EXPRESSION OF EPIDERMAL PROTEINS RESEMBLING CYTOKERATIN No. 2 IN HUMAN PROSTATIC EPITHELIAL CELL CULTURES

Yu. M. Bozhok and L. V. Tavokina

UDC 616.65-018.7-008.939.6]-092.4

KEY WORDS: differentiation; cytokeratins; prostate gland; tissue culture

The high frequency of malignant neoplasms of the human prostate, which is one of the main causes of male death [8], necessitates an experimental study of the mechanisms of differentiation and malignant transformation of the cells of this gland. The writers have suggested a model [1] for use in such investigations, which consists of undifferentiated cells of the primordial prostatic epithelium (PPE) of human fetuses and of cells of the definitive prostatic epithelium (DPE) which have completed differentiation.

The aim of this investigation was to discover and study the properties of proteins whose expression in our cultural system is characteristic of DPE cells but not of PPE cells. Information of this sort is essential for the study of the molecular mechanisms controlling differential gene activity in the cells of this model.

EXPERIMENTAL METHOD

PPE freed from stroma and cultures of it were obtained by methods [5, 6] from the prostatic part of the urogenital sinus of 32 male human fetuses at 10-12 weeks of development (therapeutic abortion). The source of DPE consisted of prostate glands with nodular hyperplasia, removed surgically from 28 patients. It was possible to use foci of nodular hyperplasia to obtain DPE because their epithelium in situ preserves the morphological and biochemical features of differentiation characteristic of the secretory epithelium of the normal gland [2, 4]. DPE free from stroma was obtained by Lasfargues' method [6, 10], adapted for prostatic epithelium, and cultures of DPE were obtained by Webber's method [15]. No proteolytic enzymes are used in any of the methods listed above.

Cultures of DPE and PPE were grown under identical conditions: in plastic dishes ("Falcon," USA), covered with rat tail collagen, in medium RPMI-1640 with 20% fetal calf serum ("Gibco," USA; "Serva," West Germany) at 37.5°C with 5% CO_2 in the gaseous phase.

Electrophoresis of total cell proteins or of individual fractions was carried out in vertical slabs of 10% polyacrylamide gel (PAG) in the presence of sodium dodecylsulfate (SDS) at pH 7.4. For isoelectric focusing, horizontal slabs of 5% PAG with 3% ampholines, pH 3.5-10 (LKB, Sweden), 7 M urea, 10% glycerol, 2% NP-40, and 5% β-mercaptoethanol ("Sigma," USA), were used in the second direction. The gels were stained with Serva Blue R-250. Densitograms were recorded on the ERI 65m instrument ("Carl Zeiss," East Germany).

Peptide maps of the proteins to be compared were obtained in situ by the methods of thermal cleavage at the Asp-Pro site [14] and cleavage at the Trp-X site [11] with the use of N-bromosuccinimide ("Sigma," USA). The peptides formed were detected by silver impregnation of the gels.

Samples of tissues, cultures, and proteins obtained from different fetuses or patients were analyzed individually in parallel tracks.

Many of the reagents and materials for the investigations were provided by the Small Supplies Program of the Department of Human Reproduction of the World Health Organization.

Laboratory of Neurohormonal Regulation of Reproduction, Research Institute of Endocrinology and Metabolism, Ministry of Health of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR, I. B. Zvarskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 7, pp. 88-90, July, 1989. Original article submitted July 22, 1988.

 0007-4888/89/0007-1008$12.50

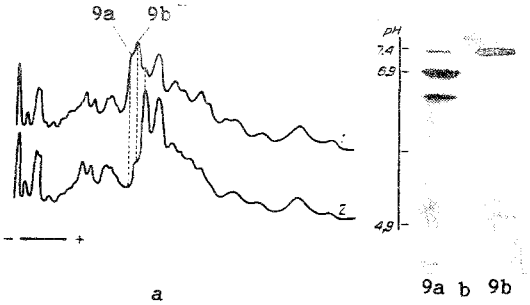

Fig. 1. Protein peaks of bands 9a and 9b on densitograms of gels in the first direction (PAG-SDS) (a) and results of isoelectric focusing of these proteins in gel in the second direction (b). 1) Extinction of electrophoretogram of proteins of DPE culture; 2) the same, of PPE culture. Here and in Fig. 2: 9a, 9b) standard for bands.

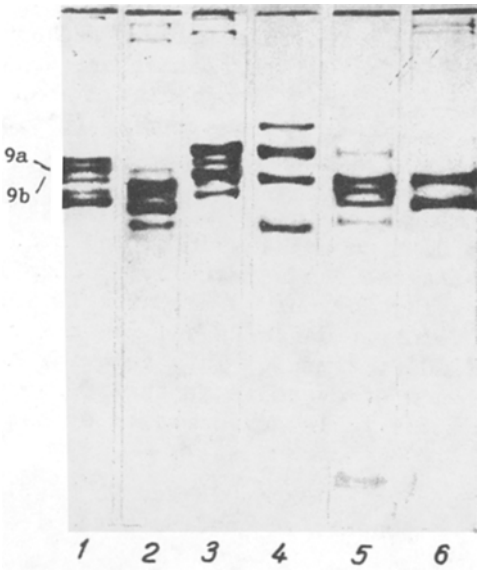

Fig. 2. PAG-SDS electrophoresis of cytokeratin preparations from different cells. 1) Cultures of DPE; 2) cultures of PPE; 3) epidermis of forearm; 4) mol. wt. markers (from top to bottom: transferrin, bovine serum albumin, catalase, ovalbumin); 5) PPE; 6) DPE.

