

Comparative Proteomic Study of Proteins in Prostate Cancer and Benign Hyperplasia Cells

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Abstract

Comparative proteomic studies of proteins in DU-145, PC-3, LNCaP and BPH-1 cultivated cells as well as in prostate tissue samples obtained from patients with benign and malignant tumors showed differences in protein profiles, in particular, high content in tissue samples of four isoforms of transgelin and profilin-1. Increased expression of protein Dj-1 was observed in cancer cells and biopsy samples of prostate cancer as opposed to BPH-1 cells and biopsy samples of tissues with benign hyperplasia. Dramatic reduction of serpin H1 was noted in the range of cell lines: BPH-1 → PC-3 → DU-145 → LNCaP. Thus, the obtained data are indicative of protein Dj-1 and serpin H1 participation in formation of the cancer phenotype in prostate cells.

Keywords: Prostate cancer; Proteomics; Two-dimensional electrophoresis

Abbreviations: PCa: Prostate cancer; BPH: Benign prostatic hyperplasia; 2DE: Two-dimensional electrophoresis

Introduction

Prostate cancer is one of the most frequent cancer diseases affecting men [1,2], due to which systemic protein studies in cultivated cell lines grown from prostate tumors as well as in samples of prostate tissue have been conducted for several decades [3-5]. DU-145, PC-3, LNCaP lines and the two-dimensional electrophoresis according to O'Farrell's method as well as other proteomic technologies have been frequently used in these investigations. Objectives of similar studies included the search of proteins involved in pathogenesis of prostate cancer. As a result, some authors described several proteins that were expected to serve either as biomarkers or as potential targets for therapeutic modalities [6-8]. So far, only the well-known prostate-specific antigen (PSA) has the clinical application, however, its drawbacks have been identified as well [9,10]. Current difficulties of proteomic analysis of prostate cancer may be caused by differences in protein profiles of cultivated cancer cells [1,11,12] as well as diversity of the cellular composition in the studied tissue samples [13]. Correspondingly, proteomic studies providing for comparison of protein profiles in lines of benign and malignant prostate cells and in prostate tissue cells is of interest but the number of such works is inconsiderable [14], though the immortalized cell line of benign prostate hyperplasia (BPH-1) was developed more than fifteen years ago [15].

This article presents results of the comparative proteomic study of proteins in cultivated cells DU-145, PC-3, LNCaP and BPH-1, as well as in prostate tissue samples obtained from patients with malignant and benign tumors.

Materials and Methods

Cell lines and tissue samples

DU-145 (ACC 261), PC-3 (ACC 465) and BPH-1 (ACC 143) cell cultures were purchased from the German Collection of Microorganisms and Cell Cultures (Germany), and samples of LNCaP line cultivated cells were kindly provided by Dr. I.G. Shemyakin

(State National Center of Applied Microbiology and Biotechnology, Obolensk, Russia). Cells were cultivated in RPMI-1640 supplemented with Hepes, sodium pyruvate, gentamycin and 20% fetal bovine serum as described earlier [16]. Cells of each line were grown in plastic flasks (Nunc, Denmark) in CO₂-incubator (Sanyo, Japan).

Biopsy samples and operation materials of prostate tissue (PCa, n = 76, BPH, n = 45) were obtained from the Urology Department of Russian Medical Academy of Postgraduate Education (Moscow, Russia). Diagnostics was performed using standard clinical, histological, and immunochemical (PSA assay) methods. Histological verification of the diagnosis was performed by means of transrectal multifocal puncture biopsy under transrectal ultrasound guidance, which provided up to 18 tissue samples from various prostate zones. All cases of prostate cancer were characterized histologically as adenocarcinoma.

Preparation of protein extracts

The cells were pre-incubated in the serum-free medium for 2 hours to remove absorbed proteins of serum used for cultivation. Then the cells were suspended in the serum-free medium mechanically taking them off the surface of plastic flasks and then the cells were precipitated by centrifugation (1,000 rpm, 5 min). The obtained pellet was washed off by the phosphate buffered saline (pH 7.4) at 4°C in order to prevent contamination. The collected cells before protein extraction were stored at -70°C.

The cells of each line (approximately 6-10 × 10⁶ cells) were

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homogenized in 200 μ l of the lysis solution containing 9 M of urea, 5% mercaptoethanol, 2% Triton X-100, 2% CHAPS, 2% Ampholine pH 3.5-10 to extract proteins. Prostate tissue samples were homogenized in four volumes of the same lysis solution. The obtained protein solutions were refined by centrifuging and immediately used for fractionation by two-dimensional electrophoresis according to O'Farrell's method in two variants.

