Cell Tissue Res (2008) 332:489–498
DOI 10.1007/s00441-008-0602-z

REGULAR ARTICLE

A novel tissue-slice culture model for non-malignant human prostate

M. Bläuer · T. L. Tammela · T. Ylikomi

Received: 30 November 2007 / Accepted: 14 February 2008 / Published online: 2 April 2008

Abstract A novel tissue culture system was established for modeling the non-neoplastic human prostate in vitro. Precision-cut prostate slices were cultivated in culture plates with a gas-permeable base in a novel serum-free mixture. Cultivated specimens was evaluated by an immunohistochemical analysis of cytokeratins 18 and 14, androgen receptor (AR), prostate specific antigen (PSA), prostate acid phosphatase (PAP), and the endothelial cell marker von Willebrand factor. Epithelial viability in the presence and absence of dihydrotestosterone (DHT) was also assessed. Satisfactory maintenance of glandular cytoarchitecture was observed in the presence of DHT with approximately half of the glands displaying a columnar or cuboidal phenotype and an intact layer of basal cells. In the absence of DHT, the corresponding percentage was significantly lower. The occurrence of involutive changes and epithelial cell death was significantly higher in the absence of DHT. Glandular and stromal cells maintained their capacity to express AR. PSA and PAP were expressed throughout the culture period, albeit at a lower level than in uncultured tissue. The viability of endothelial cells differed markedly between individual samples. During culture, the tissue slices became covered with epithelial cells originating from glands that were cut open during tissue slicing. This cell layer consisted of a stratified basal compartment overlaid by cells with a luminal phenotype. The present culture system provides a novel in vitro setting in which to study normal human prostate biology and pathobiology and may help to obviate problems related to the use of established cancer cell lines and animal models.

Keywords Prostate · In vitro · Tissue culture · Serum-free medium · Human

This study was supported by grants from competitive research funding of the Pirkanmaa Hospital District, TEKES Drug 2000, and the Juliana von Wendt Fund.

M. Bläuer (✉) · T. Ylikomi
Department of Cell Biology, University of Tampere,
Tampere FI-33014, Finland
e-mail: merja.blauer@uta.fi

T. L. Tammela
Department of Urology, Tampere University Hospital,
Tampere FI-33014, Finland

Introduction

The incidence of prostate cancer and benign prostatic hyperplasia (BPH) continues to rise in the Western world. To facilitate an understanding of the genesis of prostatic disease and to aid the development of new preventive and therapeutic regimens, in vitro models allowing experimentation on normal and pathologic prostate tissue are needed. Since the organogenesis and the differentiated function of the prostate tissue are highly dependent on androgens, the maintenance of the androgen signaling mechanism is a prerequisite for any culture model intended to mimic normal prostatic responses in vitro. Stromal-epithelial interactions are well established as being critical for mediating the effects of androgens on the epithelial cells within prostate glands (Cunha et al. 2004). Evidence has also been provided that stromal components modulate the initiation and progression of cancer (Chung and Davies 1996; Tlsty 2001). Therefore, both the epithelial and stromal compartments of the tissue should be included when aiming to model the normal function or pathobiology of the prostate in vitro.

The normal prostatic epithelium is composed of a single layer of basal cells underneath a layer of differentiated

secretory epithelial cells that line the lumina of prostate glands. The two cell types are distinguished by their characteristic expression of cytokeratins: the basal cells express cytokeratins 5 and 14, and secretory cells, cytokeratins 8 and 18. A population of proliferative cells expressing both basal and secretory features has also been identified within the epithelial parenchyma (transit amplifying cells) together with scattered stem cells and neuroendocrine cells (for a review, see Peehl 2005).

Over the years, various in vitro systems have been employed with the objective of modeling the normal development and function of the human prostate and of elucidating the cellular and molecular mechanisms underlying BPH and prostate cancer. Monocultures of primary prostatic epithelial cells derived from normal or BPH tissues tend to share the features of transit amplifying cells, and the challenge remains to create culture conditions that preserve intact the differentiated phenotype of the secretory epithelium (Liu and Peehl 2001; Peehl 2004, 2005). Differentiated prostatic secretory cells express androgen receptor (AR) together with a number of androgen-regulated proteins including prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP). The use of cultures of primary epithelial cell as models of normal prostate biology is hampered by a rapid loss of functional AR in epithelial monocultures, accompanied by a loss of androgen-sensitive cell growth and gene expression (for a review, see Peehl and Sellers 2002). Similarly, most of the established prostate cancer cell lines do not express AR or PSA or express a mutated receptor protein. Because of their metastatic origin, established cancer cell lines maintain little of the differentiated characteristics of normal secretory cells of the prostate.

As compared with epithelial monocultures in standard culture conditions, a better maintenance of in vivo characteristics of the secretory cells has been reported in co-cultures permitting epithelial-stromal interaction (Bayne et al. 1998; Hall et al. 2002). Contacts with the extracellular matrix are also known to be essential for morphological and functional differentiation of the prostatic epithelium. Correspondingly, many of the in vivo characteristics of secretory cells can be regained by the addition of extracellular components within the culture (Webber et al. 1997; Fong et al. 1991; Hudson et al. 2000). In addition, more elaborate culture models allowing spheroid formation of epithelial cells (Goossens et al. 2002; Lang et al. 2000, 2001; Takagi et al. 2007) have been shown to promote secretory cell differentiation. Organ cultures represent an in vitro model in which tissue architecture in terms of both cellular and matrix composition is identical to the tissue of origin and therefore best mimic the in vivo situation. Explants of the human prostate have been maintained under manifold static and dynamic culture conditions, but the outcome with regard to the preservation of tissue structure and function has been variable (Nevalainen et al. 1993; Margolis et al. 1999; Varani et al. 1999; Parrish et al. 2002).

The present study describes a novel organ culture model for nonmalignant human prostate. The model is based on the aseptic preparation of precision-cut tissue slices and their cultivation in dishes designed to support an optimal gas exchange in static culture. Herein, we also introduce a novel mixture of serum-free media that is able to preserve excellent tissue viability for a minimum of 7 days. An immunohistochemical analysis of cytokeratins, AR, PSA, PAP, and von Willebrand factor has been used to characterize the culture method.

