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Human Prostatic Smooth Muscle Cells in Culture: Estradiol Enhances Expression of Smooth Muscle Cell-Specific Markers

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ABSTRACT: Smooth muscle cells (SMCs) constitute a major cellular component of prostatic stroma. SMC tension plays an important role in urethral obstruction secondary to benign prostatic hyperplasia (BPH). We have developed an in vitro procedure for the propagation of human prostatic SMCs. Tissue specimens from patients undergoing radical prostatectomy or cystectomy were enzymatically disaggregated and cultured in MCDB-131 medium supplemented with horse serum, insulin, conditioned medium from the tumor cell line CRL-5813, and steroid hormones. The medium was assembled on the basis of the effects these supplements have on the growth of SMC cultures and on the expression of the two markers desmin and smooth muscle myosin. Addition of 0.1 μM of estradiol to the growth medium dramatically increased expression of these SMC-specific markers. Dihydrotestosterone (DHT) and hydrocortisone had a similar, albeit less pronounced effect. At three to five passages, about two thirds of the cells were immunohistologically positive for smooth muscle myosin or desmin. Almost all cells were positive for the myofibroblast marker smooth muscle α-actin throughout 10 passages and more. In SMC cultures, cells staining for smooth muscle myosin and desmin were found to seek direct contact to myofibroblasts. They grew in aggregates on a layer of myofibroblasts which adhered to the surface of the culture vessel. As revealed by transmission electron microscopy the cultured cells exhibited morphological features of myofibroblasts. Characteristics of smooth muscle cells, such as prominent bundles of microfilaments associated with dense bodies, basal laminae investing the cells, and numerous caveolae at the cell surfaces were regularly observed in cultures of low passages. After several passages, these features were markedly decreased and organelles of the biosynthetic system became more prominent. In summary, we present an in vitro model of prostatic SMCs and demonstrate that steroid hormones have characteristic effects on these cells. SMC cultures are expected to facilitate investigation of the functions and properties of human prostatic SMCs. *Prostate 30:117–129, 1997.*

KEY WORDS: **human prostate; smooth muscle cells; cell culture; estradiol; smooth muscle myosin; desmin**

INTRODUCTION

The stromal compartment of the prostate plays a major role in the pathogenesis of benign prostatic hyperplasia (BPH), which is the most common benign tumor in males [1]. In hyperplastic tissue the volumetric amounts of stromal tissue and glandular lu-

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men are significantly increased [2–5]. Smooth muscle cells (SMCs) are one of the main cellular components of prostatic stroma [6]. In human BPH tissue as well as in animal BPH models there is an increase in the fibromuscular portion of the gland [6–9]. Contraction of the SMCs contributes to the urethral obstruction observed in BPH patients. Therefore, SMC relaxants such as alpha receptor antagonists can be successfully used to improve BPH symptoms [10–13]. Apart from inhibitors of 5-alpha reductase, these substances are considered to be the most promising drugs for non-surgical treatment of BPH [14,15]. Their efficacy was shown to depend on the proportion of SMCs in BPH tissue [16].

The stromal compartment also plays an important role in the development and function of the prostate. Stromal cells, which are responsive to steroid hormones, regulate the development of the epithelial compartment of the prostate during embryogenesis [17–21]. They are capable of metabolizing steroids, and produce growth factors such as the keratinocyte growth factor for the epithelial cells [22–24].

In order to set up an in vitro system for the characterization of human prostatic SMCs, we have developed a technique for isolating these cells from tissue specimens and growing them in cell cultures. We investigated the growth factor requirements for serial propagation and the effects of steroid hormones on the expression of SMC-specific markers.

MATERIALS AND METHODS

Preparation of SMC Cultures

The tissue cultures were obtained from patients undergoing radical prostatectomy or cystectomy. The prostates were examined by a pathologist and subsequently specimens were excised from nonmalignant areas. The tissue specimens were cut into 1-3 mm^3 pieces and washed twice with a wash solution (calcium and magnesium-free Hank's balanced salt solution [CMF-HBSS], GIBCO, Grand Island, NY) containing 2% (v/v) dialysed horse serum (Biological Industries, Israel), 1% (v/v) penicillin-streptomycin solution (5,000 IU/ml and 5,000 μg/ml, GIBCO-BRL), and 25 ng/ml of amphotericin B (Biological Industries). Pieces obtained from 0.3–0.5 g of tissue were then incubated for 4–5 hr at 37°C in 10 ml of dissociation solution (200 U/ml of collagenase [Yakult Pharmaceutical Industries, Ltd., Yakult, Japan], 0.1 mg/ml of elastase [from porcine pancreas Type II, E. C. 3.4.21.36, Sigma Chemicals, St. Louis, MO], 40 U/ml of deoxyribonuclease I [from bovine pancreas E.C. 3.1.21.1, Sigma], 50 μg/ml of trypsin inhibitor [from soy bean, Sigma] in MCDB-131 medium supplemented with 5% [v/v] dialysed horse serum, 1% [v/v] penicillin-streptomycin solution, 25 ng/ml of amphotericin B, and 10 mM of HEPES, pH 7.2). The suspension was gently stirred by means of a magnetic bar. Released cells were poured through a 100 μm cell culture mesh and washed twice with 30-40 ml of wash solution. After each wash, the cells were collected by centrifugation for 10 min at 800*g*, and finally they were resuspended in SMC medium. Subsequently, the cell suspension was poured through a 40 μm mesh and viable cells were determined by means of trypan blue (0.4%) staining. Each gram of prostatic tissue yielded approximately 3×10^6 viable cells.

Isolated cells were cultured in collagen-coated cell culture dishes (NUNC, Denmark) at a density of 2×10^4 viable cells/cm^2. Collagen coating was achieved by covering the bottom of the flasks with a collagen solution (5 μg/cm^2, Collagen S solution, Boehringer Mannheim, Germany, diluted 1:20 with water) and subsequent evaporation to dryness. The base medium consisted of MCDB-131 (Sigma) supplemented with 15% dialysed horse serum, 10 mM of HEPES, pH 7.2, 2% penicillin-streptomycin solution, and 2% (v/v) MEM-EAGLE solution of nonessential amino acids (Biological Industries). Further compounds added to the base medium specified in the Results section and the figures were 5 μg/ml of insulin (human recombinant from yeast, Boehringer Mannheim), 10 μg/ml of transferrin (from human serum, partially iron saturated, Boehringer Mannheim), 5 ng/ml of sodium selenite (Sigma), 0.1 μM of estradiol (Sigma), 0.1 μM of dexamethasone (Sigma), and 1/3 (v/v) conditioned medium from the prostatic carcinoma cell lines CRL-5813 or LNCaP. SMC cultures were maintained in an atmosphere of 5% CO_2/95% humidified air. Every 2–3 days, the medium was changed. After reaching confluence, usually after 7–10 days, the cells were passaged into new flasks at a ratio of 1:2 using trypsin/EDTA solution (Boehringer Mannheim). The epithelial cells which were initially present in the digest had a poor attachment rate and the few cells that did attach were soon overgrown by the proliferating stromal cells.