Two-dimensional gel electrophoresis

One of these variants (IPG-2DE) included application of isoelectrofocusing (IEF) in polyacrylamide strips conjugated with immobilines (18 cm Immobiline™ DryStrip pH 3-10, GE Healthcare) on Ettan IPGphor 3. On the eve of the experiment, strips were soaked in 250 μ l of solution for rehydration (8M urea, 2% CHAPS, 2% IPG buffer, 0,28% dithiothreitol, 0,002% bromphenol blue) and were incubated during the night at the room temperature. 100 μ l of the sample containing from 50 to 500 μ g of the protein were applied to the each strip. IEF was done in the four-stage regime. After that the strips were used for fractionation in the second direction. Fractionation in the second direction (SDS-electrophoresis in specially prepared gel slabs with gradient of acrylamide concentration) was done as described earlier [17].

Another variant (IF-2DE) was a special modification of O'Farrell method which used nonequilibrium pH gradient electrophoresis (NEPHGE) as was described earlier [18]. In this variant IEF was performed in glass tubes (2.4 \times 180 mm) filled with 4% PAAG prepared on 9M urea solution containing 2% of triton X-100 and 2% mixture of ampholines. In major experiments ampholines pH 5-7 and 3.5-10 were used in the ratio 4/1. In order to determine the distribution of proteins in marginal areas more precisely ampholines pH 4-6 or ampholines pH 7-9 were applied instead of ampholines pH 5-7 in the same ration. Protein extracts (100-150 μ l) were applied to the «acid border» of the each gel column, and IEF was done 3 hr at 1,400 V per hr. (Model 175, BioRad, USA). After IEF gel columns were used for fractionation in the second direction that was done as in the first variant.

Protein visualization by Coomassie Blue R-250 and sodium nitrate staining as well as analysis of two-dimensional (2D) electrophoregrams were performed as described earlier [17,18] with minor modifications. At least three 2D electrophoregrams were obtained for each biopsy specimen, whereas the study of cultured cells proteins was performed using at least ten 2D electrophoregrams. Molecular masses of proteins in the electrophoretic fractions were determined using kits of highly purified recombinant proteins SM0661 (10– 200 kDa), SM0671 (10– 170 kDa) (Fermentas, USA).

Densitometry and image analysis

Densitometry of 2D electrophoregrams and/or their individual fragments (rectangles) was performed after scanning (Epson Expression 1680 scanner) or filming by means of a digital photcamera (Nikon 2500 or Canon PowerShot A1000 IS). The computer handling of the resultant densitometry images of protein fractions was performed using the Melanie ImageMaster software, versions 6 and 7 (Genebio, Switzerland). The statistical analysis was performed using the nonparametric Wilcoxon–Mann–Whitney test.

Mass spectrometric analysis and identification of proteins

Some fractions chosen for identification were cut out from 2DE slab gels and the proteins were hydrolyzed by trypsin. The extracted tryptic

peptides were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) as described previously [19] with some modifications [20]. A sample (0.5 μ l) was mixed with the same volume of 20% acetonitril solution containing 0.1% of trifluoroacetic acid and 20 mg/ml 2,5-dihydroxybenzoic acid (Sigma, USA), and was air-dried. Mass-spectra were obtained on MALDI-TOF-mass-spectrometer Reflex III (Bruker, USA) with UV-laser (336 nm) in the regime of positive ions in the range 500-8,000 Da and their calibration was done in accordance with the known peaks of trypsin autolysis.

During MS/MS analysis mass-spectra of fragments were registered on MALDI-TOF mass-spectrometer Bruker Ultraflex in the tandem regime (TOF-TOF) with detection of positive ions. Fragmentation of ions was induced by helium supply in the area of the initial part of the free ion drift trajectory (inert gas pressure $2 \cdot 10^{-7}$ Pa). Accuracy of mass fragments measurements did not exceed 0.05 %. Only C-signals of terminal peptide fragments disrupted by the peptide bond (γ -ions) were observed on the mass-spectrum.

Protein identification was done with the help of Mascot software, option Peptide Fingerprint (Matrix Science, USA), with the accuracy of mass measurement MH^+ equal to 0.01% (with a possibility to modify cysteines by acrylamide and methionine oxidation).