Materials and methods

Tissue samples

Samples of prostatic tissue were obtained from patients undergoing transurethral (three patients) or open (one patient) resection for BPH, or radical cystoprostatectomy (seven patients) for invasive cancer of the urinary bladder at Tampere University Hospital. The mean age of the patients was 68.5 years (range: 60–77 years). For transport to the laboratory, the tissue specimens were placed in ice-cold Medium-199 with Earle's salts (M199; Gibco, Paisley, Scotland) supplemented with antibiotic/antimycotic solution (Gibco). Pieces of the specimens were fixed in 4% paraformaldehyde for histological and immunohistochemical examination. The culture method was optimized with regard to slice thickness, medium constituents, and other culture conditions. Four samples from patients undergoing radical cystoprostatectomy were processed to study the effect of DHT supplementation on tissue viability after 7 days of cultivation in the established culture conditions. This study was conducted with the approval of the ethical committee of Tampere University Hospital and informed consent to use prostatic tissue for experimental purposes was obtained from each patient.

Preparation of precision-cut slices

For the preparation of precision-cut prostate slices, the Krumdieck tissue slicer (Alabama Research and Development, Mundford, Ala.) was used. The tissue specimens were cut into pieces with a diameter of approximately 5 mm on an ice-cooled working platform. Each piece was then separately encased in 2% Agar (Bacto Agar, Becton Dickinson, Sparks, Md.) inside a mold-plunger assembly specifically devised for tissue embedding prior to slicing with the Krumdieck slicing apparatus (Alabama Research and Development). The embedded samples were then transferred into the microtome filled with pre-cooled

M199 supplemented with antibiotics, and 300-μm slices were cut at a cycle speed of 30 slices per minute.

Tissue-slice culture

With the aid of a transfer pipette, the freshly cut tissue slices were transferred into hydrophilic Greiner Lumox culture dishes (diameter 50 mm; Greiner Bio-One) containing 1.5 ml culture medium. Four to six slices were placed in each dish. The culture medium was a 1:1 mixture of M199: K-SFM (keratinocyte serum-free medium, Gibco) supplemented with antibiotics and antimycotics as above. Only the basal media were used without fetal calf serum (FCS) or growth factor supplementation. Parallel cultures of each sample with or without 1 nM dihydrotestosterone (DHT, Fluka) were prepared. Plain ethanol vehicle (0.1%) was included in the control cultures. The media were renewed every 2 days, and fresh DHT (or vehicle) was provided. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. After 7 days, the medium was replaced with 4% paraformaldehyde, and the slices were allowed to fix in situ for 15 min. The slices were then removed with a scalpel and transferred into fixation bottles for an overnight fixation after which the specimens were dehydrated and embedded in paraffin according to routine procedures. Sections (5 μm thick) were cut for histochemical and immunohistochemical analyses.

Immunohistochemistry

Immunohistochemical analysis was used to assess the ability of the culture method to maintain tissue integrity. The following monoclonal antibodies were used at the dilutions indicated: anti-cytokeratin 14 (1:50), anti-cytokeratin 18 (1:50), anti-PAP (1:4,000), anti-PSA (1:500), and anti-Ki67 (1:500; all DAKO, Glostrup, Denmark); anti-AR (1:50; NeoMarkers, Fremont, Calif.); anti-von Willebrand factor (1:5,000; Sigma, St. Louis). Controls included omission of the primary antibodies and use of nonimmunized-mouse IgG. The staining was performed with a broad-spectrum Zymed Histostain-Plus kit (Zymed Laboratories, South San Francisco, Calif.) as previously described (Bläuer et al. 2005) with the following modifications to the manufacturer's protocol: primary antibodies were incubated overnight at 4°C and biotinylated secondary antibody for 20 min at room temperature. All washes were repeated three times (5 min each). The sections were lightly counterstained with hematoxylin.

Analysis of epithelial histology

Glandular structures within four specimens cultured for 7 days with or without 1 nM DHT were classified into three categories according to the cellular structure of the epithelium: (1) columnar or cuboidal secretory epithelium, (2) flattened epithelium and/or stratified squamous metaplasia, (3) dead secretory epithelium. The means±SEM were calculated for each category in controls and DHT-treated samples. Statistical differences between means were assessed by using the paired-sample *t*-test. Correlation was considered statistically significant at a *P*-value of <0.05.

Results

Tissue viability and structure

A novel tissue-slice culture model was developed for maintaining the differentiated morphology and protein expression of normal human prostate in vitro (Fig. 1a). Tissue slices of approximately 300 μm in thickness were cultivated within culture dishes whose base was gas-permeable. Together with a minimal amount of medium applied in each dish, the culture model permitted favorable gas exchange from both sides of the tissue slice. Tissue structure was monitored during culture by phase-contrast microscopy. Excellent tissue viability and structure could be maintained for a minimum of 7 days. Figure 1b shows a representative prostate slice with intact glandular structures after 13 days of cultivation. At the outer margins of the slices, sheets of epithelial cells could be regularly seen growing on the surface of the culture dish (Fig. 1c).

Histological evaluation of freshly cut prostate slices showed that the slicing process caused little damage to tissue structure (Fig. 2a). The 300-μm thickness of the slices enclosed a number of intact glands permitting analysis of possible changes in their cytoarchitecture upon cultivation. No considerable change in the thickness of the slices occurred during culture (Fig. 2b). Within 7 days of culture, the surface of the slices was covered with a sheet of epithelial cells (Fig. 2b). Immunohistochemical staining of cytokeratin 14 in Fig. 2c and of cytokeratin 18 in Fig. 2d shows that epithelial outgrowth is a process in which glands that have been cut open during slicing turn inside-out to cover the outer surface of the tissue sample. Basal cells within the surface epithelium were able to proliferate, as indicated in Fig. 2e by the numerous nuclei immunopositive for Ki67. The outer-most single-cell layer of secretory cells retained its expression of AR (Fig. 2f) and PAP (Fig. 2g). PSA immunoreactivity could not be observed, other than in infrequent scattered cells as depicted in Fig. 2h.