Preparation of Myofibroblast Cultures

Prostatic fibroblast cells were derived from small pieces of prostatic tissue, placed on the bottom of cell culture flasks, and cautiously covered with medium (Dulbecco's modified minimal essential medium [DMEM], supplemented with 20% fetal calf serum [FCS] and antibiotics). After a few days, cells began to emerge from the pieces and started growing on the surface of the culture vessel. For subculturing, the cells were detached with trypsin/EDTA solution and transferred at a ratio of 1:3–1:4. After two to three

TABLE I. Immunostaining of Cultured Prostatic Stromal Cells

Antibody cell type	Anti-prolyl-4-hydroxylase (anti-fibroblast)	Anti-vimentin	Anti-smooth muscle α-actin	Anti-smooth muscle myosin	Anti-desmin[a]
Skin fibroblasts	+	+	−	−	−
Prostatic myofibroblasts	+	+	+	−	−
SMCs	+	+	+	+	+

[a]Desmin staining only in confluent cultures.

passages the residual epithelial cells, which in these conditions had a very low plating efficiency, got lost. On account of their positive staining with anti-smooth muscle α-actin antibody the remaining cells were identified as myofibroblasts (see Results and Table I).

Conditioned Medium From Tumor Cell Lines

Tumor cell lines LNCaP and CRL-5813 were obtained from the American Type Culture Collection (ATCC). LNCaP cells are androgen-sensitive tumor cells derived from a lymph node metastasis of a prostate cancer patient [25]. CRL-5813 cells, initially named NCI-H660, were isolated from a lymph node metastasis during studies performed to establish small cell lung cancer cell lines [26,27]. It is a small cell carcinoma cell line expressing neuroendocrine markers. The ATCC established the prostatic origin of these tumor cells. LNCaP and CRL-5813 cells were grown in SMC base medium. The conditioned medium used for SMC cultures was obtained by growing subconfluent (50–75% confluence) cultures in base medium for 3–5 days. The cells were separated by centrifugation thereafter and the medium was stored at 4°C until use.

Immunohistochemical Staining

Cells to be examined for cytoskeletal markers and prolyl-4-hydroxylase were grown on chamber slides (26 × 21 mm) until they reached confluence. Then the cells were gently washed with phosphate-buffered saline (PBS), pH 7.4, and fixed in acetone for 5 min at room temperature. Nonspecific binding sites were blocked by incubation in block solution (1.5% [v/v] horse serum, 1% [w/v] bovine serum albumin [BSA] in PBS) for 1 hr at room temperature. Cytoskeletal proteins were stained with the monoclonal mouse antibodies anti-myosin (smooth muscle, clone hSM-v; Sigma), anti-desmin (clone 33; Sanbyo), anti-α-actin (smooth muscle, clone $1A_4$; Sigma), and anti-vimentin (Vim 3B4; DAKO, Denmark). Antibody solutions were diluted 1:400, 1:100, 1:500, and 1:150, respectively, in block solution. The fibroblast enzyme prolyl-4-hydroxylase (E. C. 1.14.11.2) was stained with the anti-fibroblast antibody 5B5 (DAKO, dilution 1:66). Slides were covered with antibody solutions for 2 hr at room temperature. A monoclonal antibody of the same isotype but with unknown specificity (DAKO) was used as a negative control. After being washed thoroughly three times with PBS, the slides were further developed with a modified ABC method [28] using an anti-mouse IgG streptavidin kit (Vectastain, Vector). The slides were first incubated with biotinylated horse anti-mouse IgG antibody solution (dilution 1:200 in block solution) for 30 min at room temperature followed by incubation with horseradish peroxidase-streptavidin complex (dilution 1:100 in block solution) for 30–60 min. The slides were developed for 10 min at room temperature in 3,3′-diaminobenzidine tetrahydrochloride (DAB) solution in the presence of hydrogen peroxide and subsequently washed in water. Cells were slightly counterstained with hematoxylin solution for about 10 sec, washed in tap water, dehydrated in a series of ethanol solutions, dried, mounted with gel mount (Biomedica, Austria), and sealed with varnish after drying for about 1 hr. Cells exhibiting brownish-black cytoplasmic staining with either of the antibodies were considered positive.

Proliferation Assay

Cells were seeded into 24-well cell culture plates in base medium at a density of 16,000 cells per well. On the following day, designed as day 0, the base medium was replaced with other media; then the cells were cultured as described and the media were replaced again on day 2. On days 3 and 4 the cell mass was measured by assessing the ATP content of the cultures with an ATP proliferation assay (Cyto-Pro-480 Bio-Orbit, Finland). The cells were washed with PBS and lysed with somalyse solution. ATP content was determined with a luminometer according to the instructions of the manufacturer and calculated in relation to that of control samples, which were cultured in base medium throughout the experiment.