Results and Discussion

Comparative analysis of protein fractions

Three types of cultivated cell lines (Du-145, PC-3, LNCaP) were chosen as routinely used models of prostate cancer (as androgen-dependent or hormone insensitive with different metastatic potential) and one cell line (BPH-1) as a model of benign prostatic hyperplasia. These cell lines are non-isogenic and it could influence results of the comparative analysis of protein profiles.

On the typical silver stained 2D electrophoregrams of proteins obtained from cultivated cell lines and from prostate tissue samples 550–600 fractions with molecular masses (Mm) from 200 to 10 kDa were detected (Figure 1). As can be seen on 2D electrophoregrams of the studied samples, the distributions of protein fraction were different but there was the whole range of common fractions corresponded to known enzymes as well as to various structural proteins. An identity from 50 to 150 common fractions was established by mass-spectrometry on 2D electrophoregrams of protein extracts of cultivated cells and prostate tissue samples. Full information about identified proteins there are in our special database (<http://ef.inbi.ras.ru>). Figure 1 shows twelve of these fractions that were further chosen as reference points for 2D electrophoregrams comparative analysis (the numeration is presented in accordance with the Mm reduction). This choice was done considering that the marked protein fractions reported to be involved in carcinogenesis and are already studied either as biomarkers or as potential targets for therapeutic interventions (Table 1). In case if MALDI-TOF MS analysis resulted in the amino acid sequence coverage less than 40% the confirming identification was done by MALDI-TOF MS/MS.

A comparative analysis of protein fractions (6-9 and 11) in the region "Rectanle 1" on 2D electrophoregrams of protein samples from DU-145 cells was performed using IPG-2DE (Figure 2A) and IF-2DE (Figure 2B), and it was shown that the IPG-2DE provides rather better protein fractions division.