The cellular structure of intact glands was analyzed immunohistochemically in a freshly cut tissue slice (Fig. 3a,b) and slices incubated for 7 days in vitro in the presence (Fig. 3c,d) or absence (Fig. 3e,f) of DHT. Staining with anti-cytokeratin 18 in Fig. 3a,c shows the excellent

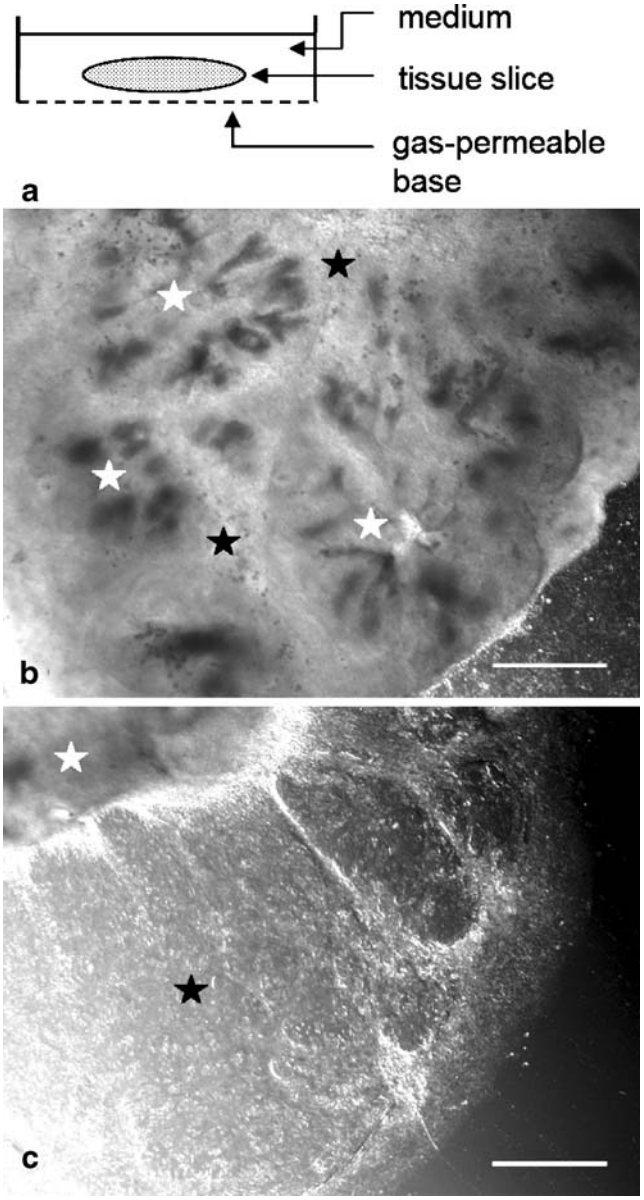

Fig. 1 Prostate slice culture. **a** Representation of the culture model. **b** Phase-contrast image of a 300-μm-thick prostate slice incubated for 13 days. Glandular structures (*white stars*) within the more translucent stroma (*black stars*) are well-preserved. **c** Outgrowth of a sheet of epithelial cells (*black star*) from the edge of the slice (*white star*). *Bars* 100 μm

preservation of columnar secretory epithelium in cultures supplemented with DHT. Staining of basal-cell-specific cytokeratin 14 revealed an intact layer of basal cells both before (Fig. 3b) and after (Fig. 3d) cultivation. Figure 3e,f represents typical glandular structure in tissue slices deprived of DHT. Glandular cells expressing cytokeratin 18 appeared as thin strands of cells around the lumina (Fig. 3e). Cytokeratin 14 -positive basal cells grew characteristically in multiple layers in the absence of DHT (Fig. 3f). Glands morphologically resembling those in uncultured tissue were also present (data not shown).

Effect of DHT on tissue viability and structure

The preservation of glandular cytoarchitecture in the presence and absence of 1 nM DHT was analyzed in four tissue specimens after 7 days of culture (Fig. 4). The viability and morphology of secretory cells were shown to be dependent on DHT supplementation. In the abscence of DHT, the mean percentage ± SEM of glands with columnar or cuboidal secretory cells was 30.7±5.9%, whereas in its presence, the corresponding value was significantly higher (P=0.036) amounting to 50.5±2.9%. The mean percentage of glands with involutive changes was 41.3±6.3% and 33.8±3.8% in the absence and presence of DHT, respectively (P=0.215). A majority of these most probably represented indentations of the surface epithelium. The percentage of glands with dead secretory epithelium was significantly higher (P=0.002) in the absence of DHT (28.0±5.6%) than in cultures supplemented with DHT (16.8±4.8%). In corresponding uncultured tissue samples, the percentage of glands displaying columnar/cuboidal morphology was 96.6±2.3%, and flat/metaplastic epithelia were seen in 3.4±2.8% of glandular structures. Dead epithelia were not detected.

The cultures were routinely terminated after 7 days. In two solitary cases, however, tissue was fixed after 4 days or at 14 days in DHT-supplemented culture conditions. After 4 days, as many as 84.4% of glands retained their columnar/cuboidal phenotype and not more than 15.6% displayed involutive changes. Glandular death was not detected. After 2 weeks, the preservation of epithelial structures resembled that of 1-week cultures with the percentages for columnar/cuboidal, flat/metaplastic, and dead glandular morphologies being 61.5%, 25.0%, and 13.5%, respectively.

Expression of AR, PSA, PAP, and Ki67 in cultured prostate slices

Immunohistochemical analysis of AR and the secretory proteins PSA and PAP was used to study the preservation of the differentiated phenotype of secretory epithelia within prostate tissue slices (Fig. 5a,c,e,g: uncultured tissue; Fig. 5b,d,f,h: tissue slices cultured for 7 days). Cultured tissue slices retained their ability to express AR not only in secretory cells but also in stromal cells (Fig. 5b). The intensity of immunohistochemical staining for AR was lower in cultured slices (Fig. 5b) than in their uncultured counterparts (Fig. 5a). The cultured slices also maintained a low level of expression for PSA (Fig. 5d) and PAP (Fig. 5f). Only a few Ki67-positive cells could be detected in uncultured (Fig. 5g) and cultured (Fig. 5h) tissue slices.

