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Secretion of prostatic specific antigen, proliferative activity and androgen response in epithelial–stromal co-cultures from human prostate carcinoma

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Summary

We investigate the proliferative activity, prostatic specific antigen (PSA) secretion, morphology and androgen response of human prostate tumour epithelial cells co-cultured with stromal cells in a bicameral system. Stromal and epithelial cells were isolated from prostate adenocarcinoma by enzyme digestion and cultured in defined media. Immunocytochemistry for prostate carcinoma tumour antigen (PCTA-1) was performed for culture purity evaluation. Also, the morphology of the epithelial cells in co-culture was evaluated by electron microscopy. PSA was determined by microparticle enzyme immunoassay (MEIA) automatized protocol and the proliferation was evaluated by a commercial spectrophotometric kit, based on formazan salt formation. Both cell cultures showed more than 90% of purity. The epithelial cell co-cultures showed marked membrane processes and cell interdigitations. The proliferative activity of the epithelial cells was increased in presence of stromal cells. Also, PSA secretion was significantly increased and maintained for at least 14 days, whereas the androgen response for PSA secretion was evidenced only in co-culture condition. Primary co-cultures of epithelial and stromal cells from human prostate carcinoma are able to maintain, for a prolonged time, proliferative and secretory properties as well hormone response, and represent a valuable tool for cellular and molecular studies on prostate cancer.

Keywords: prostate cancer, paracrine regulation, primary cell culture, PSA secretion

Introduction

Prostate adenocarcinoma is an important leading cause of male cancer deaths worldwide. The tumour growth rate varies, and patient survival reaches 5 or more years after diagnosis. The aetiology of prostate cancer as well as benign hyperplasia (BHP) is not completely understood. Early detection improves prognosis, but in most cases the diagnosis is late, mainly because of absence of symptoms at the initial state of the pathology and lack of simple and confident methods for early detection (Garnick, 1993; Wilding, 1992; Gittes, 1991).

There is cumulative evidence that supports a paracrine interaction between different prostatic cell types (Cunha *et al.*, 2003). This interaction is believed to be essential for the normal function of the prostate (Hayward *et al.*, 1992;

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Sung & Chung, 2002). Generally, the prostate is organized in glandular acini with their respective ducts, surrounded by stromal cells. The prostate acini are constituted by luminal epithelial cells on a layer of aligned basal cells. Surrounding these basal cells there is a basement membrane, which separates acini from stroma. The luminal epithelial cells are highly polarized both morphologically and functionally showing a bidirectional secretory activity. Most products, especially prostatic specific antigen (PSA), are secreted mainly toward the glandular lumen (Rui & Purvis, 1988). The prostate adenocarcinoma is characterized by epithelial cell transformation, loss of normal epithelial organization and functional polarity. For this reason, PSA, among other products, is secreted toward basal compartment, increasing its plasmatic level. Also, there is an increase in the proliferative activity of the luminal epithelial cells and a significant decrease in the basal cell number. The degree of this disorganization can be classified by the Gleason score (Gleason & Mellinger, 1974). Later on, the basal membrane is disarranged and malignant epithelial cells invade the stromal tissue. At this point, the malignant cells can be transported by the blood stream and cause metastasis (Davies & Eaton, 1991; Gittes, 1991; Ruijter *et al.*, 1999). It has been observed that morphological and functional polarity of the luminal epithelial cells depend on the maintenance of tissue organization, specially the paracrine relationship between epithelial and stromal cells (Hayward *et al.*, 1992). Although, the epithelial cells are the main target for prostate carcinogenesis. Recent evidence suggests that the prostatic epithelium interact with its surrounding stroma in a hormone and temporally related manner, both in normal and pathological tissue (Sung & Chung, 2002). It has been shown that this cell interaction is determinant in invasive, tumorigenic and metastatic properties of prostate cancer cells. The specific paracrine signals between these two cell compartments are not known.

Recently, animal models have been used to induce experimental prostate carcinoma. Injecting malignant cells in immunosuppressed rats or mice cause experimental prostate cancer (Redding *et al.*, 1992; Jungwirth *et al.*, 1997). Another experimental model widely used in the last years is the culture of cell lines derived from human prostate adenocarcinoma. The most used cell lines are LNCaP-FGC (androgen dependent) and LNCaP-LNO, DU-145, and PC-3 (androgen independent). In these systems, the expression of different receptors and the effect of several hormones as well as the effect of some drugs have been studied (Limonta *et al.*, 1992; Dondi *et al.*, 1994; Moretti *et al.*, 1996). Unfortunately, most of these cell lines are usually from metastatic origin and may not represent the actual properties of the tumour cells. On the contrary, primary cultures and subcultures of epithelial cell from prostate carcinoma grow rapidly but secretory activities and hormone response decrease after few days. In the present work we studied PSA secretion, morphology, proliferative activity and hormone response in an established two-compartment co-culture system of epithelial and stromal cells from human prostate carcinoma.

Material and methods

Reagents

All reagents, unless otherwise indicated, were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Prostatic tissue

The prostatic tissue was obtained from patient undergoing radical prostatectomy for adenocarcinoma, derived from our Institutional Hospital. The tissue was received in sterile culture medium containing RNase inhibitors. Within 60 min, the samples were brought to the laboratory and, if present, soft hyperplasic tissue was separated from the harder malignant nodes. Some tissue sections were processed by routine histological technique for Gleason classification (Gleason & Mellinger, 1974). This step is very important because allows the actual classification of the sample used for cell isolation. In this study were included eight prostate samples with a Gleason score range of 4–6. This proposal was approved by Bioethics Committee of our Institution.