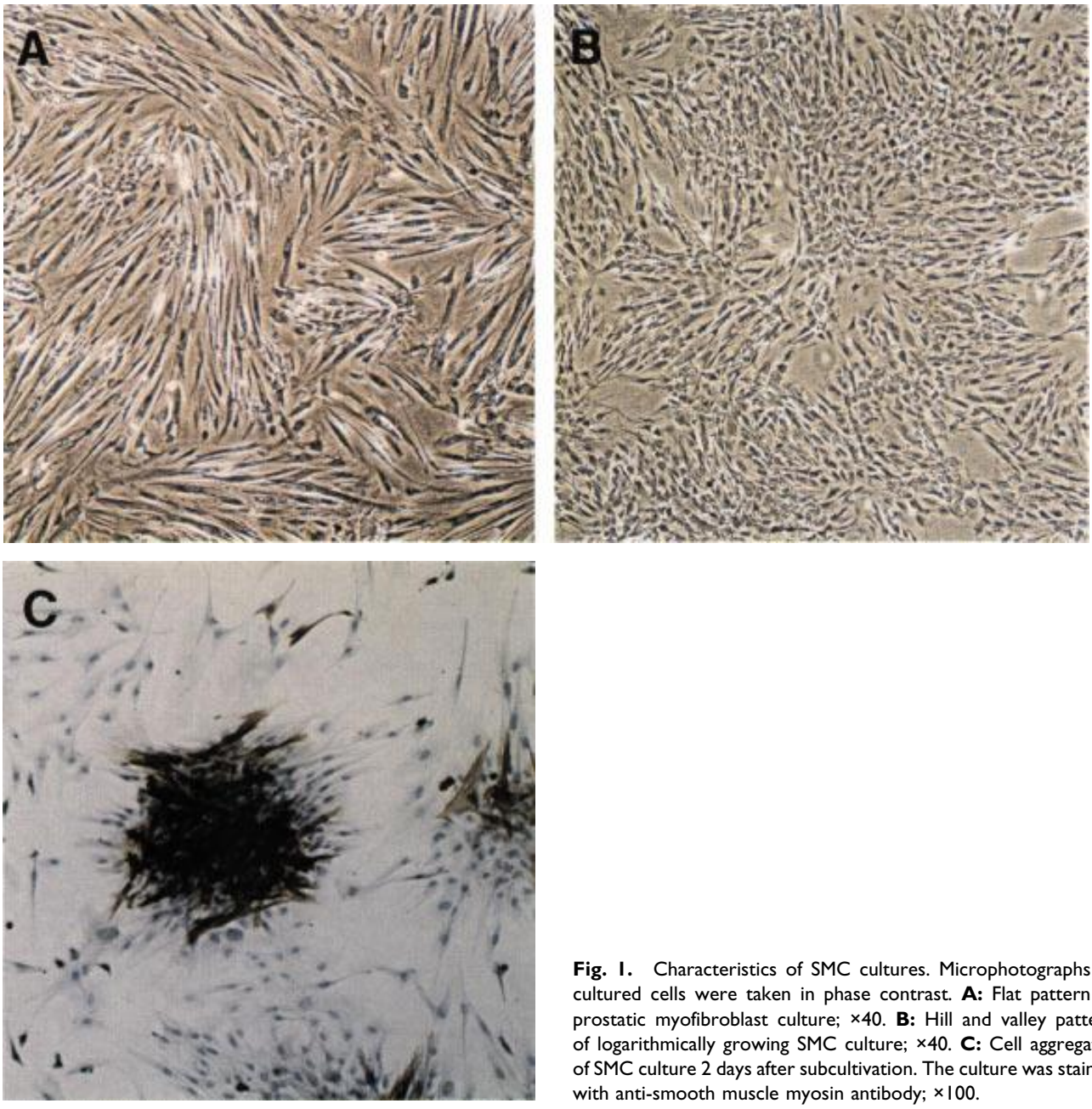

Fig. 1. Characteristics of SMC cultures. Microphotographs of cultured cells were taken in phase contrast. **A:** Flat pattern of prostatic myofibroblast culture; ×40. **B:** Hill and valley pattern of logarithmically growing SMC culture; ×40. **C:** Cell aggregates of SMC culture 2 days after subcultivation. The culture was stained with anti-smooth muscle myosin antibody; ×100.

Transmission Electron Microscopy

Cultured cells and biopsy specimens were prepared for electron microscopy according to standard protocols. They were fixed in 2.5% (v/v) glutaraldehyde in PBS, pH 7.4, for 1–2 hr at 4°C, followed by several rinses in distilled water for altogether 30 min. After postfixation with OsO_4 (1% w/v in water) for 4–8 hr at 4°C, the samples were rinsed for about 30 min in distilled water, dehydrated in a graded ethanol series, and embedded in Epon. Ultrathin sections (80 nm) were stained at 25°C with aqueous uranyl acetate (0.5% w/v) for 40 min followed by lead citrate solution for 5 min. The samples were examined with an electron microscope at 60 and 80 kV (Zeiss EM 10A, Zeiss, Oberkochen Germany).

RESULTS

Immunohistochemical Characterization of SMC Cultures

SMC cultures were established from nonmalignant prostate specimens obtained from patients undergoing radical prostatectomy or cystectomy as described

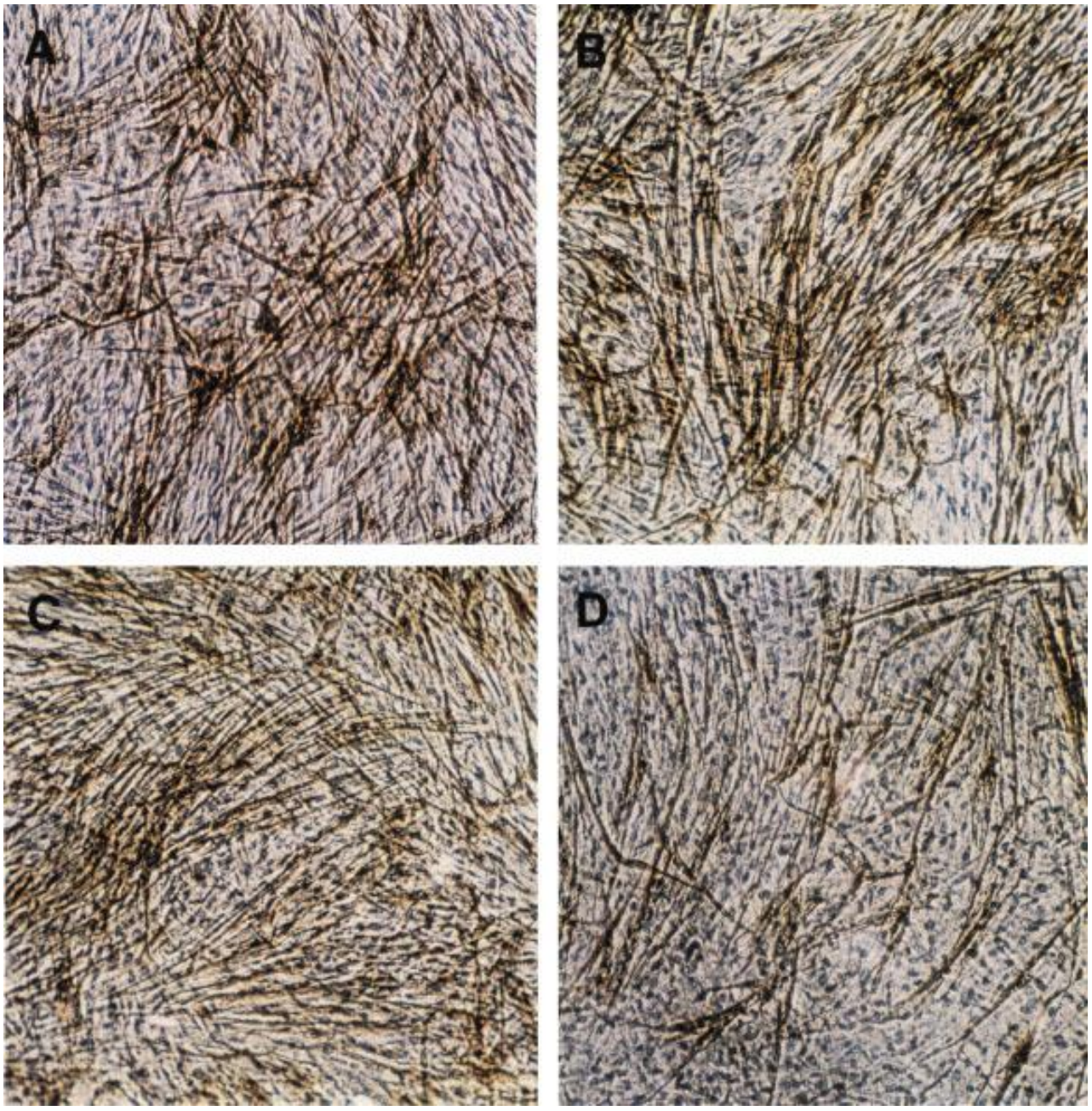

Fig. 2. Growth of prostatic SMCs on feeder layers. Aliquots of an SMC culture seeded onto irradiated (150 gray, γ-source) or unirradiated prostatic fibroblasts, onto skin fibroblasts, or directly onto plastic. After incubation for 10 days, cultures were stained with anti-smooth muscle myosin antibody. ×100. **A:** SMCs grown directly on plastic surface. **B:** SMCs grown on monolayer of unirradiated prostatic myofibroblasts. **C:** SMCs grown on monolayer of irradiated prostatic myofibroblasts. **D:** SMCs grown on monolayer of skin fibroblasts.