In the one 4-day culture examined, the expression of AR, PSA, and PAP was more intense than in cultures terminated after 1 week. After 14 days, a moderate AR and PAP immunoreactivity could be detected in scattered

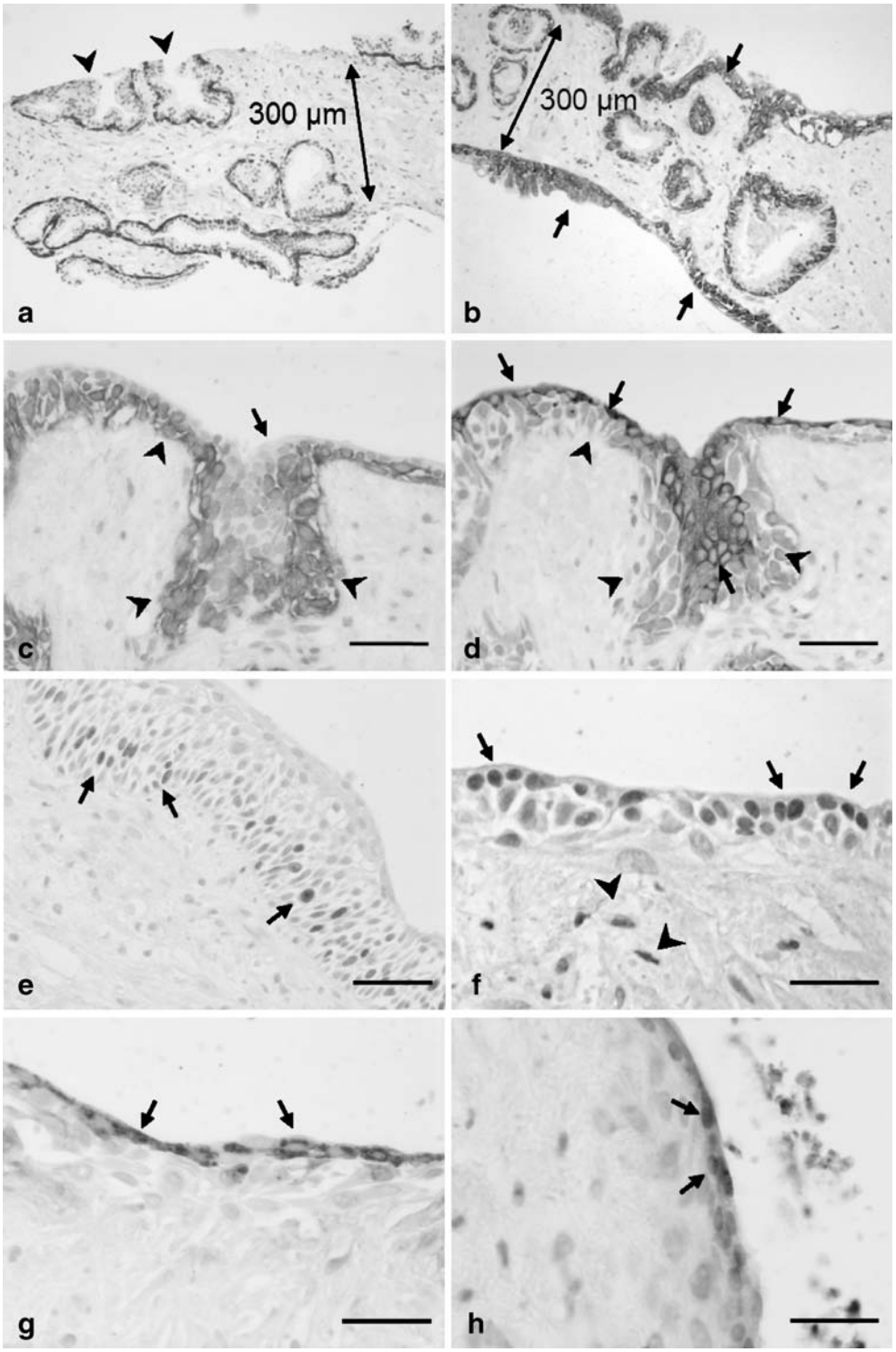

Fig. 2 Cellular structure of prostate slices in transverse sections. The tissue slicer had been set to cut 300 µm slices. **a** Freshly cut slice. Basal cells were stained with anti-cytokeratin 14 (*arrowheads* glands cut open at the surface of the slice). **b** Slice incubated for 7 days and stained with anti-cytokeratin 14. Epithelial cells from cut glands have spread out to cover the surface of the slice (*arrows*). **c**, **d** After 7 days of incubation, the epithelium of a cut gland is turning inside-out onto tissue surface. **c** The basal cells expressing cytokeratin 14 (*arrowheads*) are covered with an unstained layer of cells *arrow*). **d** In a successive section, the outmost layer of secretory cells stains positively for cytokeratin 18 (*arrows*), whereas basal cells remain unstained (*arrowheads*). **e** Proliferating basal cells on the tissue surface express Ki67 in their nuclei (*arrows*). **f** AR expression persists in secretory cells (*arrows*) growing on the tissue surface (*arrowheads* AR-positive stromal cells). **g** The outermost layer of cells expresses PAP (*arrows*). **h** PSA immunoreactivity was seen in a few isolated cells on the tissue surface (*arrows*). *Bars* 50 µm (**c**–**e**), 20 µm (**f**–**h**)

glandular structures. Here, PSA was no longer observed (data not shown).

Maintenance of endothelial cells

Histological examination and immunohistochemical staining of the endothelial cell marker, von Willebrand factor, revealed maintenance of endothelial cells of the microvasculature and those of larger vessels within the cultured specimens. Figure 6 illustrates the expression of von Willebrand factor in representative prostate slices before (Fig. 6a) and after (Fig. 6b) cultivation for 7 days. A marked individual variation in the survival of endothelial cells was observed: in some specimens, all cells appeared