Isolation of epithelial and stromal cells from prostate carcinoma
Epithelial cells Small pieces of prostatic tissue (1 mm^3) were incubated during 45 min at 37 °C in culture medium to eliminate blood cells. After washing, the fragments were digested in collagenase (2.5 mg/mL), hyaluronidase (1 mg/mL) and deoxyribonuclease (0.01 mg/mL), for 2–3 h at 37 °C in a shaking water bath. Every 1 h, the dispersed stromal cells were separated from the digesting fragments, and pooled. After this enzymatic digestion, the resulting tight and large epithelial cell aggregates were washed with fresh culture medium and further digested in collagenase for another 8–12 h in the same conditions as before. The resulting small aggregates of prostatic cancer cells were mechanically dispersed, washed and plated in cell culture inserts with polyethylene terephthalate (PET) bottom, 1 μm pore size, 12-well format (Falcon, Becton Dickinson Co, NJ, USA) at a density of 0.5×10^6 cells/mL. The inside volume was 1.0 mL and the outside volume was 2.0 mL

Stromal cells The pooled stromal cells were washed and plated on 12-well culture plates (Falcon, Becton Dickinson Co., NJ, USA) at a density of 10^6 cell/mL in 2 mL of medium.

Cell co-cultures in bicameral system

Cell co-cultures from prostate carcinoma (epithelial and stromal cells) were established according to the method described by Janecki & Steinberger (1987). Briefly, the epithelial aggregates were plated on the insert chamber with translucent PET bottom impregnated with extracellular matrix (Matrigel) (the upper chamber of the bicameral

system) using supplemented Dulbecco's Modified Eagle's and HAM F-12 (1 : 1) media mixture. The stromal cells were plated on 12-well culture plates (the lower chamber of the bicameral system) impregnated with extracellular matrix using the same media (Fig. 1). During the first days of culture, the media were supplemented with 5% of foetal bovine serum (FBS) (Gibco, Invitrogen Co., CA, USA). After first medium change (2 or 3 days depending on monolayer confluence), the cell cultures were washed and fresh medium without FBS and supplemented with human transferrin 5 mg/L, insulin 2 mg/L, epidermal growth factor (EGF) 10 μg/L, vitamin A and E 200 μg/L, hydrocortisone 10 nM, sodium selenite 2 μg/L, and when indicated with dihydrotestosterone (DHT) 10 nM, was added. After first medium change, the epithelial and stromal cell cultures were set together, the media were collected every 48 h and the co-cultures maintained for 2 weeks.

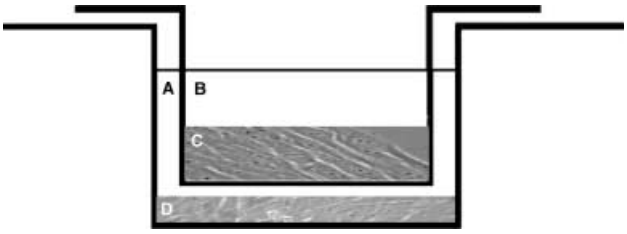

Figure 1. Schematic drawing of the bicameral co-culture system. The epithelial cells (C) were cultured on the insert chamber with translucent PET bottom (B) (the upper chamber of the bicameral system). The stromal cells (D) were cultured on regular culture plate (A) (the lower chamber of the bicameral system).

Morphological characterization of the cultures

Immunocytochemistry The cells were grown on coverslips, rinsed in PBS and fixed in paraformaldehyde 3% (Darmstadt, Merck, Darmstadt, Germany) – sucrose 2% in PBS for 30 min at room temperature. Then, the samples were blocked with PBS containing glycine 20 mM and bovine serum albumin 1% (BSA). The cellular composition of the different cultures was determined immunocytochemically using specific rabbit antibodies against prostate carcinoma tumour antigen (PCTA-1) (Sta Cruz Biotechnology Inc., CA, USA) (epithelial cells) and against vimentin (stromal cells) (Sta Cruz Biotechnology Inc., CA, USA). Then, antirabbit-FIT-C (Sta Cruz Biotechnology Inc., CA, USA) was used to evidence specific antibody binding. The cell cultures were evaluated on a Zeiss Fluorescence Microscope. PCTA-1 is a specific membrane antigen of prostate epithelial cells which is significantly overexpressed in malignant tumours (Su *et al.*, 1996).

