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HUMAN BENIGN AND MALIGNANT PROSTATIC NEOPLASMS: CYTOPLASMA PROTEIN STUDIES

Cytosol and plasma membrane proteins of human benign and malignant prostatic neoplasms were analyzed by one-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The samples of normal prostatic tissue, benign prostatic hyperplasia (BPH) and prostatic carcinoma (PCA) were obtained after transurethral resection or radical prostatectomy. The electropherograms were developed by silver nitrate staining and quantitative analysis was performed by video densitometer and software Gel-Pro® Analyzer (Media Cybernetics, USA). No significant changes in plasma membrane protein patterns of studied tissues were observed. However, qualitative and quantitative differences among cytosol proteins of human normal prostate, BPH and PCA were found. A significant increase of 32 kD component expression in PCA compared with normal and BPH tissues seemed to be specific for the malignant phenotype. The changes in 62 and 93 kD protein contents, although to different extents, both in BPH and PCA predict that the early events for progression from either normal to BPH or normal to PCA are similar.

INTRODUCTION

The prostate gland is the most common site of neoplastic disorders in men. The mechanisms of benign and malignant growth of the prostate cannot be ascribed to genetic changes of the epithelial cells alone. Despite the magnitude of morbidity and mortality associated with this disease, very little is known regarding the mechanisms involved in prostate tumorigenesis. A variety of growth factors and their receptors, steroidal hormones and their receptors, proteases, and other factors are involved in normal prostatic morphogenesis and function, but their role in BPH and PCA remains poorly understood [1, 6, 9, 10, 13].

Currently, tumor grade and stage are used to estimate prognosis. Despite modern clinical and pathological techniques, there may be a wide

variation in the behavior of tumors of a given stage and grade. These reasons underscore the necessity to develop better prognostic methods in individual patients and to search for new markers related to malignant phenotype [2, 3].

It is interested in exploring the nature of human prostatic cellular proteins with the aim of identifying any differences therein that may occur in pathologies such as BPH and PCA. Previously the specificity of nuclear protein changes accompanying human prostatic hyperplasia and malignancy was identified [4]. The purpose of the present studies was to determine the pattern of cytoplasma proteins in human prostate neoplasms.

MATERIALS AND METHODS

Fresh-frozen samples of normal prostate, benign prostatic hyperplasia and prostatic carcinoma were obtained either by transurethral resection or radical prostatectomy. The specimens were collected immediately after surgery and frozen at -70°C. The report of pathological evaluation was obtained for each case. A total 31 samples, twelve from normal prostate, twelve from BPH and seven from PCA (adenocarcinoma prostatae with stage C and D) were used.

Cellular fractionation

Cytosol and plasma membrane fraction were obtained from tissue homogenate after nuclei removing. Briefly, the samples of normal or neoplastic material were minced by fine dissection and homogenized in 10 volumes of 0.25 M sucrose, 5 mM $MgCl_2$, 0.5% Triton X-100, 1 mM phenylmethylsulphonyl fluoride (PMSF) and 50 mM Tris-HCl, pH 7.4 with a motor driven Potter homogenizer. The efficiency of homogenization was monitored by phase microscopy. The homogenate was spun down at $800 \times g$ for 10 min to remove nuclei. The centrifugation was repeated to pellet any remaining nuclei and the supernatant was centrifuged at 100,000 x g for 60 min to separate plasma membrane organelles from cytosol fraction.

One-dimensional SDS-polyacrylamide gel electrophoresis

Samples (4 mg protein/1 ml) were mixed with 1 vol of 4% SDS, 10% 2-mercaptoethanol, 10% glycerol, 125 mM Tris-HCl, pH 6.8 and boiled

for 5 min. Electrophoresis was performed in polyacrylamide slab gels containing 8% acrylamide (pH 8.8) and 0.1% SDS with stacking gel (pH 6.8) according to Laemmli [7], and 50 μg of proteins were loaded per lane. Electrophoresis was carried out at 60 V in the stacking gel and then 120 V until the marker dye front reached the bottom of the separating gel. The gel slabs were stained using silver nitrate according to Wray et al., [14]. The molecular weight of protein bands were calculated by comparison with the mobilities of the standard proteins (Sigma Chemical Co.): myosin (205 kD), β-galactosidase (116 kD), phosphorylase b (97.4 kD), albumin (66 kD), ovalbumin (45 kD), carbonic anhydrase (29 kD), soybean trypsin inhibitor (20.1 kD), α-lactalbumin (14.2 kD).