EXPERIMENTAL RESULTS

Differences in the region of mol. wt. 63-65 kD were constantly observed between spectra of total proteins of cultured DPE and PPE cells obtained after electrophoresis in 10% PAG with SDS (Fig. 1a). Electrophoretograms of cultures of DPE were characterized by the presence of two intense bands: 9a (mol. wt. 65 kD) and 9b (mol. wt. 63 kD). On electrophoresis of cultures of PPE the band 9a was absent, and band 9b was palely stained. Calculations based on densitograms showed that bands 9a and b together accounted for up to 8-10% of the total protein of the DPE cells.

To continue the analysis of the proteins of these bands, the gels were developed unfixed after electrophoresis in 5 M sodium acetate with 5% β-mercaptoethanol. The protein bands were cut out under a stereomicroscope and washed in 5% β-mercaptoethanol. Proteins of bands 9a and 9b, isolated in this way and incorporated in PAG were insoluble in water, 5% β-mercaptoethanol, and 2% NP-40, but they were soluble in 1% SDS or a mixture of 7 M urea, 2% NP-40, and 5% β-mercaptoethanol. The protein nature of these bands was confirmed by digestion with trypsin and pronase. No polysaccharides could be found in the composition of bands 9a and 9b, using the standard method with periodic acid and Schiff's reagent.

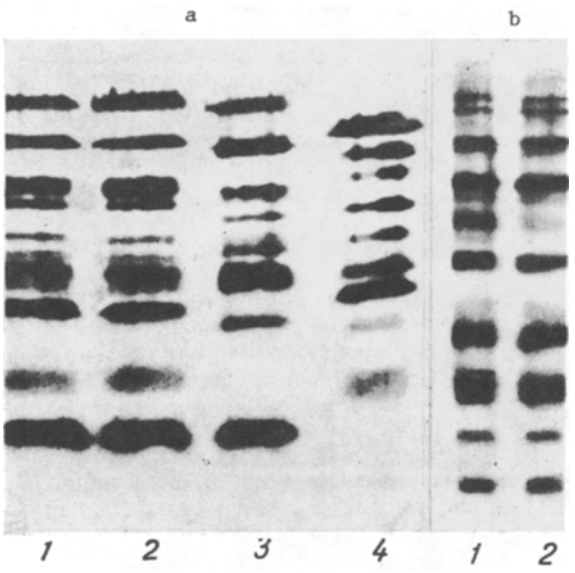

Fig. 3. Peptide maps in situ obtained by methods of cleavage at Asp-Pro (a) and Trp-X (b). 1) Epidermal protein with mol. wt. of 65 kD; 2) protein of band 9a; 3) protein of band 9b; 4) protein of band 12 of DPE cultures. Arrows on maps (b) indicate peptides varying in intensity of staining.

If fragments of PAG containing proteins of bands 9a and 9b, washed in water, were incubated for 1.5 h in 7 M urea in 0.05 M phosphate buffer, pH 5.65 (22°C) in the absence of β-mercaptoethanol, the proteins of these bands lost their solubility in SDS and their electrophoretic mobility even after heating for only 2 min with 1% SDS to 100°C. The solubility and electrophoretic mobility of proteins of bands 9a and 9b were restored after treatment with 5% β-mercaptoethanol with 1% SDS (100°C, 2 min). This behavior of the proteins tested may perhaps be connected with the presence of cysteine in their composition, facilitating cross-linkage through the SS-bond, given a favorable conformation of the protein molecules acquired by them in acid solutions of urea.

Isoelectric focusing of proteins of bands 9a and 9b, isolated after PAG-SDS electrophoresis, showed that the principal components of band 9a have pI of 7 and 6.6, and proteins of band 9b have pI of 7.5 (Fig. 1b).

Proteins of bands 9a and 9b differ in their mol. wt. values from known markers of differentiation of human prostatic epithelium in situ: prostatic acid phosphatase (mol. wt. 102 kD, subunits 50 kD), specific prostate antigen (mol. wt. 35 kD), and prostatic binding protein (mol. wt. 54 kD). Meanwhile, proteins of bands 9a and 9b are similar in mol. wt., pI, and a high content in these cells, with certain of the cytokeratins — a group of intermediate filament proteins characteristic of epithelial cells and possessing marked organ specificity [12]. In fact we found that proteins of bands 9a and 9b can be obtained from DPE cultures together with other cytokeratins by modifying the standard procedure for isolation of cytokeratins [9] by successive extraction of the cells with Triton X-100 and 1.5 M KCl (Fig. 2).