Electron microscopy Epithelial cell cultures grown on cell culture inserts were rinsed in PBS and fixed at room temperature for 60 min in glutaraldehyde 4% in phosphate

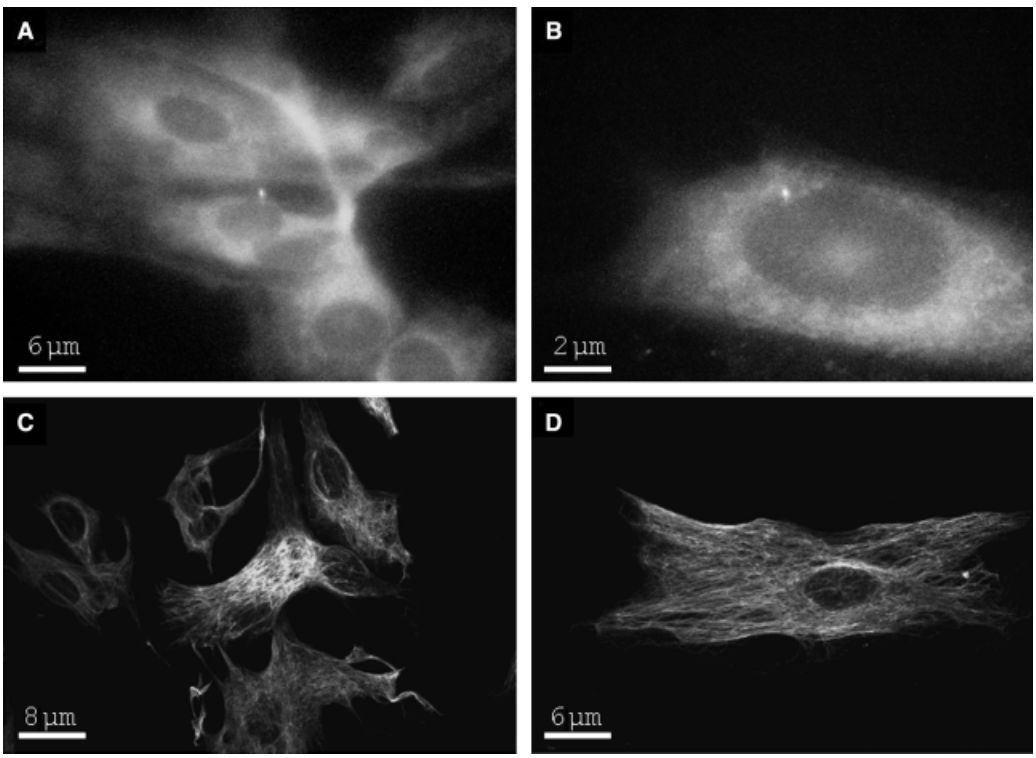

Figure 2. Immunocytochemistry of cell cultures. The cells were treated with rabbit antibodies against PCTA-1 (epithelial) and vimentin (stromal) and then, detected with FITC-conjugated anti-rabbit IgGs. (A, C) Low magnification showing most cells strongly stained. (B, D) High magnification showing the cell distribution of the fluorescence. Bars represent indicated size.

buffer 100 mM, pH 7.4. Then, they were washed several times with PBS and post-fixed in osmium tetroxide 1% (Merck). After dehydration with increasing concentration of ethanol and embedded in EPON 812, the cell morphology was evaluated on a Zeiss EM 109 electron microscope.

Prostatic specific antigen determination

Prostatic specific antigen (seminal glycoprotein from epithelial prostatic origin) was used as functional marker of prostatic epithelial cells. PSA was determined in conditioned culture media, using a commercial kit based on the biochemical principle of microparticles immunoanalysis (microparticle enzyme immunoassay; MEIA) (Abbott Laboratories, Abbott Park, Illinois, USA) (Sanchez de la Muela *et al.*, 1995). Briefly, specific PSA antibodies attached to a glass fibre matrix are bound to sample PSA and a second antibody conjugated with alkaline phosphatase is added. Finally, 4-methylumbelliferyl phosphate is used as a substrate and the fluorescent product is evaluated in automatized equipment (Axsym, Abbott Lab.). PSA standards of 4.0 ng/mL and 25 ng/mL were used as controls. Data are expressed as nanogram of PSA per milligram of protein in culture medium.

Determination of proliferative activity

The proliferative activity was assayed using a spectrophotometric assay based on mitochondrial dehydrogenases determination with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Carmichael *et al.*, 1987). Briefly, the mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple MTT formazan crystals, which can be dissolved in acidified isopropanol. The resulting purple solution is spectrophotometrically measured. Changes in the cell number are directly proportional to the absorbance (OD) at 570 nm in a cell-type specific manner. The resulting purple solution is spectrophotometrically measured. For calibration purpose, separate cultures at different cell densities were assayed by the MTT protocol and cell counting. A correlation of cell density vs. absorbance was established. The starting cell density was 10^5 cells/mL.

Statistic analysis

The statistic evaluation of data was performed using ANOVA analysis and nonparametric test of Kruskal–Wallis followed by Dunn's post-test. Statistic significance was considered for $p < 0.05$. All experiments were repeated at least six times and results are expressed as mean ± SD.

Results

Cell morphology and culture composition

The epithelial cell cultures showed more than 90% of cell positive for PCTA -1 (specific for epithelial prostatic cells), and most of them were strongly stained (specific for epithelial cancer cells) (Fig. 2A,B). The stromal cell cultures were almost devoid of stained cells for PCTA-1 (data not shown), but when assayed for vimentin, more than 85% were positive (Fig. 2C,D). At the level of electronic microscopy, the morphology of epithelial cells co-cultured with stromal showed multiple projections of cell membranes (Fig. 3A) compared with plain characteristic borders of flat cells in conventional cultures (data not shown). Medium and high magnifications (Fig. 3B,C) showed details of membrane interdigitations.