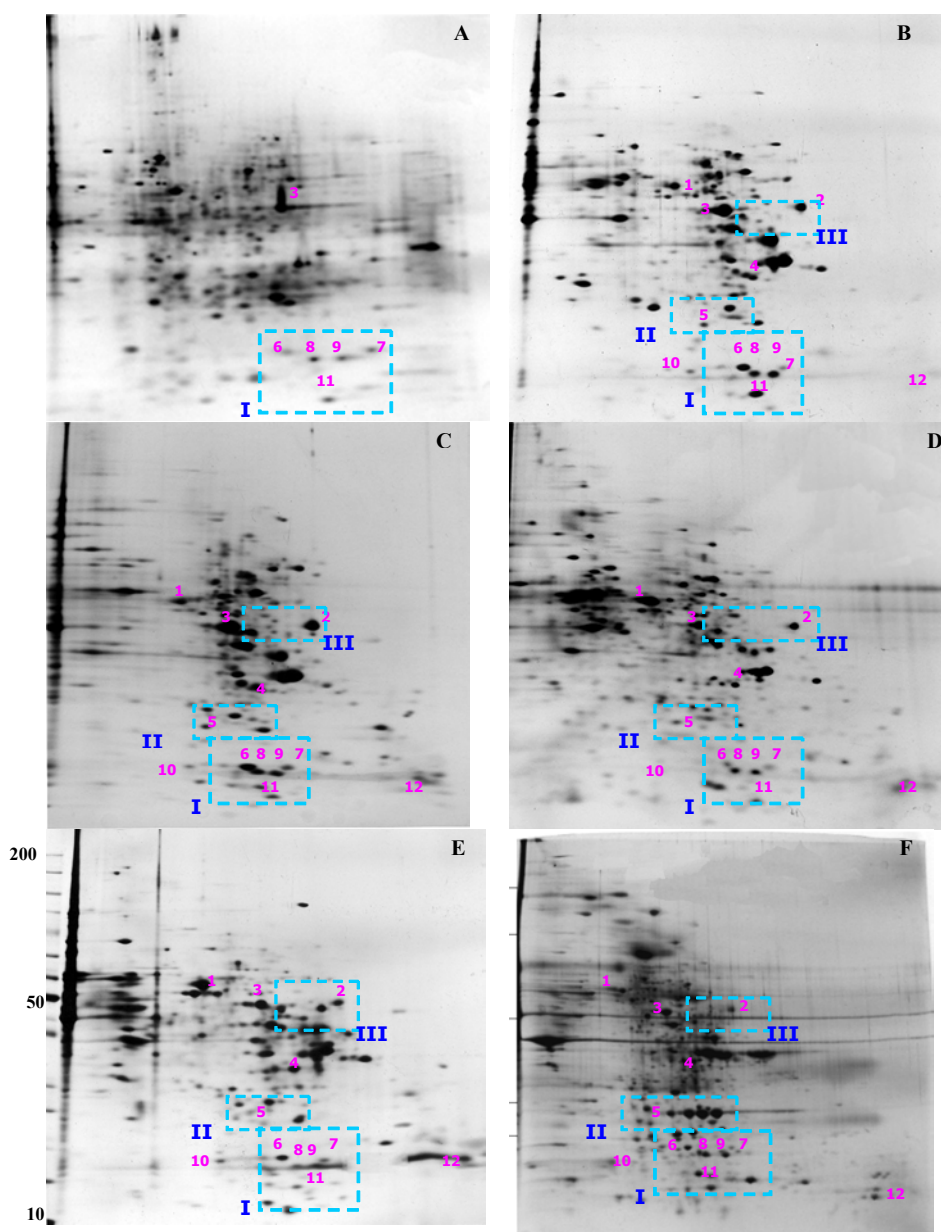


Figure 1: Silver-stained 2D electrophoregrams of proteins, extracted from cultivated cell lines and specimens of prostate tissues. A - DU-145 (IPG-2DE), B - DU-145 (IF-2DE), C - PC-3, D - LNCaP, E - BPH-1, F – sample of prostate tissues with malignant tumor. In Figure 1E Protein-Markers of Mm (200-10 kDa) are shown on left border. 1-12 – Twelve common proteins that identified on 2D electrophoregrams of protein preparations by using MALDI-TOF MS and MALDI-TOF MS/MS (Details in Table 1). I, II, III – “Rectangles” in 2D electrophoregrams marked out for the further comparative analysis (the explanatory in the text).

Moreover, the comparison of whole results for Du-145 cells obtained using IPG-2DE (Figure 1A) and IF-2DE (Figure 1B) showed that the first variant provided successful fractionation and identification of a large number of proteins from pI 4.0 to 8.5, whereas application of the second variant allowed to detect a smaller number of protein fractions. But the IF-2DE provided registration of proteins with higher pI values up to 11.0. At the same time it is worthy of note that the experimental pI values of protein fractions obtained by IPG-2DE and, in some cases, by IF-2DE were close to calculated values (Table 1). However, for protein fractions 2 (Eukaryotic translation elongation factor 1 alpha) and 4 (Porin), that were revealed by IF-2DE, the experimental pI values

were significantly different from the expected values. Thus, it is evident that IF-2DE variant does not provide for achievement of calculated pI for some proteins, i.e. this method combined the possibilities of two modifications of O’Farrell’s method, including IEF or nonequilibrium pH gradient electrophoresis (NEPHGE) in the first dimension.

The observed quantitative variations of protein fractions located in the region “Rectangle I” did not allow making a conclusion about determinative changes during malignant transformation. In particular, fraction 6 (Cofilin 1) on 2D electrophoregrams of benign hyperplasia cell samples was quantitatively similar or even higher than those on 2D electrophoregrams of malignant cells. These results were corresponded