in detail in Materials and Methods. Expression of the cell type markers smooth muscle α-actin [29], smooth muscle myosin [30,31], desmin [32], vimentin [33,34], and prolyl-4-hydroxylase [35] was investigated immunohistochemically in our prostatic SMC cultures and compared to prostatic and skin fibroblast cultures (Table I). All types of cells stained for the fibroblast marker prolyl-4-hydroxylase and the mesenchymal cell marker vimentin. Smooth muscle α-actin was detected not only in SMC cultures but also in prostatic fibroblast cultures. In contrast, skin fibroblast cultures were α-actin negative. The fact that this marker

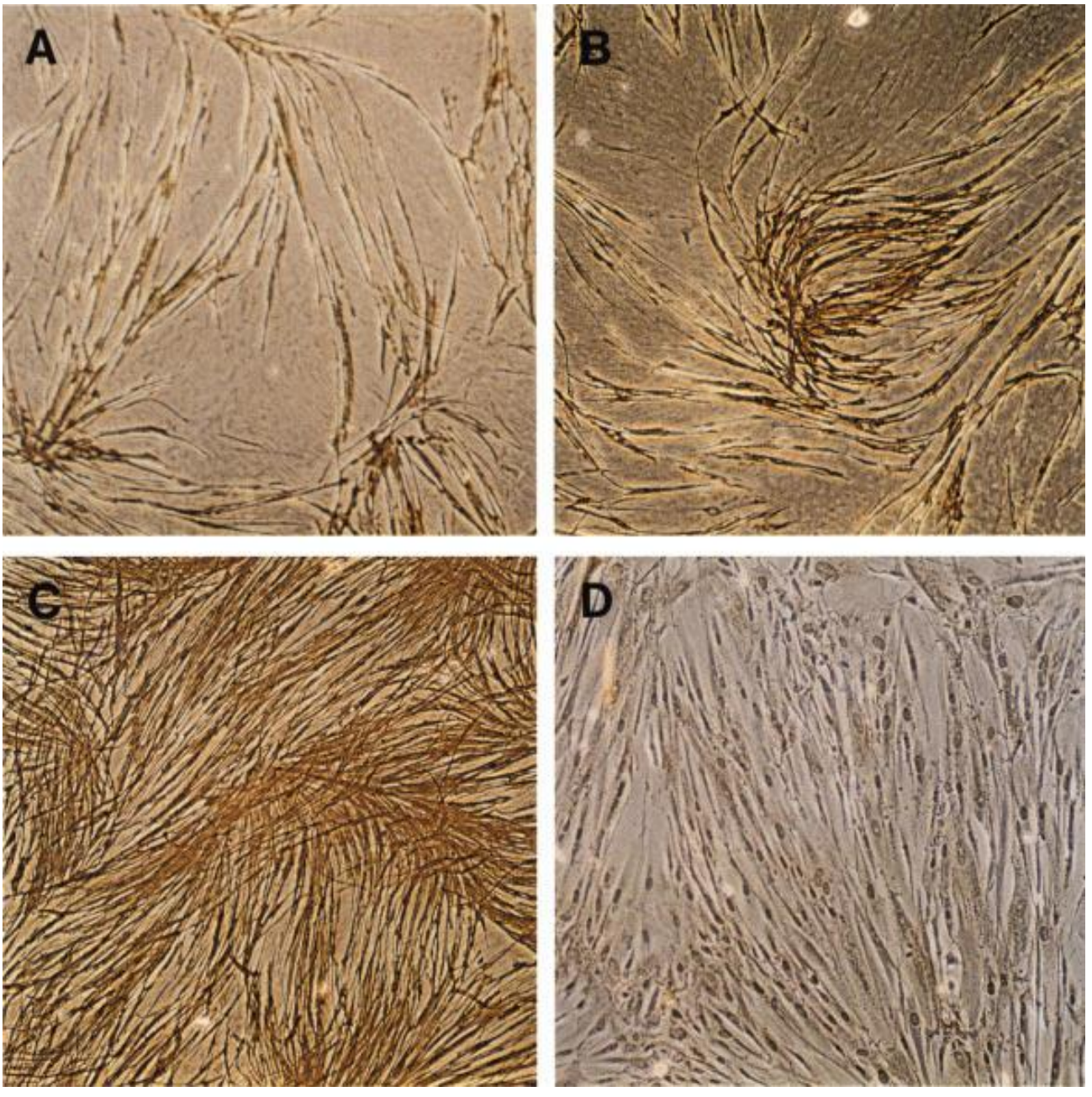

Fig. 3. Effect of estradiol and DHT on desmin expression. SMC cultures were grown in selective medium with or without the addition of steroid hormones. Confluent cultures were stained with anti-desmin antibody. ×100. **A:** Without steroid hormone supplementation. **B:** Supplementation with 100 nM of DHT. **C:** Supplementation with 100 nM of estradiol. **D:** Control myofibroblast culture.

is expressed by prostatic fibroblasts provides evidence that these cells are so-called myofibroblasts. In SMC cultures, antibodies against smooth muscle myosin and the muscle cell marker desmin specifically stained SMCs. Desmin was expressed only in confluent SMC cultures, whereas proliferating SMCs were negative for it. Conversely, smooth muscle myosin was expressed independent of cell density. These data demonstrate that the marker smooth muscle α-actin permits discrimination between fibroblasts (negative) and myofibroblasts (positive), whereas smooth muscle myosin and desmin can be employed to distinguish between myofibroblasts (negative) and SMCs (positive).