PSA secretion in separated epithelial and stromal cell cultures from prostate carcinoma

The epithelial and stromal cells were isolated from prostate tumours that showed similar Gleason score (4–6) (Fig. 4A) in order to obtain equivalent cultures. The epithelial cells were plated on 24-well plates, in presence of 10 nM of DHT. After the cells reached 70% of confluence, the media were changed every 48 h and

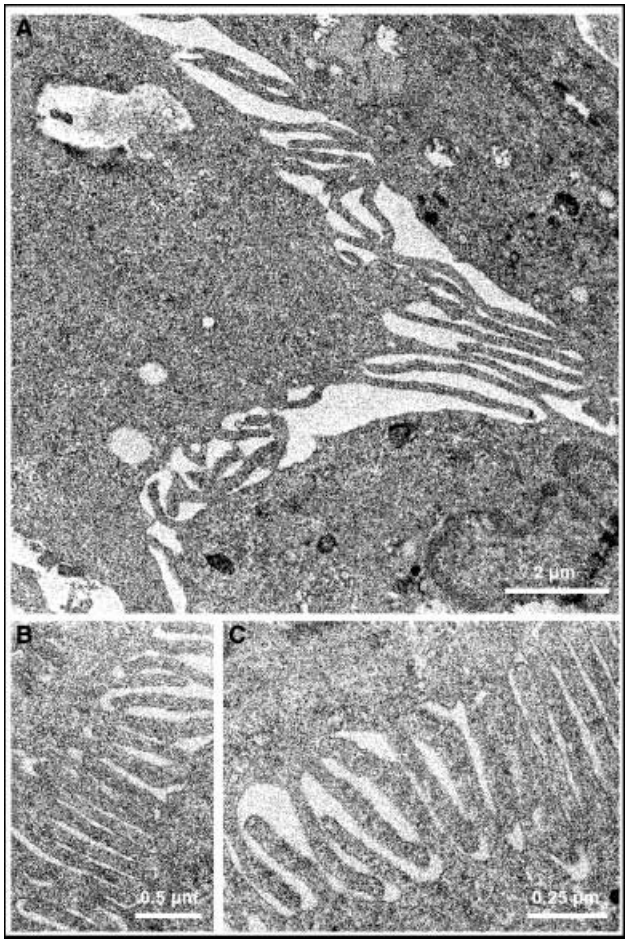

Figure 3. Electron microscopy analysis of epithelial cell morphology in co-culture system. The epithelial cells, cultured on insert transwells and maintained in co-culture with stromal prostatic cells, were processed for electron microscopy and morphology evaluation. (A) Low magnification showing multiple cell processes. (B, C) High magnification showing details of the interdigitations between membranes of neighbouring cells. Bars represent indicated size.

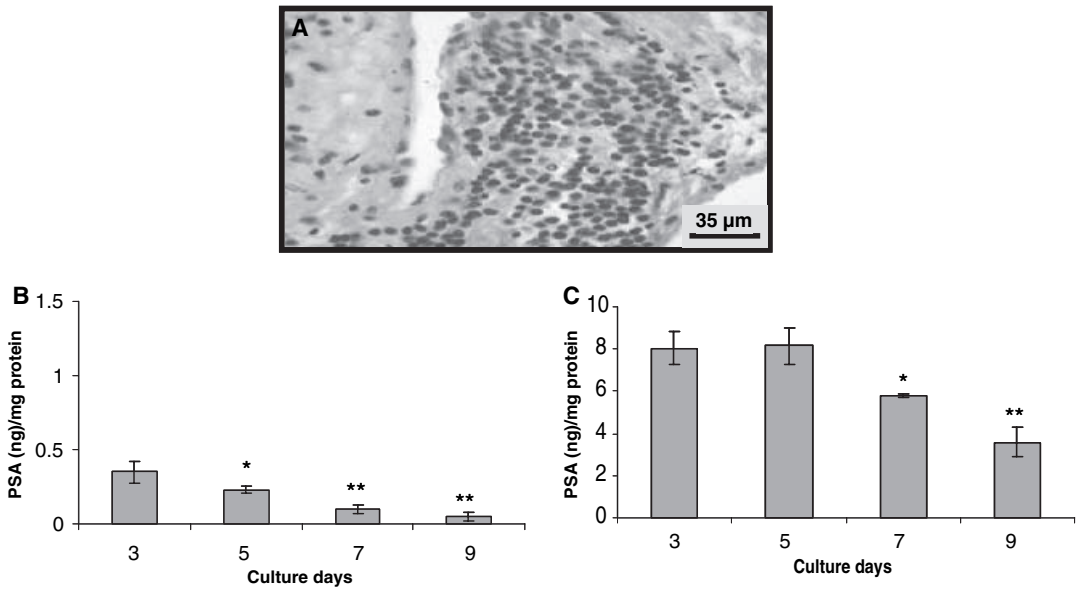

Figure 4. Prostatic specific antigen (PSA) secretion in epithelial and stromal cell cultures from human prostate adenocarcinoma. The epithelial and stromal cells were isolated and separately cultured. Both cultures were maintained in presence of 10 nM of DHT. Secreted PSA was evaluated every 48 h in both cultures. (A) Histology control of the prostatic tissue indicating the average differentiation degree of the tumour used in these experiments (Gleason score 4–6) (Bar represents 35 µm). (B) PSA secretion in stromal cell culture. (C) PSA secretion in epithelial cell cultures. Values represent mean ± SD of six different experiments $^{*}p < 0.05$, $^{**}p < 0.001$.

collected for PSA assay. The cultures showed a maintained PSA secretion until at least 1 week, declining slightly thereafter (Fig. 4C). Whereas the stromal cells, cultured in a similar manner, secreted only basal amount of PSA to the culture medium, probably because of the little amount of residual epithelial cells (Fig. 4B).

