Upsala J Med Sci 104: 199–206, 1999

Expression of Prostasome-like Granules by the Prostate Cancer Cell Lines PC3, Du145 and LnCaP Grown in Monolayer

B. Ove Nilsson,[1] Lena Lennartsson,[2] Lena Carlsson,[3] Sten Nilsson[2] and Gunnar Ronquist[3]

From the [1]Department of Cell Biology, [2]Department of Oncology and [3]Department of Clinical Laboratory Sciences, University of Uppsala, Sweden

ABSTRACT

Prostasomes are a granular type of secretory product in the human prostate gland cells. It is not known, whether *in vitro* grown cells derived from human prostate cancers also express prostate secretory components containing granules with properties similar to the prostasomes. Therefore, we carried out the present investigation and found that cytospins of *in vitro* grown PC3, DU145 and LNCaP cells generally expressed a granular secretion. DU145 demonstrated the highest ratio of cells with granules (about 90%), while cytospins of PC3 and LNCaP contained less stained cells (50-70%). Purified granules from PC3 cells were immunoreactive with a monoclonal antibody (mAb78) originally raised against human seminal prostasomes. The PC3 granules also shared the property with human seminal prostasomes having an elevated UV260/UV280 absorbance ratio. On the other hand we found a low aminopeptidase activity in PC3 granules contrary to that of human prostasomes. Prostasomes may form a heterogeneous group with different properties due to the source from which they are isolated and perhaps it is justified to recognize them as different members of a prostasome family.

INTRODUCTION

Prostasomes are a granular type of secretory product in the prostate gland cells (2, 10). Immunostaining of the prostate epithelium with anti-prostasome antibodies displays a secretion, which is assumed to be related to the prostasomes (7, 8). When the normal prostate epithelium passes a neoplastic transformation, the secretory activity of the cells decreases and at high Gleason grades, the immunostained secretion disappears (6). As expected, the amount of secretions in the cytoplasm of the transforming prostate cells indicates their degree of differentiation.

Experimental studies of neoplastic prostate cells are often performed on *in vitro* grown cell lines, established from metastases of prostate cancers (5, 13, 14) Among the cell lines available, the most commonly used are PC3 (4), Du145 (11) and LNCaP (3). The cell lines can be applied, for instance, for evaluating whether the differentiation of the cells are influenced by some experimental condition. To judge the degree of cellular differentiation, various indicators are used, like the expression of prostate-specific antigen or androgen receptors (13).

It is not known, however, whether the *in vitro* grown cells also express prostate secretory components and whether these can be visualized by, for instance, the anti-prostasome antibodies used for detecting secretions in the cells of the prostate gland. Therefore, we immunostained cytospin specimens from monolayers of the cancer cell lines mentioned with the anti-prostasome antibody mAb78 (7, 15). Since we observed that the cells contained secretory components, we analyzed the PC3 cells to find out whether they contained granules which exhibited properties similar to the prostasomes. The procedures applied and the results obtained are reported in the present contribution.

MATERIALS AND METHODS

Growing of the cell lines PC3, DU145, and LNCaP

The human prostatic carcinoma cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). Cells were maintained in RPMI 1140 cell culture medium containing 5 μg/mL phenol red and supplemented with 10% heat-inactivated fetal calf serum, 1% glutamine, 100 μg/mL penicillin and 100 μg/mL streptomycin.

The cells were grown in Falcon Petri dishes (100 mm) at 37°C in humidified atmosphere of 5% CO_2 in air. When the cells reached 90% confluence, they were treated with trypsin-EDTA and recovered by gently scraping. Each plate yielded 2-3x10^6 cells. The cells were carefully rinsed by two centrifugations at 2000xg for 3 min.

Some of the cells were resuspended in isotonic Tris-HCl buffer and frozen at -70°C. Some other resuspended cells were prepared by cytospin on glass slides using a concentration of 5x10^4 cells/100 μL. The slides were air-dried and prepared for immunohistochemistry without delay.

The following protocol, rinsings excluded, were used for immunostaining.

Blocking with 3% BSA and 1% normal horse serum in PBS for 30 min.

Primary antibody, being undiluted mouse monoclonal IgG1 18:78 supernatant for 60 min.

Secondary antibody, being biotinylated horse anti-mouse IgG, diluted 1:200 (Sigma) for 60 min.

