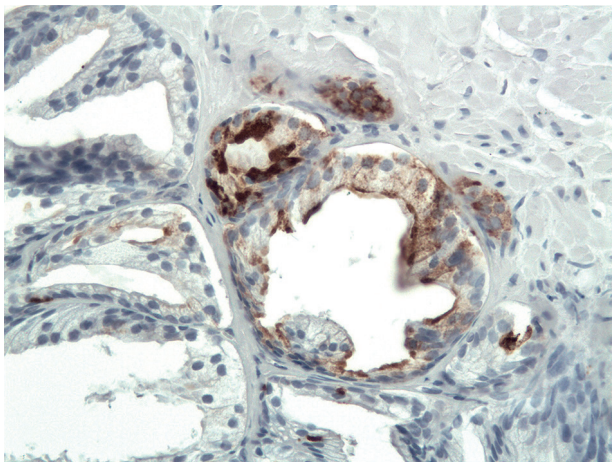

Fig. 3. Benign prostate hyperplasia. Weak immunoexpression of survivin in the cytoplasm of epithelial cells. Magnification 200×

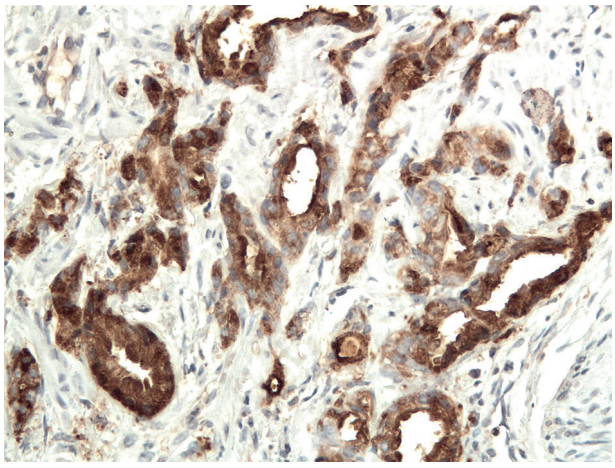

Fig. 4. Prostate cancer. Strong immunoexpression of survivin in the cytoplasm of epithelial cells. Magnification 200×

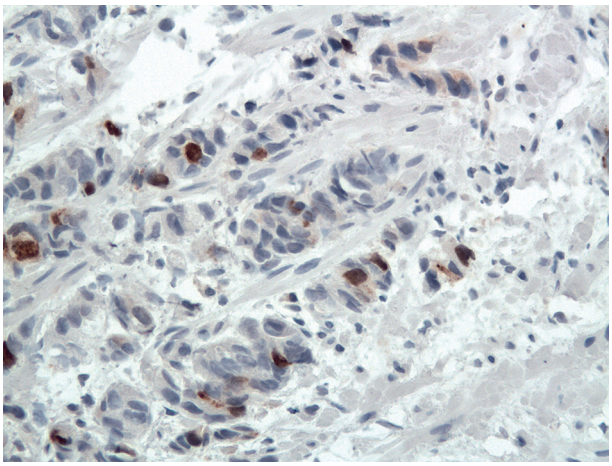

Fig. 5. Prostate cancer. Nuclear immunoexpression of survivin. Magnification 200×

Scanning Systems, Poland) to calculate the number of objects (semiautomatic function).

The percentage of Ki-67 positive cells was estimated by counting 100 cells in five monitor fields (0.029 mm^2 each), marking immunopositive cells (semiautomatic function), so that in each case 500 cells were analyzed.

Statistical methods

All values were expressed as a mean ± SD (standard deviation). The differences between groups were tested using the Mann-Whitney U test. Correlation coefficients were calculated using Spearman's method. Results were considered statistically significant if $p < 0.05$.

Results

In BPH biopsies we found 8 of 12 cases with a positive survivin reaction.

The immunoexpression of survivin protein was also positive in 26 of 28 cases of prostatic cancer. Four of these cases were low risk PCa (PSA level < 10 ng/ml and Gleason score < 6); 2 of them were survivin negative. Twenty-four cases were survivin-positive high-risk PCa. In BPH the immunoexpression of survivin was exclusively membranous/cytoplasmic (Fig. 3). Similarly, in most cases of prostate cancer the immunoexpression of survivin was membranous/cytoplasmic (Fig. 4), although in some patients a nuclear reaction for survivin was seen (Fig. 5). In both BPH and prostate cancer groups the immunoexpression of Ki-67 was exclusively nuclear (Figs. 6, 7).

The semiquantitative and morphometric data of the immunoexpression of survivin and Ki-67 antigen appear in Table I. The immunoexpression of survivin and Ki-67 in epithelial cells in the prostate cancer group was significantly increased as compared to BPH cases.

The correlations between immunoexpression of survivin and Gleason score, PSA levels and Ki-67 antigen are given in Table II. In the prostate cancer group there were positive significant correlations between immunoexpression of survivin and Gleason score as well as Ki-67 antigen. The correlation between immunoexpression of survivin and PSA levels was also positive, but it did not reach statistical significance. In BPH patients all these correlations were weak and not significant.