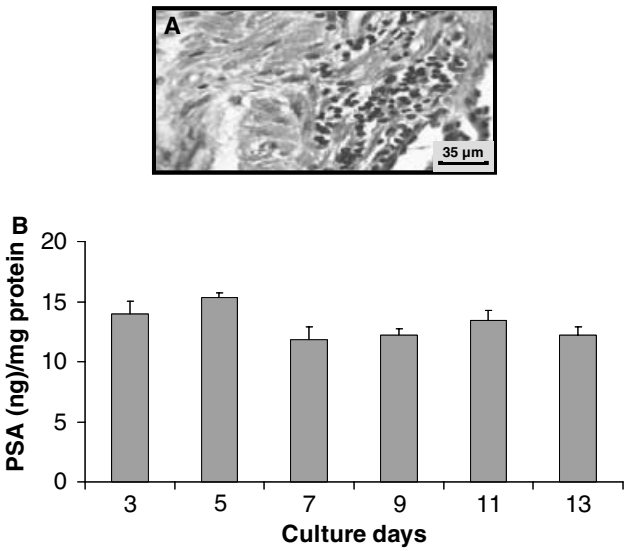

Figure 5. Prostatic specific antigen (PSA) secretion in epithelial/stromal cell co-cultures in bicameral system. The epithelial prostatic cells were plated on insert transwells and placed on 12-well plated with stromal cell monolayers. The co-cultures were maintained in presence of 10 nM of DHT. PSA was assayed in the culture medium every 48 h. (A) Histology control of the prostatic tissue indicating the average differentiation degree of the tumour used in these experiments (Gleason score 4–6) (Bar represents 35 µm). (B) PSA secretion in co-culture system. PSA was significantly higher compared with the epithelial culture alone and it is maintained for a longer period of time. Values represent mean ± SD of six different experiments. $p > 0.05$ (not statistically significant) between all bars.

PSA secretion in epithelial–stromal bicameral co-cultures from prostate carcinoma

Epithelial and stromal cells obtained from equivalent prostatic adenocarcinoma (Fig. 5A) were co-cultured. The epithelial cells were plated on appropriate cell culture inserts and placed on 12-well plates with a previously cultured stromal cell monolayer. The cells were co-cultured in presence of 10 nM of DHT. The media were collected every 48 h for PSA assay. The PSA secretion in epithelial–stromal bicameral co-cultures was considerably higher than in epithelial cultures alone and the level of PSA in culture medium was maintained for more than 10 days without significant variation (Fig. 5B).

Androgen response in epithelial cells alone and co-cultured with stromal cells

Epithelial and stromal cells obtained from prostatic adenocarcinoma with similar Gleason score (Fig. 6A) were co-cultured. The epithelial cell cultures and epithelial–stromal cell co-cultures were treated with 1–100 nM of DHT. The media were collected every 48 h for PSA assay. When cultured alone, the epithelial cells from prostate carcinoma showed no significant response to DHT regarding to PSA secretion (Fig. 6B). This response was significantly higher when the epithelial cells were co-cultured with stromal cells in the bicameral system (Fig. 6C).