№	Protein name (some synonyms), <i>gene symbols</i>	Numbers in databases: Protein NCBI / OMIM / UniProt	Sequence coverage (%)*	Mm / pI (experimental)*	Mm / pI (calculated)	Some references
1	ER-60 protease (protein disulfide isomerase, family A, member 3) <i>PDIA3</i> , <i>ER60</i>	1208427 / 602046 / P30101	30-55	57,0-60,0 / 5,80-6,12	56,8 / 5,98	Stierum et al. [21]; Shishkin et al. [22]
2	Eukaryotic translation elongation factor 1 alpha 1 (cervical cancer suppressor 3 isoform) <i>EEF1A1</i>	48734733 / 130590 / P68104	24-29	48,2-49,8 / 8,10-8,50	50,2 / 9,14	Rho et al. [24]; Yao et al. [25]
3	Alpha-enolase isoform 1 (phosphopyruvate hydratase) <i>ENO1</i>	4503571 / 172430 / P06733	35-86	43,0 – 47,0 / 6,80-7,12	42,7 / 7,01	Stierum et al. [21]; Chang et al. [23]
4	Porin (voltage-dependent anion channel 1) <i>VDAC1</i>	4507879 / 604492 / P21796	34-75	27,0-30,8 / 7,50-8,00	30,6 / 8,63	Liu et al. [26]; Thinnes [27]
5	Protein Dj-1 (parkinson protein 7) <i>PARK7</i> , <i>DJ1</i>	50513593 / 602533 / Q99497	38-70	20,0-22,0 / 6,30-6,35	20,0 / 6,33	Tillman et al. [28]; Zeng et al. [29]
6	Cofilin 1, (cofilin non-muscle) <i>CFL1</i>	15012201 / 601422 / P23528	30-65	18,0-19,3 / 7,60-7,80	18,5 / 8,22	Stierum et al. [21]; Castro et al. [30]
7	Protein NM23B (non-metastatic cells 2, protein) <i>NME2</i> , <i>NM23B</i>	12803317 / 156492 / P22392	50-61	17,5-17,9 / 8,10-8,40	17,2 / 8,55	Kidd et al. [31]; Schulz et al. [32]
8	Cyclophilin A, electrophoretic isoform 1 (peptidylprolyl isomerase A) <i>PPIA</i>	13937981 / 123840 / P62937	28-38	17,3-17,7 / 7,65-7,85	17,9 / 7,82	Rho et al. [33]; Bai et al. [34]
9	Cyclophilin A, electrophoretic isoform 2 (peptidylprolyl isomerase A) <i>PPIA</i>	13937981 / 123840 / P62937	38-50	17,3-17,7 / 8,00-8,30	17,9 / 7,82	Rho et al. [33]; Bai et al. [34]
10	Stathmin 1 (oncoprotein 18) <i>STMN1</i>	15680064 / 151442 / P16949	24-26	17,0-17,5 / 5,80-6,00	17,3 / 5,76	Polzin et al. [35]; Chung et al. [36]
11	Profilin-1 (Profilin 1) <i>PFN1</i>	4826898 / 176610 / P07737	55-67	14,0-14,5 / 7,65-7,75	15,0 / 8,44	Zou et al. [37]; Minamida et al. [38]
12	H3 histone, family 3A, <i>H3F3A</i>	55665435 / 601128 / P84243	60-71	14,3-14,5 / 11,1-11,2	15,3 / 11,3	Liu et al. [39]; Shishkin et al. [22]

* Results are shown as a range of the data obtained for identified proteins from different samples.

Table 1: Twelve common proteins that identified on 2D electrophoregrams of proteins from cultivated cells (DU-145, PC-3, LNCaP, BPH-1) and specimens of prostate tissues. Numeration as revealed on Figure 1.

to the data of Davila et al. [40] who at comparative study of proteins of BPH-1 and PC-3 cells did not detect any changes in the level of Cofilin 1, though the increased expression of Cofilin 1 was observed in the cells of colorectal and lung cancer [21,30].

Fraction 7 (NM23B, non-metastatic cells 2, protein) in various prostate biopsy samples showed the high quantitative variability up to its complete absence. Due to the fact that for more than 15 years a gene encoding protein NM23B has been considered as a potential metastasis suppressor [41], it can be supposed that biopsy samples lacking protein NM23B contain cells of the metastasizing prostate cancer. Correspondingly, the presence of protein NM23B was registered in all studied lines of malignant cells (DU-145, PC-3, LNCaP) that were initially obtained from metastases of prostate cancer.

Quantitative reduction of fraction 11 (Profilin-1) was observed in cultivated cells in the region “Rectangle I” during comparison of cell samples (Figure 3 A-D) and prostate biopsy samples (Figure 3 E-F).

High level of profilin-1 in prostate tissue samples can be related to the presence of muscle cells.

More significant differences between 2D electrophoregrams of prostate tissue and cultivated cells proteins were observed in the region “Rectangle II”. For example, the Figure 2 shows corresponding regions of 2D electrophoregrams of prostate tissue proteins (C), as well as proteins of DU-145 (D) and BPH-1 cells (E). These data make evident that only extracts of prostate tissue samples contained four major protein fractions identified as electrophoretic isoforms of transgelin (Table 2). Thus, appearance of transgelin isoforms in prostate biopsy samples is evidently related to presence of smooth muscle cells in these biomaterials.