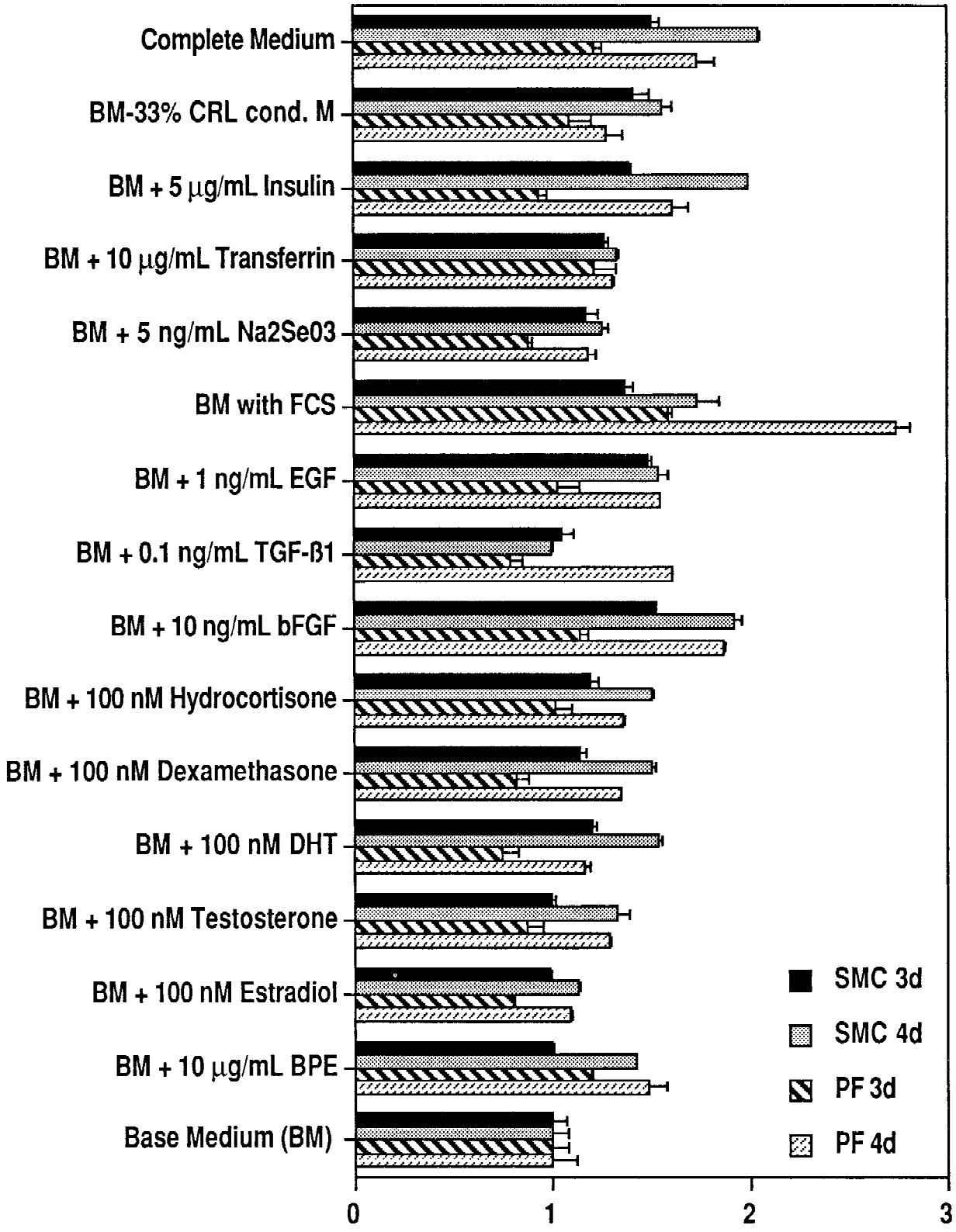

Fig. 4. Effect of growth factors, hormones, and conditioned medium on growth of SMC cultures as compared to myofibroblast cultures. Logarithmically growing cultures were treated for 3 or 4 days in base medium with the individual supplements indicated. Then the cell mass was determined by luminometric measurement of cellular ATP. SMC, prostatic SMC culture, passage 4. PF, prostatic fibroblast culture, passage 8, BPE = bovine pituitary extract.

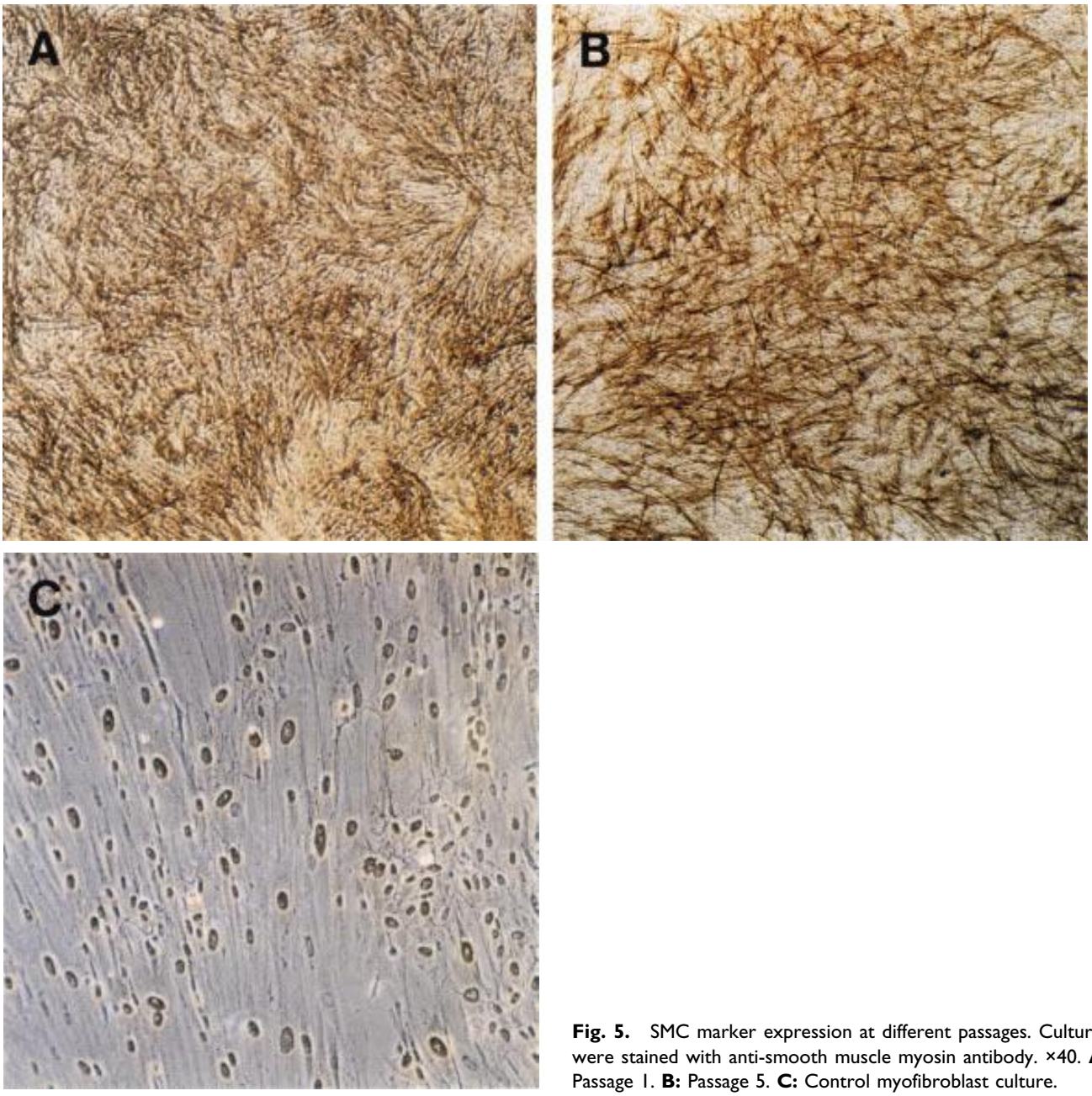

Fig. 5. SMC marker expression at different passages. Cultures were stained with anti-smooth muscle myosin antibody. ×40. **A:** Passage 1. **B:** Passage 5. **C:** Control myofibroblast culture.