Quantitative estimations of electropherograms

For quantitative analysis of protein band density a video densitometer (Biotec-Fischer, Germany) and software Gel-Pro® Analyzer (Media Cybernetics, USA) were used.

Analytical procedures

Protein was estimated using bovine serum albumin as standard by means of the modified Lowry procedure [5].

RESULTS

Cytosol and plasma membrane proteins from BPH and PCA were compared with normal prostatic tissue. The protein patterns were analyzed by one-dimensional SDS-PAGE in 8% gels followed by silver nitrate staining. As shown in Fig. 1 cytosol proteins of the examined tissues have in general many common protein constituents in molecular weight ranging from $\sim$20 to$\rangle$ 200 kD. However, they revealed some components which differed from the normal and neoplastic cells. It is interesting that protein profiles of normal and BPH tissue appeared to be relatively similar. No peculiar cytosol polypeptides were present or absent in BPH only compared with normal or PCA tissues but in PCA, when compared with normal and BPH tissues, the appearance of 32 kD component was observed. Moreover, in the molecular weight regions of 62 kD and 93 kD quantity changes

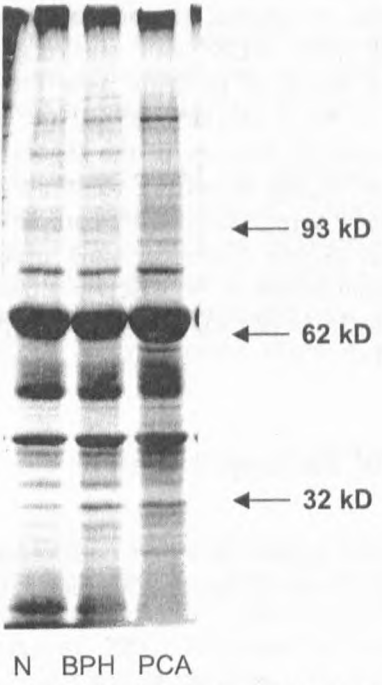

Fig. 1. Cytosol proteins electrophoretic pattern in 8% SDS-polyacrylamide gels of normal human prostate (N), benign prostatic hyperplasia (BPH) and prostatic carcinoma (PCA). About 50 μg of each protein samples per lane were loaded

were noticed. In the plasma membrane fractions obtained from normal, BPH and PCA tissues, no qualitative and quantitative changes in proteins pattern were found (data not shown).

The cytosol protein phenotypes were further explored by quantitative analysis of protein band densities using video densitometer and software Gel-Pro® Analyzer. Figure 2 shows the histograms for components in which the major quantitative variabilities were indicated. By means of quantitative analysis PCA was characterized by a significant increase of 62 kD and 93 kD proteins in comparison with normal and BPH tissues. The abundance of the 62 kD component ascended progressively from normal (hardly anything) to PCA tissues were its level appeared to be ninefold higher than in BPH. In the case of 93 kD protein eightfold and fivefold enrichment was observed in PCA in comparison with normal and BPH tissues, respectively.