Alkaline phosphatase complex (Dako Pat) for 30 min.

Substrate Vector Red (Vector Laboratories, Burlingame, CA) or DAB for appropriate times.

Positive controls were sections of human prostate gland and negative controls were obtained by omitting the primary antibody.

Preparation of PC3 granules

Frozen PC3 cells from 15-20 dishes were thawed and pooled, and the suspension of disintegrated cells was centrifuged at 1500xg for 30 min. The supernatant obtained was subjected to a second centrifugation at 6000xg for 20 min. The new supernatant, containing PC3 granules, was ultracentrifuged at 100,000xg for 2 h. The pelleted material was suspended in isotonic Tris-HCl buffer and run through a Sephadex G200 column (Pharmacia AB, Uppsala, Sweden) at 6 mL/h, and 2 mL fractions were collected. The isotonic Tris-HCl buffer was the eluant, and the eluate was read at 260 and 280 nm. The fractions (5-12) with initially elevated UV absorbance and with a positive reaction on the anti-prostasome mAb 78 were pooled and ultracentrifuged at 100,000xg for 2 h. Isotonic Tris-HCl buffer was added to the pellet, and the resulting mixture was regarded as a suspension of PC3 granules.

Aminopeptidase assay

The aminopeptidase assay was performed mainly according to a previous investigation (9); namely, 5 mg of the [$Suc(ala)_3pNA$] substrate was dissolved in 11 mL 0.2 mol/L Tris-HCl buffer (pH 8.0). A 10-100 μL sample was added to 1 mL of the substrate solution, and the absorbance read at 410 nm at 0, 5, 10 and 20 min of incubation time. Enzyme activity was expressed in Δ-absorbance units at 410 nm/min/mL (25°C).

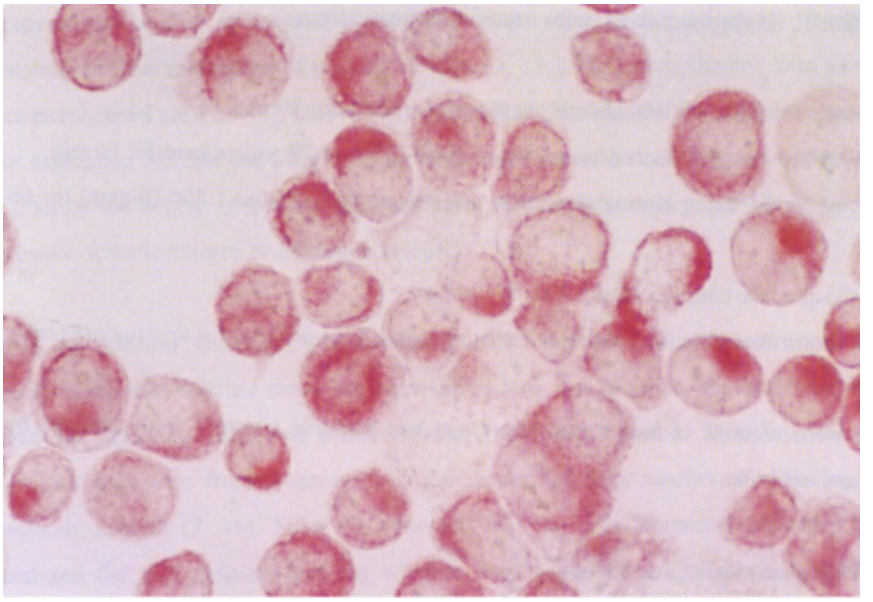

Fig.1. Cytospin of PC3 cells immunostained with mAb78. Cytoplasmic components of the cells demonstrate a granular staining which is irregularly distributed. Some cells do not contain any stained structures. Mag. 270x.

RESULTS

Immunostaining of PC3, DU145 and LNCaP cytospin cells

The general morphology of the growing monolayers was similar to that reported by others (3, 4, 11). Trypsinized monolayers prepared as cytospin specimens demonstrated round cells of various sizes. When the cells were immunostained for detecting secretory components we found that all three cell lines expressed secretory components. Most cells expressed a granular secretion but scattered cells or small groups of cells contained no or only few granules (Fig. 1). Among the cell lines tested, DU145 demonstrated the highest ratio of cells with granules (about 90%), while cytospins of PC3 and LNCaP contained less stained cells (50-70%).