It is known, that transgelins are actin-associated proteins that participate in cytoskeleton formation [42]. Accordingly, the expression changes of transgelin genes (*TAGLN*, *TAGLN2*) during cancerogenesis can have an influence on cell motility, and also on invasiveness and

metastatic ability of cancer cells [43]. However the data concerning the character of expression changes of transgelin genes at prostate cancer are conflicting. So, Prasad et al. [42] have recently reported that transgelin level is decreased in various types of prostate cancer cultured cells (except Du-145), and Lee et al. [43] have the opposite observations that level of transgelin is essentially increased in malignant prostate cells.

As the Figure 1 and Table 1 show, among the major proteins of prostate cancer cells two isoforms of Cyclophilin A (Peptidyl-prolyl cis/trans isomerase A, PPIA) have been identified. As it has been reported earlier, overexpression of PPIA is observed in prostate cancer cells, namely in Du-145 cells [44,45]. Thereby, Cyclophilin A, that is induced by hypoxia and has an influence on the folding of proteins, is important in tumorigenesis.

Among the identified proteins Peroxiredoxin 1 (PDRX1) is also

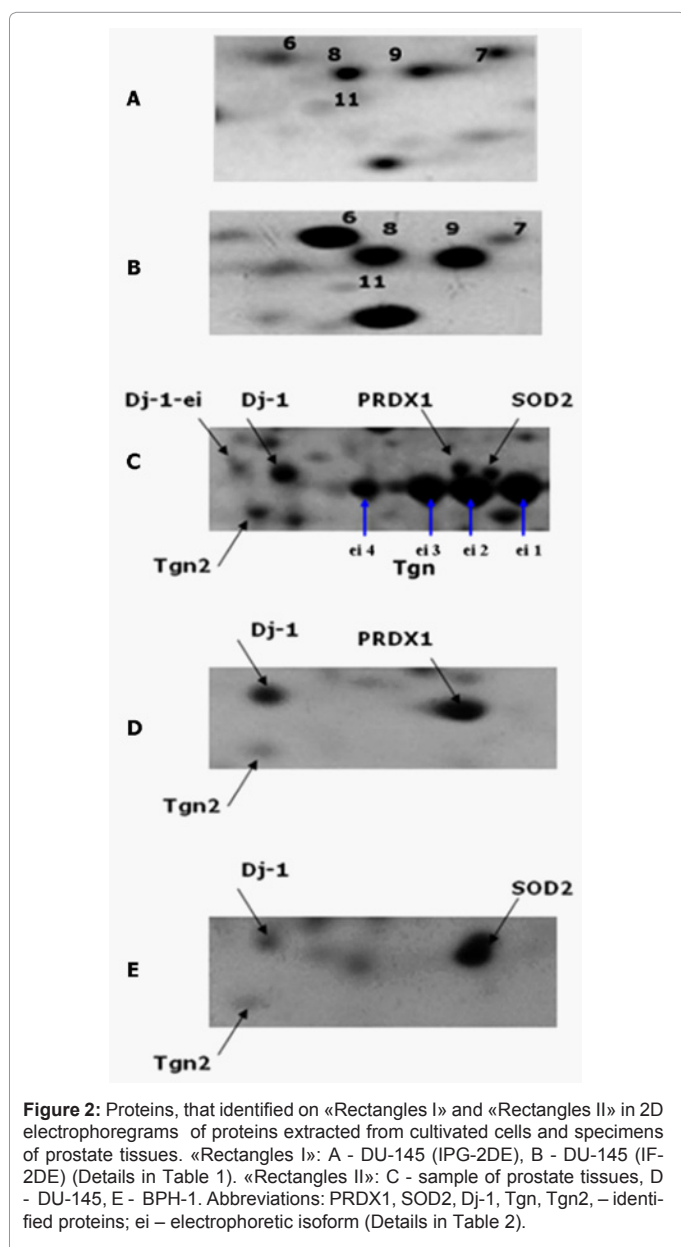


Figure 2: Proteins, that identified on «Rectangles I» and «Rectangles II» in 2D electrophoregrams of proteins extracted from cultivated cells and specimens of prostate tissues. «Rectangles I»: A - DU-145 (IPG-2DE), B - DU-145 (IF-2DE) (Details in Table 1). «Rectangles II»: C - sample of prostate tissues, D - DU-145, E - BPH-1. Abbreviations: PRDX1, SOD2, Dj-1, Tgn, Tgn2, – identified proteins; ei – electrophoretic isoform (Details in Table 2).