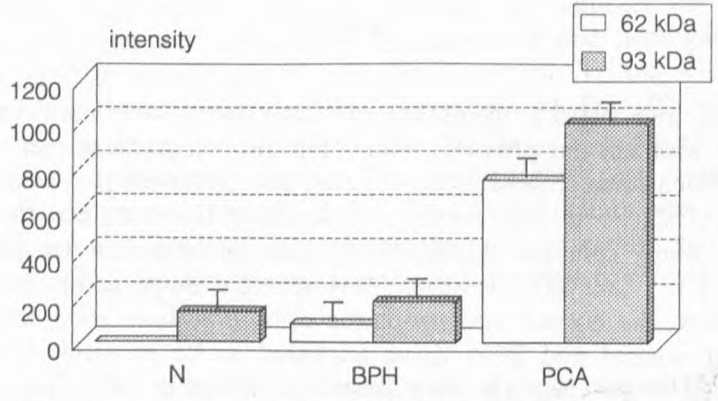

Fig. 2. Quantitative analysis of selected cytosol protein band densities of normal human prostate (N; n=12), benign prostatic hyperplasia (BPH; n=12) and prostatic carcinoma (PCA; n=7). The data are expressed as the mean ±SD

DISCUSSION

The present results indicate that a significant increase of 32 kD cytosol protein expression in PCA tissue may be specific for the malignant phenotype. However, the low number of cancer samples investigated and the degree of differentiation could have a bearing on the results. No unique cytosol or plasma membrane protein(s) for BPH, which were present in BPH only but were absent in BPH, was observed.

BPH and PCA are often indicated in prostate specimens. BPH is the most frequent benign condition found in the prostate and there are some similarities between PCA and BPH. Both require androgenic stimulation, show increased provalence with age, may co-exist and may respond to androgen deprivation. Most PCAs arise in prostate which already have BPH [8].

Two different models can be postulated for the progression of normal prostatic epithelial cells to either benign prostatic hyperplasia or prostate cancer. The first model predicts that the early events for progression from either normal to BPH or normal to PCA are similar. The second model predicts that progression for BPH and cancer undergo different events [11].

Quality and quantity changes identifying among cytosol proteins seem to confirm the first model. The quantitative variabilities of 62 kD and 93 kD components, both in BPH and PCA may suggest that BPH shares many of the cytosol protein changes observed in PCA. The specific behavior of 32 kD cytosol protein is also consistent with the first model. These observations are in agreement with our work on nuclear proteins and the work of Partin et al. [12] on nuclear matrix proteins from human prostatic neoplasms which indicated that similar phenotypic expressions were occuring in the nuclear proteins of cells progressing to BPH as in those cells progressing to prostate cancer.

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BIAŁKA CYTOPLAZMATYCZNE NOWOTWORÓW GRUCZOŁU KROKOWEGO CZŁOWIEKA

Białka frakcji cytosolowej oraz frakcji błon plazmatycznych komórek łagodnego rozrostu stercza oraz raka tego gruczołu analizowano za pomocą jednokierunkowej elektroforezy w żelu poliakryloamidowym z SDS (PAGE-SDS). Prawidłową tkankę stercza, łagodny rozrost (BPH ang. *benign prostatic hyperplasia*) oraz raka prostaty (PCA, ang. *prostatic carcinoma*) pozyskiwano w wyniku częściowej elektroresekcji przezcewkowej lub całkowitej prostatektomii. Rozdziały elektroforetyczne białek wybarwiano metodą srebrową a następnie analizowano za pomocą wideodensytometru i programu komputerowego Gel-Pro® Analyzer (Media Cybernetics, USA). Nie stwierdzono istotnych zmian jakościowych i/lub ilościowych w obrębie białek frakcji błon plazmatycznych badanych tkanek. We frakcji cytosolowej prawidłowego stercza, BPH i PCA zaobserwowano natomiast różnice natury zarówno jakościowej jak i ilościowej. Znaczący w przypadku raka prostaty, w porównaniu z tkanką prawidłową i BPH, wzrost ekspresji białka 32 kD wydaje się być specyficzny dla nowotworu złośliwego. Z kolei zmiany ilościowe białek 62 kD i 93 kD stwierdzone w BPH jak i PCA sugerują podobny przebieg wczesnych etapów karcynogenezy obu wymienionych nowotworów.