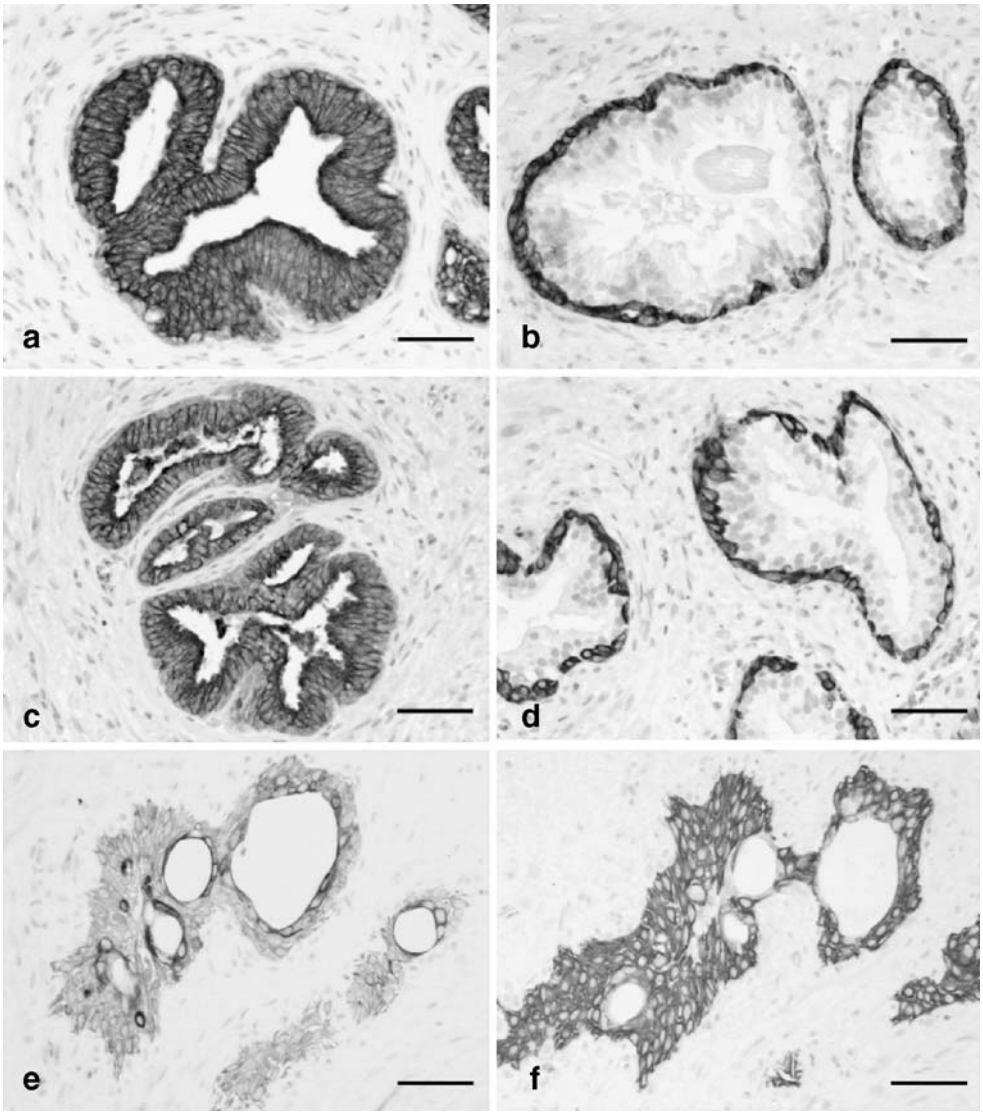

Fig. 3 Immunohistochemical analysis of cytokeratins 18 (**a**, **c**, **e)** and 14 (**b**, **d**, **f)** in prostate slices. **a**, **b** Non-cultivated tissue. **c**, **d** Tissue after 7 days in culture with DHT. **e**, **f** Tissue after 7 days in culture without DHT. **a**, **c** Cytokeratin 18 staining reveals excellent maintenance of columnar secretory epithelium in tissue cultured with DHT. **b**, **d** A single layer of basal cells surrounds the secretory compartment before and after cultivation in medium containing DHT. Stromal cells are abundant. **e** A typical glandular structure in DHT-deprived cultures. Thin strands of glandular cells expressing cytokeratin 18 surround the lumina. **f** Cytokeratin 14 staining in basal cells growing in multiple layers in the absence of DHT. Stromal cells are infrequent. Counterstained with hematoxylin. *Bars* 50 μm

intact, whereas in others, detaching endothelia were detected (data not shown).

Discussion

Precision-cut tissue slices represent an in vitro model in which the functionally important features of the tissue of origin (multicellularity together with cell-cell and cell-matrix interactions) are well preserved. Slice cultures of human tissues have been used for a wide range of applications including toxicological and pharmacological studies of liver and lung specimens (Nave et al. 2006; Kern et al. 2006) and neurophysiological experiments on brain slice preparations (González-Martinez et al. 2007). Slice cultures possess several advantages over conventional explant cultures. Automated cutting and subsequent collection of the slices in cold medium allow optimal usage of

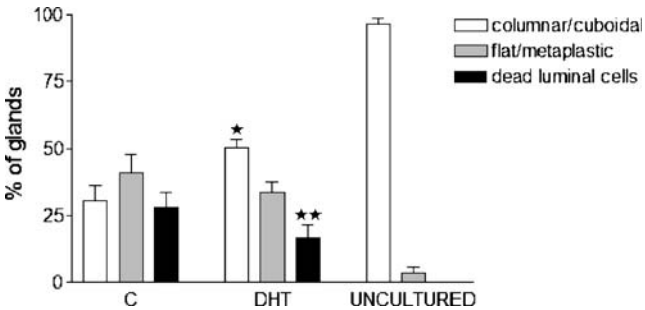

Fig. 4 Effect of DHT on glandular epithelium. Tissue slices from four specimens were maintained for 7 days in culture without (*C*) or with 1 nM DHT (*DHT*). The percentage of glands expressing columnar/cuboidal, flat/metaplastic, or dead luminal cell phenotype was assessed in representative histological sections. As a reference, the analysis was also undertaken on corresponding uncultured tissue samples. The values represent the mean±SEM. **P*<0.05, ***P*<0.005 compared with the corresponding control; paired samples *t*-test