Protein symbol	Protein name (some synonyms); gene symbols	Numbers in databases: Protein NCBI / OMIM / UniProt	Sequence coverage* (%)	Mm / pI (experimental)*
PRDX1	Peroxiredoxin-1 (Proliferation-associated gene protein); <i>PRDX1</i>	32455266 / 176763 / Q06830	41-67	22,0-22,5 / 6,95-7,00
SOD2	Superoxide dismutase [Mn], mitochondrial; <i>SOD2</i>	134665 / 147460 / P04179	42-75	22,3- 22,5 / 7,10 – 7,30
Dj-1	Protein Dj-1 (parkinson protein 7) <i>PARK7, DJ1</i>	50513593 / 602533 / Q99497	38-70	20,0-22,0 / 6,30-6,35
Tgn**	Transgelin (SM22); <i>TAGLN</i>	49168456 / 600818 / Q6F152	70-90	ei** 1 22,0 / 7,15 ei** 2 22,0 / 7,05 ei** 3 22,0 / 6,86 ei** 4 22,0 / 6,76
Tgn2	Transgelin 2 (SM22-alpha homolog); <i>TAGLN2</i>	55960374** / 604634 / P37802	43-55	19,5-21,4 / 6,20-6,25

* Results are shown as a range of the data obtained for identified proteins on 2D electrophoregrams of different samples

** Transgelin was identified on «Rectangle II» in 2D electrophoregrams of extracts from specimens of prostate tissues only; ei – electrophoretic isoform

Table 2: Proteins that identified on «Rectangle II» in 2D electrophoregrams of extracts from cultivated cells (DU-145, PC-3, LNCaP, BPH-1) and specimens of prostate tissues. Protein symbols as revealed on Figure 4.

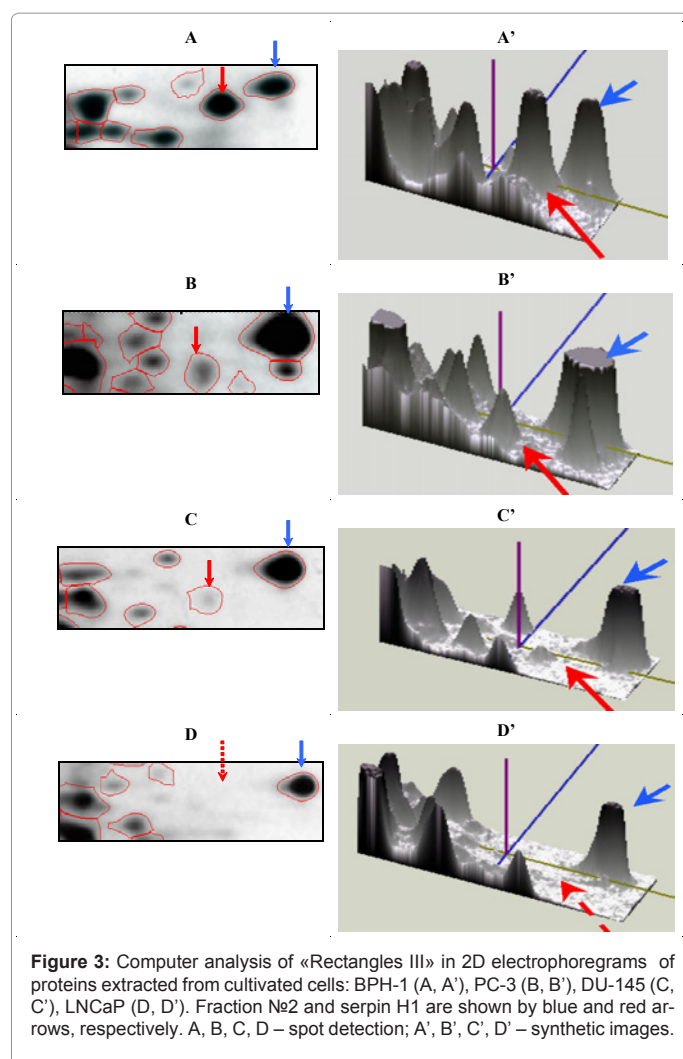
worthy of note. PDRX1 belongs to a family of peroxidases that are involved in antioxidant defense. PDRX1 regulates cell proliferation and apoptosis by its interaction with oncogene products such as c-Abl [46]. There are some data that PDRX1 controls prostate cancer growth through Toll-like receptor 4-dependent regulation of tumor vasculature [47].