Growth Characteristics of SMC Cultures

The immunohistochemical staining pattern showed that our SMC cultures contained smooth muscle cells which were positive for α-actin, myosin, and desmin, and myofibroblasts which were positive for α-actin. After subculturing, myofibroblasts spread regularly and rapidly attached to the surface of culture vessels. By comparison, SMCs attached more slowly and sought contact to myofibroblasts. The latter proliferated until they formed a dense monolayer (Fig. 1A), while SMCs grew in aggregates on the myofibroblasts and formed a ''hill and valley'' pattern (Fig. 1B). This striking growth pattern became noticeable when the aggregates were stained with the smooth muscle myosin-specific antibody (Fig. 1C).

After subculturing, the majority of SMCs settled on myofibroblasts. SMCs that had accidentally seeded directly onto the vessel surface were able to adhere, but there were no signs of proliferation. These cells were always isolated, as can be seen in Figure 1C. By the time the cultures had reached confluence, the bottom layer was completely negative for smooth muscle

myosin or desmin, which indicates that SMCs which had initially attached directly to the surface lost their ability to express this marker and became myofibroblasts or that these cells had died or detached from the surface when the cultures became confluent. We maintained a high cell density in culture and subcultured at a transfer ratio of 1:2 in order to guarantee contact between SMCs and myofibroblasts.

The importance of a close contact between SMCs and myofibroblasts also became obvious when we investigated the effect of feeder layers. Cells from an SMC culture were seeded onto monolayers of irradiated as well as unirradiated prostatic myofibroblasts and of skin fibroblasts, or onto uncoated dishes. Following incubation for 10 days, the cultures were fixed and stained with anti-myosin (Fig. 2). SMCs grew equally well on irradiated and unirradiated prostatic myofibroblasts as well as on uncoated dishes; however, there was a significant reduction in the number of cells, if they were grown on skin fibroblasts. This finding indicates that contact of SMCs with myofibroblasts supports the growth of SMCs and/or slows down their gradual transformation into myofibroblasts.

Effects of Steroid Hormones, Growth Factors, and Tumor Cell-Conditioned Media on SMC Cultures

We investigated the effect of several steroid hormones, growth factors, and tumor cell-conditioned media on the expression of SMC-specific markers on the one hand, and on cell growth on the other. Cells were grown in base medium for 24 hr. Subsequently, the medium was supplemented with several individual factors, and incubation was continued until the cultures reached confluence with the medium being changed every third day. The cultures were then stained for myosin or desmin by means of immunohistochemistry. The results revealed that expression of SMC markers was stimulated by the steroid hormones estradiol and dexamethasone at concentrations of 100 nM, and by conditioned medium from the prostatic carcinoma cell line CRL-5813. Essentially the same, albeit slightly less pronounced, effects were achieved with 100 nM of the androgens dihydrotestosterone (DHT) and testosterone and the glucocorticoid hydrocortisone as well as with conditioned medium from LNCaP cells. The dramatic increase in the expression of desmin in the presence of estradiol and the moderate increase in the presence of DHT are shown in Figure 3. SMC marker expression was inhibited by 5–30 μg/ml of bovine pituitary extract in a concentration-dependent manner, and by 1 ng/ml of epidermal growth factor (EGF) or 0.1 ng/ml of transforming growth factor (TGF)-β1 (not shown).

In order to identify growth factors that support the growth of SMC cultures, we compared the growth effects of medium supplements on SMC and pure myofibroblast cultures. The cultures were incubated in base medium for 24 hr and then supplemented with several individual factors for 3 or 4 days; subsequently growth was assessed by determining the ATP content of the cells (Fig. 4). The results showed that the growth of both cell types was stimulated by transferrin, sodium selenite, EGF, basic fibroblast growth factor (bFGF), and bovine pituitary extract. Insulin, DHT, dexamethasone, and conditioned medium from the CRL-5813 tumor cell line predominantly stimulated SMC cultures, whereas FCS showed a very strong effect on myofibroblast cultures. In 4 days, myofibroblasts in medium supplemented with FCS grew 1.6 times faster than those in medium supplemented with horse serum. TGF-β1 also stimulated the growth of myofibroblasts, however, it exerted its effect only on dense cultures.

Based on the growth data and the results of SMC marker expression, a medium for SMC cultures was designed: MCDB-131 supplemented with 15% dialysed horse serum, 2% nonessential amino acid solution, 10 mM of HEPES, pH 7.2, 5 μg/ml of insulin, 10 μg/ml of transferrin, 5 ng/ml of sodium selenite, 100 nM of estradiol, 100 nM of dexamethasone and 1/3 conditioned medium from the CRL-5813 tumor cell line, and antibiotics. In this complete medium, SMC cultures grew 1.2 times faster than myofibroblast cultures over a 4-day incubation period (Fig. 4). Transfer of myofibroblasts growing in DMEM supplemented with 10% FCS into the SMC medium caused a marked decline in growth (not shown). At three to five passages, about two thirds of the cells in SMC culture immunohistochemically stained for the SMC markers smooth muscle myosin or desmin (Fig. 5). With an increasing number of passages, expression of these markers gradually decreased. However, up to 10 passages and more almost all the cells were still found to stain for the myofibroblast marker smooth muscle α-actin.

Ultrastructure of Cultured SMCs

As revealed by electron microscopy, human prostatic SMCs grown in culture exhibited ultrastructural features of myofibroblasts (Fig. 6). The ultrastructural appearance of the cells changed with increasing passage numbers. Characteristics of SMCs, such as prominent bundles of microfilaments associated with dense bodies, basal laminae investing the cells, and densely packed caveolae at the cell surfaces, were regularly observed during the early passages (Fig. 6A,B), but were markedly decreased in a culture at