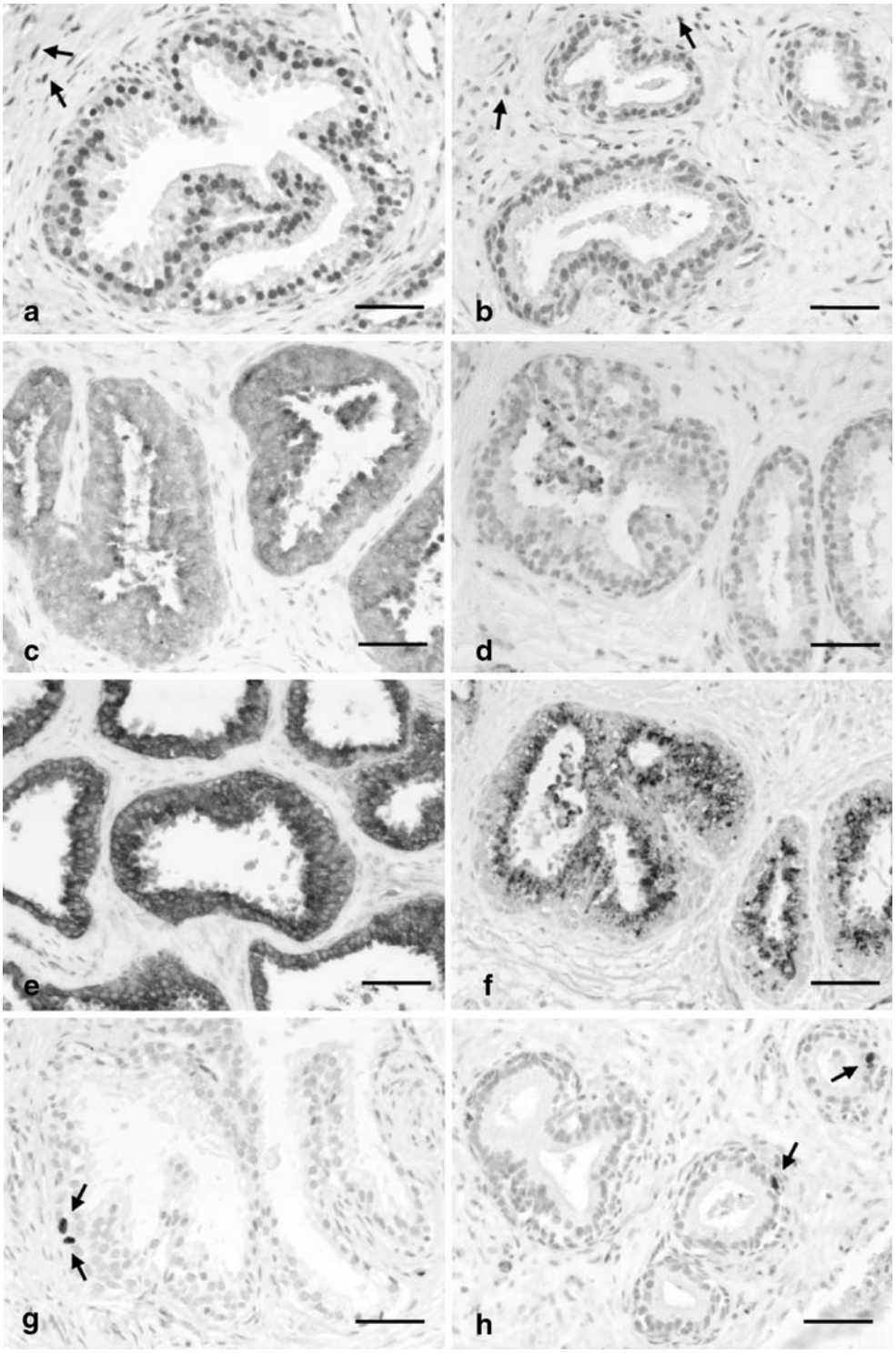

Fig. 5 Immunohistochemical analysis of AR, PSA, PAP, and Ki67 in prostate slices. **a** Intense AR immunoreactivity is detected in secretory epithelial cells and stroma (*arrows*) in control tissue. **b** After 7 days in culture, a lower level of AR expression can be seen in both compartments (*arrows* staining in stroma). **c** Secretory epithelial cells in non-cultured tissue express PSA. **d** PSA expression diminishes in culture. **e** PAP immunoreactivity in control tissue is intense throughout the secretory epithelium. **f** In cultured tissue, PAP expression becomes more apical. **g**, **h** Proliferating cells as detected by anti-Ki-67 (*arrows*) remain scarce. Counterstained with hematoxylin. *Bars* 50 μm

limited biological material, minimize tissue damage, and reduce cellular metabolism during sample preparation. The typical 250– to 300-μm thickness of a tissue slice permits rapid nutrient and gas exchange, thereby inhibiting, in the core of the tissue, the ischemic injury that regularly ensues in manually prepared explants (reviewed by Lerche-Langrand and Toutain 2000). In many applications, oxygen diffusion has been further facilitated by the use of dynamic organ culture incubators that alternately expose the slices to ambient gases and medium (Lerche-Langrand and Toutain 2000).

Here, the advantages of a dynamic culture system have been translated to a conventional static setting by the use of Petri dishes with a gas-permeable base. Together with the minimum amount of culture medium applied in each dish,

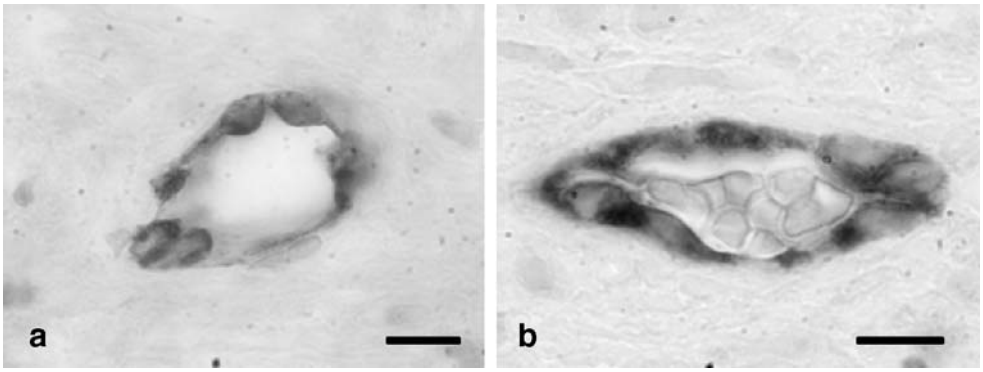

Fig. 6 Immunohistochemical analysis of von Willebrand factor in prostate slices. **a** Uncultured slice. **b** After 7 days in culture, endothelial cells remain intact. Note erythrocytes within the vessel. No counterstain. *Bars* 10 μm