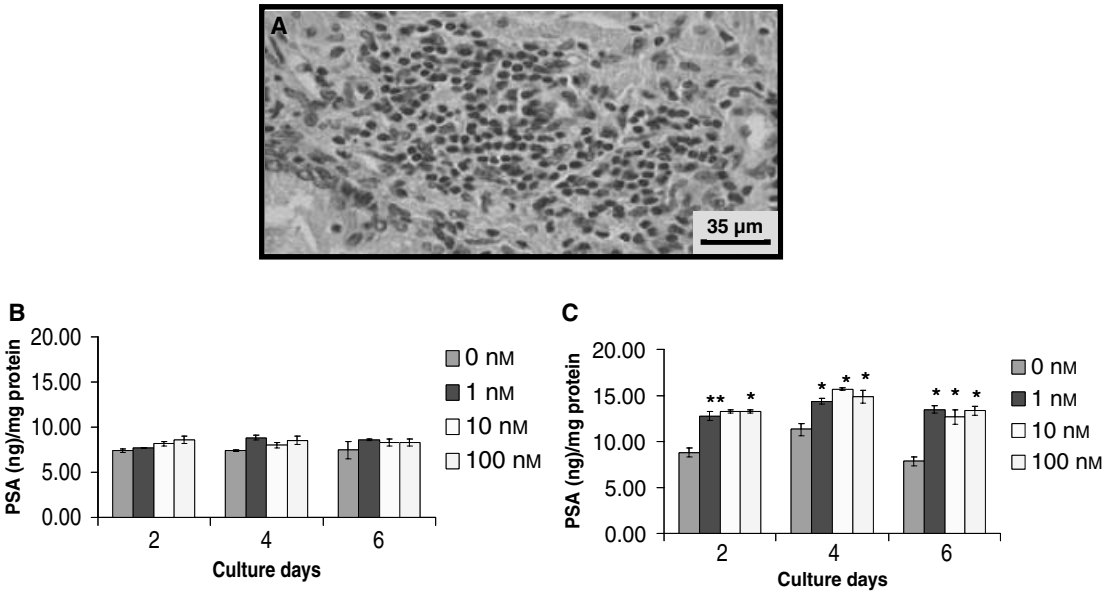

Figure 6. Androgen response of epithelial prostatic cells cultured alone or co-cultured with stromal cells in bicameral system. The epithelial cells were cultured either alone or with stromal cells and the prostatic specific antigen (PSA) secretion was evaluated in absence or presence of different concentrations of dihydrotestosterone (DHT) (1–100 nM). PSA was evaluated in the culture medium every 48 h. (A) Histology control of the prostatic tissue indicating the average differentiation degree of the tumour used in these experiments (Gleason score 4–6) (Bar represents 35 µm). (B) PSA secretion in cultures of epithelial cells alone showing no effect of DHT. (C) PSA secretion in bicameral co-cultures showing a significant increase in presence of all DHT concentrations tested. Values represent mean ± SD of six different experiments. *$p < 0.05$ between control and all DHT treatment in each day.

Proliferation activity in epithelial cells alone and co-cultured with stromal cells

The epithelial cell cultures and epithelial–stromal co-cultures were settled as described previously and the MTT cell growth assay was performed. The epithelial cell growth increased significantly when these cells were co-cultured with stromal cells (Fig. 7).

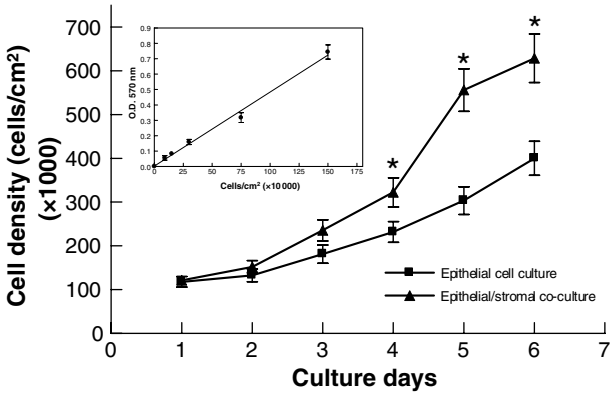

Figure 7. Growth rate in epithelial cell cultured alone or co-cultured with stromal cells in bicameral system. The epithelial cells were cultured either alone or with stromal cells and the growth rate was evaluated by spectrophotometric assay (see Material and methods). A significant increase is shown in the epithelial cell growth when co-cultured with stromal cells compared with the epithelial cells cultured alone. Values represent mean ± SD of six different experiments. *$p < 0.05$. Insert: calibration curve representing epithelial cell number vs. optical density.

Discussion

During the last years, several biological models for the study of prostate cancer have been developed, both in vivo and in vitro. Among them, cell cultures both primary and cell lines have been used. Most cell culture systems use metastatic derived cell lines, which may not represent the actual properties of the primary tumour cells. On the contrary, cumulative evidences indicate that paracrine interactions within the prostate are determinant for the normal growth regulation, tumour development and metastasis induction (Gleave *et al.*, 1991, 1992; Wong & Wang, 2000; Cunha *et al.*, 2003). For instance, the production of different types of matrix proteases, which are crucial to penetrate the extracellular matrix barriers, is a cooperative activity of epithelial and stromal prostatic cells (Greiff *et al.*, 2002). Also, Lang *et al.* (2000) using cell lines, have demonstrated that prostate stromal cells from malignant tissue influence differentially the invasive properties of normal and malignant epithelial.

The androgens are the main hormones involved in the prostate normal regulation and cancer development (Wilding, 1992; Cunha *et al.*, 2003). Testosterone and its active metabolite DHT stimulate tumour growth, at least in the initial stages of the pathology. In some cases, the tumour development becomes androgen independent presumably by receptor gene mutation. At this stage, the prostate cancer has the worse prognosis. Also, effects of prolactin, EGF, somatostatin and other growth factors on the tumour development have been studied (Kadar *et al.*, 1988; Fekete

et al., 1989; Lubrano *et al.*, 1993; Peehl, 1996; Lamharzi *et al.*, 1998). Several of these factors are produced by the stromal tissue suggesting a local regulation of the tumour development.

Our results indicate that it is possible to isolate epithelial and stromal cells with an acceptable degree of purity, assessed by immunocytochemically identification of tumour epithelial cells. We have used for this purpose, the specific membrane antigen PCTA-1. The advantage of this antigen, recently described (Su *et al.*, 1996) is that it is expressed specifically in epithelial prostatic cells and is significantly overexpressed in tumour epithelial cells. This allowed us to easily identify the relative amount of tumour cells in our cultures. Our epithelial cultures presented more than 90% of fluorescent cells, most of them strongly stained, whereas the stromal cultures were almost devoid (<5%) of stained cells. On the contrary, the histological evaluation of the prostatic tissue allowed us to determine the differentiation degree of the samples used for cell isolation. This is a very critical point, because the prostate carcinoma may have several tumours and previous diagnosis biopsies may not represent accurately the tissue segment that we obtain for cell isolation. The reproducibility of our system is based, in an important extent, in the equivalency of Gleason score of the samples used.