Of the two bands studied, it was the proteins of band 9a that were most interesting because they are virtually absent in cultured PPE cells and the cytokeratin fraction isolated from them (Fig. 2). It follows from the data in a catalogue of human cytokeratins [12] that cytokeratin No. 2 (mol. wt. 65 kD), belonging to the subgroup of basic cytokeratins, is closest to proteins of band 9a in the value of their mol. wt. This cytokeratin is expressed in man in small quantities in the epidermis of the skin, the epithelium of the anal canal, the exocervix and upper third of the vagina [7, 12]. It is not found in the prostatic epithelium of adult men [3]. We likewise found no significant amounts of protein in the region of mol. wt. 64-66 kD on the electrophoretograms of cytokeratin fractions of uncultured DPE and PPE cells (Fig. 2). Meanwhile, the fraction of cytokeratins isolated from fragments of forearm skin from volunteers, as might be expected, contained a protein band coinciding in position in the gel with the 9a band of DPE cultures (Fig. 2). In the value of mol. wt. (65 kD) and relative content in the cytokeratin fraction, this protein (or group of proteins) of the skin is similar

to cytokeratin No. 2. It is located in the epidermis, for it was not found on electrophoretograms of connective-tissue samples of the same fragments of forearm skin from which the cytokeratin fraction was obtained.

The epidermal protein with mol. wt. of 65 kD from forearm skin, like the band 9a proteins, is immobilized in PAG as a result of treatment, by the method described above, with an acid solution of urea and its solubility is restored by heating with 5% β-mercaptoethanol in the presence of 1% SDS.

Band 9a proteins of DPE cultures and epidermal protein with mol. wt. 65 kD, isolated from the cytokeratin fractions and incorporated in PAG, were analyzed by two methods of peptide mapping in situ. The peptide maps of these proteins, obtained by the thermal degradation method, coincided completely with respect to all peptides formed (Fig. 3a). Band 9b proteins used as the control or certain others gave different patterns of distribution of the peptides in the gel. Peptide maps of band 9a proteins and of epidermal protein with mol. wt. 65 kD, obtained with the aid of N-bromosuccinimide, coincided in number and position of the 14 peptides formed, but differed in intensity of silver staining of two of them (Fig. 3b). This latter phenomenon may be due to phosphorylation of one of the proteins compared, which is often observed among cytokeratins.

These investigations thus showed that DPE cells in culture, unlike PPE cells, synthesize large quantities of proteins which are extremely similar to an epidermal protein (or proteins) with mol. wt. of 65 kD, previously identified as cytokeratin No. 2. Final identification of the bound 9a proteins is not yet possible because of the absence of antibodies specific for cytokeratin No. 2. However, the fact that such a strange manifestation of differentiation of prostatic cells in vitro as expression of an epidermal protein with mol. wt. of 65 kD, not characteristic of it in situ, itself makes our suggested model interesting for the study of mechanisms controlling cell differentiation. Band 9a proteins are probably unique markers of DPE cells in culture, for proteins with mol. wt. 65-68 kD are not found in cytokeratin fractions of monolayer cultures of human epithelial cells of a different origin, including epidermal cells [13].

LITERATURE CITED

1. Yu. M. Bozhok, S. S. Kireeva, and S. V. Varga, Éksp. Onkol., No. 1, 15 (1985).
2. V. V. Vyalik and V. G. Pinchuk, Pathological Anatomy and Ultrastructure of Nodular Hyperplasia and Carcinoma of the Prostate Gland [in Russian], Kiev (1977).
3. T. Achtstatter, R. Moll, B. Moore, et al., J. Histochem. Cytochem., 33, 415 (1985).
4. G. Aumüller, C. Pohl, R. L. Etten, et al., Virchows Arch. Abt. B Zellpathol., 35, 249 (1981).
5. Yu. M. Bozhok and L. V. Dziuba, Prostate, 3, 1 (1982).
6. Yu. M. Bozhok, S. S. Kireeva, and L. V. Tavokina, Prostate, 8, 221 (1986).
7. B. Czernobilsky, R. Moll, W. W. Franke, et al., Pathol. Res. Pract., 179, 31 (1984).
8. M. J. Droller, Urol. Clin. N. Am., 7, 579 (1980).
9. W. W. Franke, H. Denk, R. Kalt, et al., Exp. Cell Res., 131, 299 (1981).
10. E. Lasfargues and L. Ozzelo, J. Natl. Cancer Inst., 21, 1131 (1958).
11. M. A. Lischwe and D. Ochs, Anal. Biochem., 127, 453 (1982).
12. R. Moll, W. W. Franke, D. L. Schiller, et al., Cell, 31, 11 (1982).
13. W. G. Nelson and T.-T. Sun, J. Cell Biol., 97, 244 (1983).
14. J. Rittenhouse and F. Marcus, Anal. Biochem., 138, 442 (1984).
15. M. M. Webber and O. G. Stonington, J. Urol. (Baltimore), 114, 246 (1975).