diffusion of oxygen from both sides of the sample is facilitated. Tissue viability and cellular integrity are further supported by the chemical environment provided by the novel mixture of keratinocyte medium and M199, both of which have been separately used in organ cultures of the human (Nevalainen et al. 1993; Nevalainen et al. 1997; Varani et al. 1999; Parrish et al. 2002) and rat (Martikainen 1987; Martikainen and Isaacs 1990) prostate. The mixture of the two formulations has been chosen here because of our earlier observation of its ability to maintain primary human prostatic epithelial cells in a tight cobblestone arrangement in monolayer culture (unpublished observation). Outgrowing sheets of epithelial cells have also been regularly seen here, wherever epithelial cells from cut glands contact the bottom surface of the culture dish. Growth of stromal fibroblasts has not been detected, suggesting that the medium is highly selective for epithelial cell growth on tissue culture plastic. Within tissue slices, however, the medium has been shown to favor not only glandular epithelial, but also stromal and endothelial cell survival and to support the maintenance of an in vivo-like tissue morphology. Whereas, in many previous studies, a loss of glandular cytoarchitecture as manifested by basal cell proliferation and the disappearance of secretory cells has been detected after 4 days of culture (Parrish et al. 2002; Varani et al. 1999), under the present culture conditions, excellent tissue structure is maintained for a minimum of 7 days. The maintenance of a single layer of basal cells may be attributed, at least in part, to the serum-free composition of the culture medium as, in the prostate tissue-slice model of Parrish et al. (2002), the addition of FCS has been found to promote squamous metaplasia. The exact factors contributing to the remarkable ability of K-SFM/M199 to promote prostate tissue survival remain unclear. As K-SFM is of proprietary composition, the precise formulation of the medium remains obscure. Survival of the tissue slices without added growth factors suggests that, under the present culture conditions, the appropriate growth factors might be produced within the tissue itself.

In agreement with previous in vitro studies, the survival of glandular epithelia has been shown to be dependent on DHT supplementation (Nevalainen et al. 1993, 1997; Parrish et al. 2002; Varani et al. 1999). In the presence of 1 nM DHT, half of the glands inside tissue slices display columnar or cuboidal phenotype. Whether a higher dose of DHT or the addition of growth factors (e.g., epidermal growth factor) would further increase this proportion remains to be studied. Of note, histological sections of cultured tissue slices include cross sections of the undulating de novo epithelium on the tissue surface. As the epithelial sheet has a squamous, often stratified, appearance, the analysis may be biased toward a higher percentage of glands displaying the flat/metaplastic phenotype.

In the absence of DHT, a significantly higher proportion of dead or deficient secretory epithelia has been detected, resembling the effects of castration in vivo (Ohlson et al. 2005). Of interest, under the present culture conditions, approximately 30% of glands retain the columnar or cuboidal phenotype, even in the absence of DHT. In the clinic, androgen ablation is the standard therapy for metastatic prostate cancer and is known to result in increased apoptosis and rapidly decreased proliferation in primary tumor cells and in adjacent non-malignant prostate glands (Ohlson et al. 2005). As, in the present culture model, no other growth- or differentiation-promoting agents apart from DHT have been used, the method may serve as a tool to elucidate the effects of various biological factors on prostate tissue in vitro.

Considerable cell proliferation has been shown to occur solely on the tissue surface where the epithelium migrates to cover the surface of the tissue. In intact glandular structures, Ki67 expression remains scarce, which is in agreement with the known slow proliferation rate (turnover time of 2 years) of the normal prostatic epithelium (Peehl 2005). Interestingly, the surface epithelium has been shown to consist of a stratified basal cell compartment covered with secretory cells. The secretory cells retain their characteristic expression of cytokeratin 18, AR, and PAP throughout the experimental period, and a few cells have

been observed to express PSA. Although epithelial outgrowth as such is a well-documented phenomenon (Parrish et al. 2002; Varani et al. 1999), maintenance of the secretory phenotype of the epithelium covering tissue explants has not been previously described. Its preservation may be taken as a further indication of the survival- and differentiation-promoting quality of the novel medium mixture applied herein.

Vascular endothelial cells are an integral component of the tissue microenvironment. These cells are known to produce a number of active substances having manifold effects on the normal physiology and metabolism of organs and tissues (Pirtskhalaishvili and Nelson 2000). Endothelial cells and their paracrine factors are suggested to play a significant role in the pathophysiology of prostate cancer (Pirtskhalaishvili and Nelson 2000; Izawa and Dinney 2001). This is the first report to demonstrate viable vascular endothelia in human prostate explants. A marked interindividual variation in their survival has, however, been observed.

Although the significance of tissue slices as experimental models of manifold human tissues is currently well appreciated, their use in prostate research has remained limited. Recently, however, human prostate slices have been employed to validate, in the tissue context, checkpoint marker-protein responses observed in primary epithelial cell cultures following irradiation, thereby elucidating one of the mechanisms predisposing prostate epithelium to malignant transformation (Kiviharju-af Hällström et al. 2007). Enclosing the structural and functional features of the tissue in vivo, prostate slices of non-malignant and malignant tissues provide a unique tool not only to study the mechanisms of cancer, but also to test novel means for its prevention and cure. The model may also be used experimentally to address questions related to the role of vitamins and other dietary factors in normal prostate physiology and in the etiology of prostate pathologies (Tuohimaa et al. 2004). Development of the present slice culture model has been undertaken as part of a search for an in vitro test system for novel anti-androgenic drugs. End points yet to be validated for this purpose include morphological parameters and the epithelial expression of androgen-regulated proteins and apoptosis markers.

In conclusion, the development of in vitro models displaying normal tissue structure and function of the human prostate are critical for exploring the mechanism of prostate cancer and for preclinical testing of novel preventive and therapeutic regimens. The tissue-slice culture model presented here provides an in vitro setting in which the tissue architecture and differentiated phenotype of normal human prostatic tissue is well preserved. As the model promotes the survival of the glandular epithelial compartment and that of stromal and endothelial cells, it provides a unique in-vivo-like microenvironment for studies of human prostate function and dysfunction and may help to circumvent problems encountered with the use of continuous cell lines and animal models.

Acknowledgements We thank Mirja Hyppönen for her excellent technical assistance and Heini Huhtala for statistical advice.