The bicameral co-cultures of epithelial cells with corresponding stromal prostatic cells showed important morphological changes in the epithelial cells at the level of electronic microscopy. Multiple cell processes and interdigitations were seen at the plasma membranes between neighbouring cells. These phenotypic characteristics are related with invasive and metastatic properties of the malignant cells (Scott *et al.*, 2000). The co-culture of stromal and epithelial cells from the same prostatic tumours is one of the main differences with some other studies in which stromal cells and different prostatic cell lines are co-cultured (Lang *et al.*, 2000). In that study, prostatic cell lines are used to study the influence of stromal cells isolated from different patients, which may not be completely representative of the epithelial–stromal interaction in prostatic cancer. On the contrary, our co-culture conditions increased significantly PSA secretion suggesting that this process is regulated by paracrine interactions. Moreover, the androgen response of the epithelial cells was detected mainly in co-culture conditions. This result suggests that alterations in paracrine epithelial–stromal interactions may be relevant in the lost of the androgen response that is seen at certain level of the prostate cancer development.

Regarding to the growth rate of the epithelial cell cultures, we have found that the proliferative activity increased markedly in presence of stromal cells confirming data reported in the literature regarding the influence of growth factors produced by stromal cells on the proliferative activity of the corresponding epithelium (Gleave *et al.*, 1991). Using a similar co-culture system, but with cells obtained from prostatic hyperplasia, Bayne *et al.*, have reported changes in morphology, androgen receptor and PSA expression, and 5-alpha-reductase expression and activity of hyperplasic epithelial cell when cultured with fibroblast of the same sample (Bayne *et al.*, 1998a,b; Habib *et al.*, 2000). Because the prostate BHP is not related with the prostatic adenocarcinoma, our results with cancer cells indicate that in this pathological process, at least part of this paracrine interaction is maintained. Moreover, these cells interactions might be increased abnormally in prostatic carcinogenesis, as suggested by in vivo studies (Cunha *et al.*, 2003). It is concluded that the bicameral co-culture system, in which prostatic epithelial and stromal cells from adenocarcinoma are separated by a PET membrane impregnated with extracellular matrix proteins, allowed these different cell types to maintain some of their paracrine relationships. This condition resemble more closely the in vivo condition of the tumour regarding to the secretory and proliferative activities as well as hormone response and cell morphology than primary cultures of epithelial cells alone or cell lines cultures. For these reasons, and considering the scanty amount of reports based on prostate primary cell cultures, the co-culture conditions reported here may represent a valuable tool for cellular and molecular studies on prostate cancer.

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References

Bayne, C. W., Donnelly, F., Chapman, K., Bollina, P., Buck, C. & Habib, F. K. (1998a) A novel coculture model for benign prostatic hyperplasia expressing both isoforms of 5 alpha-reductase. *Journal of Clinical Endocrinology and Metabolism* **83**, 206–213.

Bayne, C. W., Ross, M., Donnelly, F., Chapman, K., Buck, C., Bollina, P. & Habib, F. K. (1998b) Selective interactions between prostate fibroblast and epithelial cells in co-culture maintain the BPH phenotype. *Urologia Internationalis* **61**, 1–7.

Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D. & Mitchell, J. B. (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of radiosensitivity. *Cancer Research* **47**, 943–946.

Cunha, G. R., Hayward, S. W., Wang, Y. Z. & Ricke, W. A. (2003) Role of the stromal microenvironment in carcinogenesis of the prostate. *International Journal of Cancer* **107**, 1–10.

Davies, P. & Eaton, C. L. (1991) Regulation of prostate growth. *Journal of Endocrinology* **131**, 5–17.

Dondi, D., Limonta, P., Moretti, R., Marelli, M., Garattini, E. & Motta, M. (1994) Antiproliferative effects of luteinizing hormone-releasing hormone (LHRH) agonists on human androgen-independent prostate cancer cell line DU 145: evidence for an autocrine-inhibitory LHRH loop. *Cancer Research* **54**, 4091–4095.

Fekete, M., Redding, T., Comaru-Scahlly, A., Pontes, J., Connelly, R., Srkalovic, G. & Schally, A. (1989) Receptors for luteinizing hormone-releasing hormone, somatostatin, prolactin, and epidermal growth factor in rat and human prostate cancers and in benign prostate hyperplasia. *Prostate* **14**, 191–208.

Garnick, M. B. (1993) Prostate cancer: screening, diagnosis, and management. *Annals of Internal Medicine* **118**, 804–818.

Gittes, R. F. (1991) Carcinoma of the prostate. *New England Journal of Medicine* **324**, 236–245.

Gleason, D. F. & Mellinger, G. T. (1974) Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. *Journal of Urology* **111**, 58–64.

Gleave, M., Hsieh, J. T., Gao, C. A., von Eschenbach, A. C. & Chung, L. W. (1991) Acceleration of human prostate cancer growth in vivo by factors produced by prostate and bone fibroblasts. *Cancer Research* **51**, 3753–3761.

Gleave, M. E., Hsieh, J. T., von Eschenbach, A. C. & Chung, L. W. (1992) Prostate and bone fibroblasts induce human prostate cancer growth in vivo: implications for bidirectional tumor-stromal cell interaction in prostate carcinoma growth and metastasis. *Journal of Urology* **147**, 1151–1159.