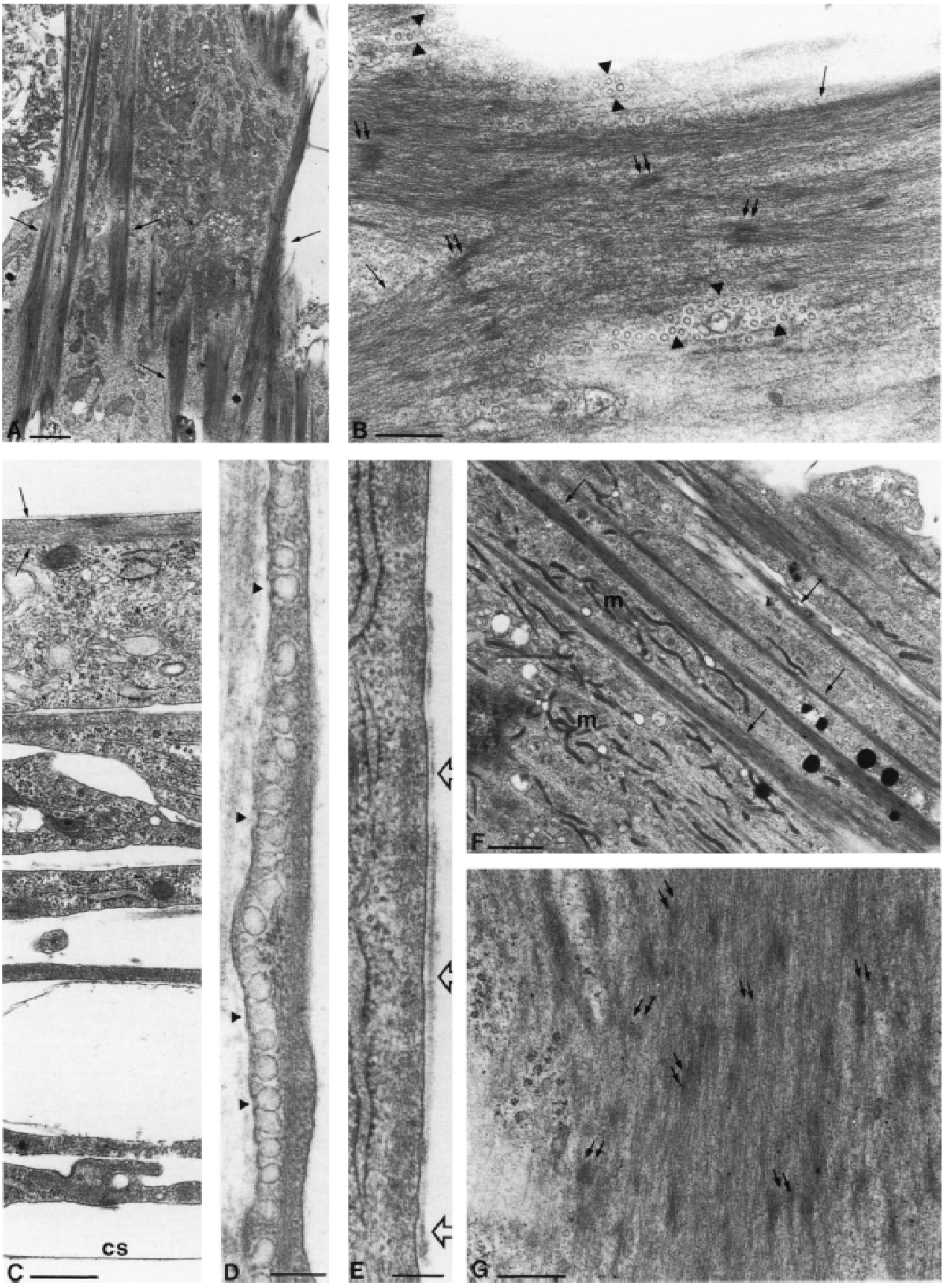

Fig. 6.

passage 7. On the other hand, organelles of the biosynthetic system including rough endoplasmic reticulum, Golgi apparatus, and secretory granules became more prominent at high passages. The cells grew in two or more layers (Fig. 6C). At passage 1 or 2 and 23 days in culture, caveolae and microfilaments were still present in the cells (Fig. 6D), but microfilaments were less densely packed and basal laminae appeared fragmented (Fig. 6E). Endoplasmic reticulum and Golgi membranes occupied wide cytoplasmic areas in these cells. In addition mitochondria were numerous and regularly exhibited a conspicuous dense matrix (Fig. 6F). After seven passages, the cells usually lacked caveolae and basal laminae were no longer apparent, but dense body-associated microfilaments could still be observed (Fig. 6G).

DISCUSSION

One of the major cellular components of prostatic stroma are the SMCs. We have developed a cell culture system for the growth of these cells which were obtained from human prostatic tissue. In our cell cultures, about half to two thirds of the cells stained for smooth muscle myosin and desmin at three to five passages. Practically all cells were positive for the myofibroblast marker smooth muscle α-actin. SMC content gradually decreased with increasing number of subcultivations. However, the cells remained positive for smooth muscle α-actin for more than 10 passages. These observations, as well as our electron microscopic data, indicate that SMC-specific proteins are gradually depleted in SMC cultures resulting in transformation of the culture into a myofibroblast culture. Therefore, it was impossible to establish a pure culture of prostatic SMCs. In order to enrich stromal cell cultures with SMCs, it was necessary to stimulate SMC growth without stimulating myofibroblast and fibroblast growth, and/or to halt downregulation of SMC marker expression. In accordance with this principle, we have developed a medium which is favorable for growth of SMCs. Horse serum has been previously reported to suppress proliferation of fibroblasts and myofibroblasts. Therefore, we used horse serum instead of FCS, which is a strong growth stimulator for fibroblasts. Other growth-stimulating components added to the SMC culture were insulin, dexamethasone, and conditioned medium from prostatic tumor cells. Both CRL-5813 and LNCaP cells were found to secrete factors into their medium which stimulate SMC proliferation. CRL-5813 cells, which had a stronger effect than LNCaP cells, were derived from a lymph node metastasis and were described as small-cell neuroendocrine tumor cells [27]. A stimulatory effect of prostate tumor cells on SMCs also is supported by the results of Kooistra et al. [36] who observed phenotypic differences between stromal explant cultures from BPH and tumor tissue. Their primary cultures from malignant tissue contained significantly more desmin and smooth muscle α-actin-positive cells than the cultures from nonmalignant lesions.

Our SMC cultures were similar to those of Ricciardelli et al. [37] who cultured SMCs from the prostates of guinea pigs. Their cultures also showed the characteristic ''hill and valley'' growth pattern and expression of desmin. As in our cultures, desmin expression decreased with time and the cells acquired a more myofibroblastoid phenotype. Recently, culturing of SMCs from BPH tissue has been reported [38]. More than 90% of these cultures were positive for smooth muscle α-actin, which, however, does not discriminate between SMCs and myofibroblasts.

Besides growth factors, steroid hormones showed a stimulatory effect in our SMC culture. Estradiol and dexamethasone, followed by DHT and hydrocortisone, another glucocorticoid, were the most potent ones. Estradiol exerted an influence on the expression of SMC markers rather than on growth of the SMC cultures. We measured only a minimal growth-stimulating effect of estradiol, whereas the influence of DHT on culture growth was more pronounced. These results suggest that estradiol either is capable of halting downregulation of smooth muscle myosin and desmin expression and thus slows down transformation of SMC cultures into myofibroblast cultures or it favors growth of cells expressing SMC myosin over the growth of myofibroblasts. Ricciardelli et

Fig. 6. Ultrastructure of cultured SMCs. SMC cultures at different passages were analyzed by transmission electron microscopy. **A,B:** Passage 0 (14 days in culture). Tangential sections through cultured SMCs show prominent microfilament bundles (arrows). In addition, dense bodies (double arrows) and caveolae (arrowheads) are clearly identified. A: ×600. Bar = 2 μm. B: ×24,000. Bar = 0.5 μm. **C:** Passage 1 (23 days in culture). Multiple layers of cultured SMCs are seen in a section perpendicular to the culture surface (cs). Microfilament bundles (arrows) are located merely in the cell periphery whereas most of the cells' volume is occupied by components of the biosynthetic system (e.g., endoplasmic reticulum, Golgi apparatus). ×12,000. Bar = 1 μm. **D:** Passage 1 (23 days in culture), **E:** passage 2 (23 days in culture), perpendicular sections. Some of the cultured SMCs exhibit caveolae (arrowheads) and/or a discontinuous basal lamina (open arrows). D: x50,000. Bar = 0.2 μm. E: ×48,000. Bar = 0.2 μm. **F:** Passage 1 (23 days in culture), tangential section. Arrows mark microfilament bundles; note the numerous mitochondria (m) showing a dense matrix. ×4,800. Bar = 2 μm. **G:** Passage 7 (66 days in culture), tangential section. At this developmental stage cultured SMCs only occasionally possess dense bodies (double arrows) associated with the microfilaments. ×24,000. Bar = 0.5 μm.

al. [39] reported that estradiol stimulates DNA synthesis in cultured guinea pig SMCs. DHT showed the opposite effect in that SMC culture system.