References

Bayne CW, Donnelly F, Chapman K, Bollina P, Buck C, Habib FK (1998) A novel coculture model for benign prostatic hyperplasia expressing both isoforms of 5a-reductase. J Clin Endocrinol Metab 83:206–213

Bläuer M, Heinonen PK, Martikainen PM, Tomás E, Ylikomi T (2005) A novel organotypic culture model for normal human endometrium: regulation of epithelial cell proliferation by estradiol and medroxyprogesterone acetate. Hum Reprod 20:864–871

Chung LWK, Davies R (1996) Prostate epithelial differentiation is dictated by its surrounding stroma. Mol Biol Rep 23:13–19

Cunha GR, Cooke PS, Kurita T (2004) Role of stromal-epithelial interactions in hormonal responses. Arch Histol Cytol 67:417–434

Fong C-J, Sherwood ER, Sutkowsky DM, et al (1991) Reconstituted basement membrane promotes morphological and function differentiation of primary human prostate epithelial cells. Prostate 19:221–235

González-Martinez JA, Bingaman WE, Toms SA, Najm IM (2007) Neurogenesis in the postnatal human epileptic brain. J Neurosurg 107:628–635

Goossens K, Deboel L, Swinnen JV, Roskams T, Manin M, Rombauts W, Verhoeven G (2002) Both retinoids and androgens are required to maintain or promote functional differentiation in reaggregation cultures of human prostate epithelial cells. Prostate 53:34–49

Hall JA, Maitland NJ, Stower M, Lang SH (2002) Primary prostate stromal cells modulate the morphology and migration of primary prostate epithelial cells in type I collagen gels. Cancer Res 62:58–62

Hudson DL, O'Hare M, Watt FM, Masters JR (2000) Proliferative heterogeneity in the human prostate: evidence for epithelial stem cells. Lab Invest 80:1243–1250

Izawa JI, Dinney CPN (2001) The role of angiogenesis in prostate and other urologic cancers: a review. Can Med Assoc J 164:662–670

Kern MA, Haugg AM, Eiteneuer E, Konze E, Drebber U, Dienes HP, Breuhahn K, Schirmacher P, Kasper HU (2006) Ex vivo analysis of antineoplastic agents in precision cut tissue slices of human origin: effects of cyclooxygenase-2 inhibition in hepatocellular carcinoma. Liver Int 26:604–612

Kiviharju-af Hällström TM, Jäämaa S, Mönkkönen M, Peltonen K, Andersson LC, Medema RH, Peehl DM, Laiho M (2007) Human prostate epithelium lacks Wee1A-mediated DNA damage-induced checkpoint enforcement. Proc Natl Acad Sci USA 104:7211–7216

Lang SH, Stower M, Maitland NJ (2000) In vitro modelling of epithelial and stromal interactions in non-malignant and malignant prostates. Br J Cancer 82:990–997

Lang SH, Sharrad RM, Stark M, Villette JM, Maitland NJ (2001) Prostate epithelial cell lines form spheroids with evidence of glandular differentiation in three-dimensional Matrigel cultures. Br J Cancer 85:590–599

Lerche-Langrand C, Toutain HJ (2000) Precision-cut liver slices: characteristics and use for in vitro pharmaco-toxicology. Toxicology 153:221–253

Liu AY, Peehl DM (2001) Characterization of cultured human prostatic epithelial clells by cluster designation antigen expression. Cell Tissue Res 305:389–397

Margolis L, Hatfill S, Chuaqui R, Vocke C, Emmert-Buck M, Linehan WM, Duray PH (1999) Long term organ culture of human prostate tissue in a NASA-designed rotating wall bioreactor. J Urol 161:290–297

Martikainen P (1987) Maintenance of adult rat ventral prostate in organ culture. Anat Rec 218:166–174

Martikainen P, Isaacs J (1990) Role of calcium in the programmed death of rat prostatic glandular cells. Prostate 17:175–187

Nave R, Fisher R, Zech K (2006) In vitro metabolism of ciclesonide in human lung and liver precision-cut tissue slices. Biopharm Drug Dispos 27:197–207

Nevalainen MT, Valve EM, Ping W, Nurmi M, Härkönen PL, Martikainen PM (1993) Hormone regulation in human prostate in organ culture. Cancer Res 53:5199–5207

Nevalainen MT, Valve EM, Ingleton PM, Nurmi M, Martikainen PM, Härkönen PL (1997) Prolactin and prolactin receptors are expressed and functioning in human prostate. J Clin Invest 99:618–627

Ohlson N, Wikström P, Stattin P, Bergh A (2005) Cell proliferation and apoptosis in prostate tumors and adjacent non-malignant prostate tissue in patients at different time-points after castration treatment. Prostate 62:307–315

Parrish AR, Sallam K, Nyman DW, Orozco J, Cress AE, Dalkin BL, Nagle RB, Gandolfi AJ (2002) Culturing precision-cut human prostate slices as an in vitro model of prostate pathobiology. Cell Biol Toxicol 18:205–219

Peehl DM (2004) Are primary cultures realistic models of prostate cancer? J Cell Biochem 91:185–195

Peehl DM (2005) Primary cell cultures as models of prostate cancer development. Endocr Relat Cancer 12:19–47

Peehl DM, Sellers RG (2002) Epithelial cell culture: prostate. In: Atala A, Lanza R (eds) Methods of tissue engineering. Academic Press, San Diego, pp 247–261

Pirtskhalaisvili G, Nelson JB (2000) Endothelium-derived factors as paracrine mediators of prostate cancer progression. Prostate 44:77–87

Takagi A, Watanabe M, Ishii Y, Morita J, Hirokawa Y, Matsuzaki T, Shiraishi T (2007) Three-dimensional cellular spheroid formation provides human prostate tumor cells with tissue-like features. Anticancer Res 27:45–53

Tlsty TD (2001) Stromal cells can contribute oncogenic signals. Semin Cancer Biol 11:97–104

Tuohimaa P, Tenkanen L, Ahonen M, Lumme S, Jellum E, Hallmans G, Stattin P, Harvei S, Hakulinen T, Luostarinen T, Dillner J, Lehtinen M, Hakama M (2004) Both high and low levels of blood vitamin D are associated with a higher prostate cancer risk: a longitudinal, nested case-control study in the Nordic countries. Int J Cancer 108:104–108

Varani J, Dame MK, Wojno K, Schuger L, Johnson KJ (1999) Characteristics of nonmalignant and malignant human prostate in organ culture. Lab Invest 79:723–731

Webber MM, Bello D, Kleinman HK, Hoffman MP (1997) Acinar differentiation by non-malignant immortalized human prostatic epithelial cells and its loss by malignant cells. Carcinogenesis 18:1225–1231