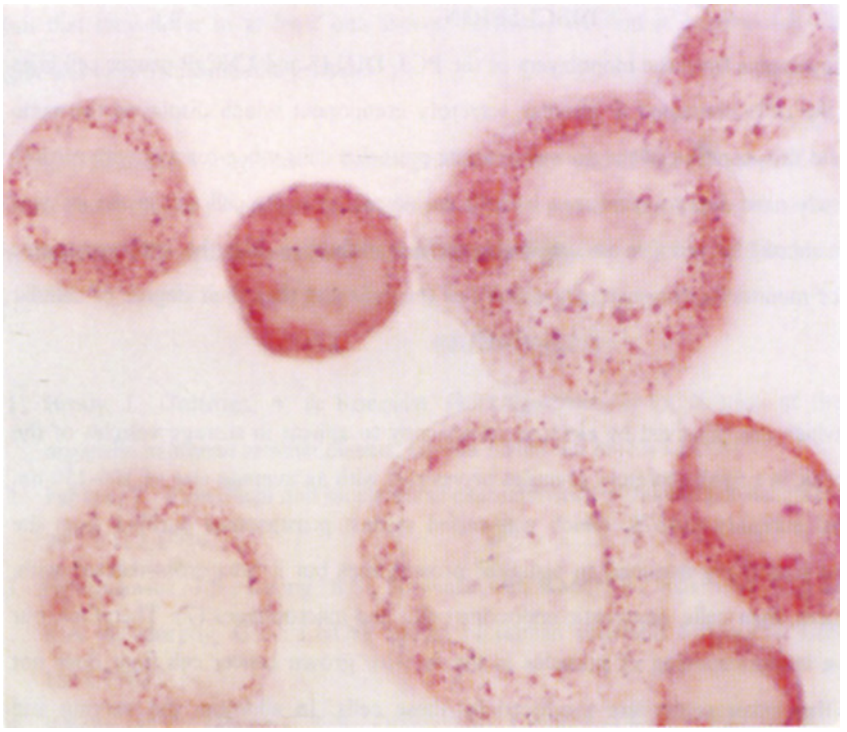

Fig.2. Cytospin of PC3 cells immunostained with mAb78. In the cytoplasm, many positively stained granules are observed. The nuclei are unstained. Mag. 710x.

The size of the granules varied, most of them had a diameter of 200-500 nm, but some had a diameter of 1 um (Fig. 2). The number of cytoplasmic granules varied among the cells from nearly filling the whole space between the nucleus and the cell membrane to being localized at some small region of the cells.

Biochemical features of PC3 granules

The granules appeared in the same position in the chromatogram of Sephadex G200 as did human seminal prostasomes (2). The PC3 granules as well as the human seminal prostasomes demonstrated an elevated UV absorbance at 260 nm in relation to that of 280 nm. However, the rather high aminopeptidase activity in human seminal prostasomes (9) was exchanged for a low activity in the PC-3 granules.

DISCUSSION

Immunostaining of cytospins from the monolayers of the PC3, DU145 and LNCaP cancer cell lines demonstrated that the cells contained a granular secretory component which displayed the same antigen epitope as did the secretion of the normal prostate epithelial cells when reacted with mAb78 (6). Since the antibody used detects secretions in both the normal prostate cell and in the *in vitro* grown cell lines, it should be possible to use anti-secretion antibodies, like the anti-prostasome antibody mAb78, for monitoring the secretory activity of the cells and thus their degree of cellular differentiation.

The prostasomes, which are observed by electron microscopy to appear in storage vesicles of the prostate epithelial cells, are small secretory granules or vesicles with an average size of 100-150 nm (1). The monoclonal antibody mAb78, which was raised against prostasomes purified from the seminal plasma, was tentatively assumed to visualize prostasomes but it also cross-reacted with, among other structures, liver cells, pancreatic endocrine cells, and macrophages (7). Therefore, our finding of a positive immunostaining of granules in the *in vitro* grown cancer cell lines does not neccessarily imply that prostasomes are produced by these cells. In addition, the varying and comparatively large size of the stained components in the cytospin cells suggests that the mAb78 also recognized additional secretory components of the cells, perhaps also a precursor to a granular component. Therefore, we grew PC3 cells and used them as a source for purification and biochemical characterization of their granules to find out whether the granules had some properties in common with human prostasomes.