Greiff, A. H., Fischer, W. M. & Sehgal, I. (2002) Paracrine communication between malignant and non-malignant prostate epithelial cells in culture alters growth rate, matrix protease secretion and in vitro invasion. *Clinical and Experimental Metastasis* **19**, 727–733.

Habib, F. K., Ross, M. & Bayne, C. W. (2000) Development of a new in vitro model for the study of benign prostatic hyperplasia. *Prostate (Supplement)* **9**, 15–20.

Hayward, S. W., DelBuono, R., Deshpande, N. & Hall, P. A. (1992) A functional model of adult human prostate epithelium. The role of androgens and stroma in architectural organization and the maintenance of differentiated secretory function. *Journal of Cell Science* **102**, 361–372.

Janecki, A. & Steinberger, A. (1987) Bipolar secretion of androgen-binding protein and transferrin by Sertoli cells cultured in a two-compartment culture chamber. *Endocrinology* **120**, 291–298.

Jungwirth, A., Schally, A. V., Pinski, J., Halmos, G., Groot, K., Armatis, P. & Vadillo-Buenfil, M. (1997) Inhibition of in vivo proliferation of androgen-independent prostate cancers by an antagonist of growth hormone-releasing hormone. *British Journal of Cancer* **75**, 1585–1592.

Kadar, T., Ben-David, M., Pontes, J., Fekete, M. & Schally, A. (1988) Prolactin and luteinizing hormone-releasing hormone receptors in human benign prostatic hyperplasia and prostate cancer. *Prostate* **12**, 299–307.

Lamharzi, N., Halmos, G., Armatis, P. & Schally, A. (1998) Expression of mRNA for luteinizing hormone-releasing hormone receptors and epidermal growth factor receptors in human cancer cell lines. *International Journal of Oncology* **12**, 671–675.

Lang, S. H., Stower, M. & Maitland, N. J. (2000) In vitro modelling of epithelial and stromal interactions in non-malignant and malignant prostates. *British Journal of Cancer* **82**, 990–997.

Limonta, P., Dondi, D., Moretti, R., Maggi, R. & Motta, M. (1992) Antiproliferative effects of luteinizing hormone-releasing hormone agonists on the human prostatic cancer cell line LNCaP. *Journal of Clinical Endocrinology and Metabolism* **75**, 207–212.

Lubrano, C., Toscano, V., Petrangeli, E., Spera, G., Trotta, M., Rombola, N., Frati, L., Di Silverio, F. & Sciarra, F. (1993) Relationship between epidermal growth factor and its receptor in human benign prostatic hyperplasia. *Journal of Steroid Biochemistry and Molecular Biology* **46**, 463–468.

Moretti, R., Marelli, M., Dondi, D., Poletti, A., Martini, L., Motta, M. & Limonta, P. (1996) Luteinizing hormone-releasing hormone agonists interfere with the stimulatory actions of epidermal growth factor in human prostatic cancer cell lines, LNCaP and DU 145. *Journal of Clinical Endocrinology and Metabolism* **81**, 3930–3937.

Peehl, D. M. (1996) Cellular biology of prostatic growth factors. *Prostate (Supplement)* **6**, 74–78.

Redding, T., Schally, A., Radulovic, S., Milovanovic, S., Szapeshazi, K. & Isaacs, J. (1992) Sustained release formulations of luteinizing hormone-releasing hormone antagonist SB-75 inhibit proliferation and enhance apoptotic cell death of human prostate carcinoma (PC-82) in male nude mice. *Cancer Research* **52**, 2538–2544.

Rui, H. & Purvis, K. (1988) Hormonal control of prostate function. *Scandinavian Journal of Urololgy and Nephrology* **107**, 32–38.

Ruijter, E., Van de Kaa, C., Miller, G., Ruiter, D., Debruyne, F. & Schalken, J. (1999) Molecular genetics and epidemiology of prostate carcinoma. *Endocrine Reviews* **20**, 22–45.

Sanchez de la Muela, P., Povo Castilla, J., Bobillo de Lamo, M., Rosales, F. & Perez-Piqueras, J. L. (1995) Microparticle immunoenzymatic assay for detection of prostate specific antigen: characterization of the technique and comparative analysis with a monoclonal immunoradiometric assay. *Military Medicine* **160**, 416–419.

Scott, L. J., Clarke, N. W., Sharrard, M. S., George, N. J. & Lang, S. H. (2000) Invasive phenotype of primary human prostatic epithelial cells. *Prostate Cancer and Prostatic Diseases* **3** (S1), S8.

Su, Z. Z., Lin, J., Shen, R., Fisher, P. E., Goldstein, N. I. & Fisher, P. B. (1996) Surface-epitope masking and expression cloning identifies the human prostate carcinoma tumor antigen gene PCTA-1 a member of the galectin gene family. *Proceeding of the National Academy of Science USA* **93**, 7252–7257.

Sung, S. Y. & Chung, L. W. (2002) Prostate tumor-stroma interaction: molecular mechanisms and opportunities for therapeutic targeting. *Differentiation* **70**, 506–521.

Wilding, G. (1992) The importance of steroid hormones in prostate cancer. *Cancer Surveys* **14**, 113–130.

Wong, Y. C. & Wang, Y. Z. (2000) Growth factors and epithelial–stromal interactions in prostate cancer development. *International Review of Cytology* **199**, 65–116.

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