In our study we could not reveal reliable changes of these proteins (Transgelin, PPIA, PDRX1) between the investigated cancer cells and BPH-1.

Dj-1 protein in biopsy samples and cultivated cell lines

Practically all analyzed prostate cancer biopsy samples contained Dj-1 protein in the form of two electrophoretic fractions that differed in amount and pI values. During MALDI MS identification for the major Dj-1 fraction the of amino acid sequence coverage by detected tryptic peptides was 70.4%, and for the minor - 42.0% (Table 2). The major fraction was thus identified as the main isoform and the minor fraction was considered as the minor electrophoretic isoform designated as Dj-1-ei. Identification of several Dj-1 isoforms in breast cancer has been already reported in the literature [48], but no information about Dj-1 isoforms in prostate cells is available. At the same time, it is known that this protein can undergo certain post-translational modifications (Tyr67 phosphorylation, Cys106 sulfination, Lys148 acetylation) (record Q99497 UniProt). These modifications may cause formation of electrophoretic isoforms with pI values lower than the main isoform has. Thus, observed Dj-1-ei isoform, possibly, corresponds to a product of post-translational modification of the Dj-1 protein present in prostate tissue.

In the region “Rectangle II”, in addition to the protein fraction



5 (protein Dj-1) specified above, the following proteins have been identified: transgelin 2 (Tgn 2), mitochondrial superoxide dismutase (SOD2), and peroxiredoxin 1 (PRDX1) (Figure 3, Table 2). Comparison of the levels for those protein fractions with the level for Dj-1 protein on 2D electrophoregrams of DU-145 (as well as other cancer prostate cells) and BPH-1 cells showed that the level of Dj-1 protein in cancer cells is considerably increased (Figure 2 D and E). In addition to this, comparative densitometry shows that the level of Dj-1 protein in adenocarcinoma biopsy samples was 5-10 fold higher than the level of this protein in hyperplasia tissue samples. In order to minimize possible individual variations, the comparative study of Dj-1 concentration in biopsy material included analysis of PCa and BPH tissue samples obtained from various prostate regions of one and the same patient. The used collection was composed of biomaterials of four patients.

In summary, the results of the comparative analysis of protein fractions in regions “Rectangle II” revealed the possibility of increased synthesis of Dj-1 protein in prostate cancer and its involvement in carcinogenesis. These results were corresponded to the literature data about the ability of protein Dj-1 to affect progression of some malignant prostate neoplasms due to intensification of cell proliferation [49]. Dj-1 protein is secreted by cancer cells to blood and is studied as

a biomarker for some types of cancer [50]. Taking into account our results, it is possible to suppose the diagnostic value of this protein for prostate cancer.

Serpin H1 in cultivated cell lines

The comparative computer analysis of the region “Rectangle III” that contained fraction 2 (Eukaryotic translation elongation factor 1 alpha) allowed to identify an additional protein fraction the level of which was dramatically reduced in the range of cell lines BPH-1 → PC-3 → DU-145 → LNCaP (Figure 3). This quantitatively changing fraction was identified by MALDI MS (with 40-50% coverage) as inhibitor of serine proteases - serpin H1 (synonym: HSP47). In the range of cell lines BPH-1 → PC-3 → DU-145 → LNCaP the values of quantitative ratio serpin H1 / fraction 2 were $1.13 \pm 0.05 \rightarrow 0.09 \pm 0.03 \rightarrow 0.05 \pm 0.01 \rightarrow < 0.01$. Proteomic analysis of prostate tissue biopsy samples allowed to register serpin H1 on several 2D electrophoregrams of proteins from benign hyperplasia samples as a minor fraction as compared to fraction 2. Practically, in prostate cancer biopsy samples serpin H1 could not be identified. Fraction 2 on all 2D electrophoregrams was distinctly observed. These data to a certain degree contradict the report about increase of the serpin H1 level in ulcerative colitis-associated carcinomas [51]. However, there are materials stating that serpin H1 is a biomarker of stromal tumor cells [52] and normal fibroblasts are stained by antibodies to serpin H1 more intensely than cancer cells [53]. Thus, it is possible that the observed low level of serpin H1 in cancer cells of studied lines is caused by the fact that they were obtained from metastases of prostate adenocarcinomas and might be significantly different from stromal cells of cancer tumors.

Summarizing the obtained data, it may be concluded that they are indicative of involvement of Dj-1 protein and serpin H1 in formation of the cancer phenotype in prostate cells.

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