Discussion

Survivin, an anti-apoptotic protein, inhibits the processing of initiator caspase-9 as well as caspase-3 and caspase-7, terminal effectors of apoptosis. Additionally, survivin counteracts pro-apoptotic stimuli induced by interleukin-3, Fas, Bax, tumor necrosis factor and anticancer drugs [13, 17-21]. Besides the anti-apoptotic properties, survivin regulates cell division [22-24]. In prostate cancer aberrantly activated signal transducer and activator of transcription 3

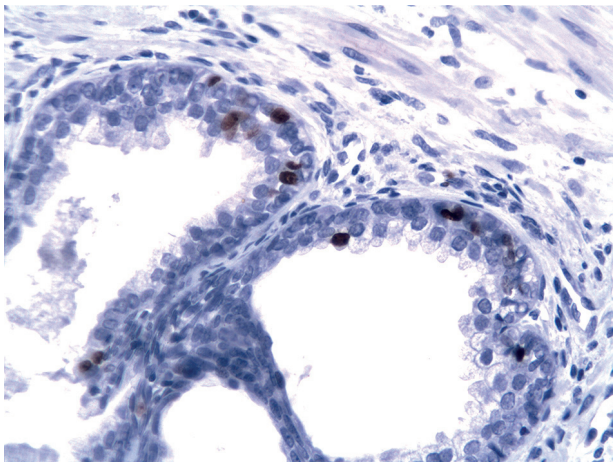

Fig. 6. Weak immunoexpression of Ki-67 protein in benign prostate hyperplasia. Magnification 200×

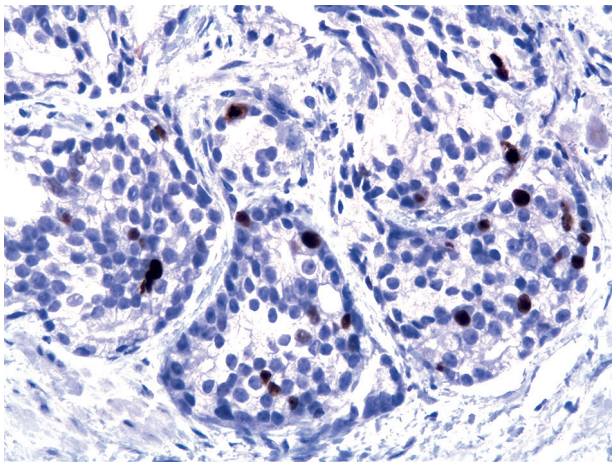

Fig. 7. Numerous nuclei marked as Ki-67 positive in patient with prostate cancer. Magnification 200×

(Stat3) is probably involved in direct activation of survivin and other proto-oncogenes [25].

In the present study we confirmed the findings of Shariat *et al.* [26] that the immunoexpression of survivin is significantly increased in prostate cancer patients as compared to controls, whereas Kishi *et al.* revealed that survivin mRNA was in PCa significantly higher than that found in control tissues [13]. Koike *et al*. quantified the gene expression levels of survivin and its isoforms in PCa and BPH and found that in prostate biopsy samples, the survivin expression in PCa was significantly greater than that in BPH or PCa after androgen deprivation therapy [15]. In the results of other investigators, plasma-derived exosomal survivin was also significantly increased in the PCa-derived samples, compared to those from BPH [1]. Similarly, in the paper of Bialkowska-Hobrzanska *et al*. survivin mRNA was in PCa up to 13.5-fold higher as compared to control tissue [16].

As might be expected, in our study the percentage of Ki-67 positive nuclei was significantly higher in prostate cancer patients in comparison with BPH individuals. However, the most important findings in the present paper are correlative data. We revealed a strong positive correlation between the immunoexpression of survivin and Gleason score in the PCa group. This result supports the observations that survivin correlates with the parameters of aggressiveness in prostate cancer. In the study of Kishi *et al.* in PCa samples survivin expression was significantly higher in cancers with a high Gleason score, high pathological T stage, positive surgical margin and vessel invasion [13]. Similarly, Koike *et al.* and Shariat *et al*. found that survivin expression was also associated with high Gleason score [15, 26]. Contrary to us and cited authors, Khan *et al*. [1] and Bialkowska-Hobrzanska *et al*. [16] did not reveal any correlations between survivin and Gleason score. These differences may depend on methodological reasons, as to evaluate survivin these researchers used various methods.

Moreover, we found a significant positive correlation between immunoexpression of survivin and the Ki-67 index. Similarly, other authors demonstrated that survivin expression is positively related to PCa proliferation activity measured by the PCNA labeling index [13]. However, to our best knowledge in

Table I. Semiquantitative data of immunoexpression of survivin and morphometric data of Ki-67 score in prostate cancer and controls

Number of cases	Controls (BPH) (n = 12)	Prostate cancer (n = 28)	P value
Survivin (mean score)	0.92 ±0.82	2.25 ±1.76	< 0.02
Ki-67 (%)	0.97 ±0.53	12.32 ±5.25	< 0.001

Table II. Correlations between immunoexpression of survivin and Gleason score, PSA levels and Ki-67 score in prostate cancer as well as in controls

Correlation between	Controls (BPH) (n = 12)	Prostate cancer (n = 28)
Immunoexpression of survivin and Gleason score	–	r = 0.61, p < 0.005
Immunoexpression of survivin and PSA levels	r = 0.49, p = 0.1 (NS)	r = 0.36, p = 0.06 (NS)
Immunoexpression of survivin and Ki-67 (%)	r = 0.32, p = 0.31 (NS)	r = 0.54, p < 0.004

NS – not significant

the available literature no data have documented such a relationship using Ki-67 antibody. Finally, we noticed in PCa cases a positive correlation between the immunoexpression of survivin and PSA serum levels, but this relationship was not significant. Interestingly, in a previous study [13] the survivin expression in PCa patients with a short preoperative prostate specific antigen doubling time (less than 2 years) was also significantly higher than those with a moderate (2-4 years) or long (greater than 4 years) doubling time.

In conclusion, we can confirm that in prostate cancer the immunoexpression of survivin is augmented as compared to BPH and positively correlated with parameters of tumor aggressiveness.

The authors declare no conflict of interest.

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