The analyses of the PC3 granular material obtained after preparative ultracentrifugation and chromatography revealed that the preparation was immunoreactive with mAb78 which was raised against human seminal prostasomes. The PC3 granules also shared the property with human seminal prostasomes having an elevated UV260/UV280 absorbance ratio. Further, PC3 prostasome-like granules promoted the forward motility of human spermatozoa, that is, they had similar functional effects on sperm cells as have human seminal prostasomes (12). On the other hand, we found a low aminopeptidase activity in the PC3 granules. This is dissimilar to human seminal prostasomes indicating that the PC3 granules have some properties in common with human seminal prostasomes

but that they differ in at least one aspect. Anyhow, we find it justified to characterize the PC3 granules as prostasome-like granules.

Thus, prostasomes may form a heterogeneous group with different properties due to the source from which they are isolated. Therefore we assume that there exists a prostasome family which as yet includes PC3 prostasomes, native prostasomes from the prostatic fluid and seminal prostasomes.

REFERENCES

1. Brody, I., Gottfries, A. & Ronquist, G.: Ultrastructural localization of the prostasome - an organelle in human seminal plasma. Upsala J Med Sci 88:63-80,1983.
2. Fabiani, R.: Functional and biochemical characteristics of human prostasomes. Upsala J Med Sci 99:73-112,1994.
3. Horoszewicz, J.S., Leong, S.S., Kawinski, E., Karr, J.P., Rosenthal, H., Chu, T.M., Mirand, E.A. & Murphy, G.P.: LNCaP model of human prostatic carcinoma. Cancer Res 43:1809-1818,1983.
4. Kaighn, M.E., Narayan, K.S., Ohnuki, Y., Lechner, J.F. & Jones, L.W.: Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Invest Urol 17:16-23,1979.
5. Murphy, G.P. (1980): Models for prostate cancer. Alan R. Liss, Inc., New York.
6. Nilsson, B.O., Egevad, L., Jin, M., Ronquist, G. & Busch, C.: Distribution of prostasomes in neoplastic epithelial prostate cells. Prostate 39:36-40,1999.
7. Nilsson, B.O., Jin, M., Einarsson, B., Persson, B.-E. & Ronquist, G.: Monoclonal antibodies against human prostasomes. Prostate 35:178-184,1998.
8. Nilsson, B.O., Jin, M. & Ronquist, G.: Immunolocalization of prostasomes in the human prostate. Upsala J Med Sci 101:149-158,1996.
9. Ronquist, G.: Zinc-ion stimulation of ATP cleavage by prostasomes from human seminal plasma. Urol int 43:334-340, 1988.
10. Ronquist, G. & Brody, I.: The prostasome: Its secretion and function in man. Biochim Biophys Acta 822:203-218,1985.
11. Stone, K.R., Mickey, D.D., Wunderli, H., Mickey, G.H. & Paulson, D.F.: Isolation of a human prostate carcinoma cell line (DU 145). Int J Cancer 21:274-281,1978.

12. Wang, J., Lundqvist, M., Carlsson, L., Nilsson, B.O., Lundkvist, Ö. and Ronquist, G.: Prostasome-like granules from the PC-3 prostate cancer cell line increase the motility of washed human spermatozoa and adhere to the sperm. Hum Reprod 2000.
13. Webber, M.M., Bello, D. & Quader, S.: Immortalized and tumorigenic adult human prostatic epithelial cell lines: Characteristics and applications Part I. Cell markers and immortalized nontumorigenic cell lines. Prostate 29:386-394,1996.
14. Webber, M.M., Bello, D. & Quader, S.: Immortalized and tumorigenic adult human prostatic epithelial cell lines: Characteristics and applications Part 2. Tumorigenic cell lines. Prostate 30:58-64,1997.
15. van Weerden, W.M., van Kreuningen, A., Elissen, N.M.J., Vermeij, M., de Jong, F.H., van Steenbrugge, G.J. & Schröder, F.H.: Castration-induced changes in morphology, androgen levels, and proliferative activity of human prostate cancer tissue grown in athymic nude mice. Prostate 23:149-164,1993.

Adress for reprints: B. Ove Nilsson
Section of Human Anatomy
Biomedical center
Box 571
SE-751 23 UPPSALA, Sweden