In previous studies, stromal cells of the prostate were shown to possess androgen and estrogen receptors [18,19,40–43]. The effect of androgens on prostatic cells has been studied extensively and is well established [44]. It is interesting to note that in our cell culture system estradiol showed better results concerning the maintenance of the SMC phenotype than DHT. Estrogens have long been suspected to be involved in the etiology of BPH, since in animal models BPH-like alterations of the prostatic tissue could be induced by long-term estrogen treatment [7–9,45,46]. Furthermore, aromatase inhibitors were found to be capable of preventing these alterations [47]. This finding is supported by our results which revealed a stimulatory effect of estrogens on prostatic SMCs.

The growth pattern of SMC cultures was different from that of fibroblast or prostatic myofibroblast cultures. It was most interesting to observe that SMCs grow on myofibroblasts. In prostatic tissue SMCs and fibroblasts are also in close contact. Therefore, we speculate that cocultures of SMCs and myofibroblasts reflect the influence of these two cell types on each other in vivo. The growth pattern observed indicates that this close contact is very important for the regulation and differentiation of SMCs.

In conclusion, we have established an in vitro model of human prostatic SMCs that should facilitate characterization of these cells and be helpful in investigating the receptors expressed by these cells.

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REFERENCES

1. Shapiro E, Hartanto V, Becich MJ, Lepor H: The relative proportion of stromal and epithelial hyperplasia is related to the development of symptomatic BPH. J Urol 147:1293–1297, 1992.
2. Bartsch G, Brungger A, Schweikert U, Hintner H, Hopfl R, Rohr HP: Benign prostatic hyperplasia: A stromal disease. Urologe A 28:321–328, 1989.
3. Bartsch G, Brungger A, Schweikert U, Hintner H, Hopfl R, Rohr HP: Benign prostatic hyperplasia: Morphometric studies in relation to the hormone sensitivity of stromal tissue. Prog Clin Biol Res 386:51–65, 1994.
4. Deering RE, Bigler SA, King J, Choongkittaworn M, Aramburu E, Brawer MK: Morphometric quantitation of stroma in human benign prostatic hyperplasia. Urology 44:64–70, 1994
5. Bartsch G, Brüngger A, Schweikert HU, Hintner A, Stanzl U, Rohr HP: Benign prostatic hyperplasia: morphometric studies in relation to the pathogenesis. In (Schröder F, Ackermann R, eds): ''Prostatic Hyperplasia.'' Berlin: Walter de Gryter & Co, 1989, pp 35–53.
6. Shapiro E, Hartanto V, Lepor H: Quantifying the smooth muscle content of the prostate using double-immunoenzymatic staining and color assisted image analysis. J Urol 147:1167–1170, 1992.
7. Habenicht UF, el Etreby MF, Lewis R, Ghoniem G, Roberts J: Induction of metachromasia in experimentally induced hyperplastic/hypertrophic changes in the prostate of the cynomolgus monkey (*Macaca fascicularis*). J Urol 142:1624–1626, 1989.
8. Robinette CL: Sex-hormone-induced inflammation and fibromuscular proliferation in the rat lateral prostate. Prostate 12:271–286, 1988.
9. Tam CC, Wong YC: Ultrastructural study of the effects of 17 beta-oestradiol on the lateral prostate and seminal vesicle of the castrated guinea pig. Acta Anat Basel 141: 51–62, 1991.
10. Morita T, Ando M, Kihara K, Matsumura T, Kamai T, Ohshima H: Subtypes of alpha 1-adrenoceptors in urinary bladder and urethral smooth muscle and prostatic adenoma. Nippon Hinyokika Gakkai Zasshi 84:1629–1634, 1993.
11. Milroy E: Clinical overview of prazosin in the treatment of prostatic obstruction. Urol Int 45 (Suppl 1):1–3, 1990.
12. Lepor H: Alpha adrenergic antagonists for the treatment of symptomatic BPH. Int J Clin Pharmacol Ther Toxicol 27:151–155, 1989.
13. Lepor H: The emerging role of alpha antagonists in the therapy of benign prostatic hyperplasia. J Androl 12: 389–394, 1991.
14. Denis LJ: Future implications for the management of benign prostatic hyperplasia. Eur Urol 25 (Suppl 1):29–34, 1994.
15. Monda JM, Oesterling JE: Medical treatment of benign prostatic hyperplasia: 5 alpha-reductase inhibitors and alpha-adrenergic antagonists. Mayo Clin Proc 68:670–679, 1993.
16. Shapiro E, Hartanto V, Lepor H: The response to alpha blockade in benign prostatic hyperplasia is related to the percent area density of prostate smooth muscle. Prostate 21:297–307, 1992.
17. Cunha GR: Mesenchymal-epithelial interactions during androgen-induced development of the prostate. In: (Lash JW and Saxon L, eds.), ''Developmental Mechanisms: Normal and Abnormal''. New York: A.R. Liss, 1985, pp. 15–24.
18. Cooke PS, Young P, Hess RA, Cunha GR: Estrogen receptor expression in developing epididymis, efferent ductules, and other male reproductive organs. Endocrinology 128:2874–2879, 1991.
19. Cooke PS, Young P, Cunha GR: Androgen receptor expression in developing male reproductive organs. Endocrinology 128:2867–2873, 1991.
20. Cunha GR, Alarid ET, Turner T, Donjacour AA, Boutin EL, Foster BA: Normal and abnormal development of

the male urogenital tract—Role of androgens, mesenchymal-epithelial interactions, and growth factors. J Androl 13:465–475, 1992.
21. Chung LWK, Cunha GR: Stromal epithelial interactions: II. Regulation of prostate growth by embryonic urogenical sinus mesenchyme. Prostate 4:503–511, 1983.
22. Yan G, Fukabori Y, Nikolaropoulos S, Wang F, McKeehan WL: Heparin-binding keratinocyte growth factor is a candidate stromal to epithelial cell andromedin. Mol Endocrinol 6:2123–2128, 1992.
23. Eicheler W, Tuohimaa P, Vilja P, Adermann K, Forssmann WG, Aumuller G: Immunocytochemical localization of human 5 alpha-reductase 2 with polyclonal antibodies in androgen target and non-target human tissues. J Histochem Cytochem 42:667–675, 1994.
24. Kirschenbaum A, Wang JP, Eliashvili E, Ren M, Levine AC, Russel DW: Immunohistochemical characterization of type II 5a-reductase expression in human fetal and adult prostates. J Urol 53:299A, 1995.
25. Horoszewicz JS, Leong SS, Ming Chu T, Wajsman ZL, Friedman M, Papsidero L, Kim U, Chai LS, Kakati S, Arya SK, Sandberg AA: The LNCaP cell line—A new model for studies on human prostatic carcinoma. Prog Clin Biol Res 37:115–132, 1980.
26. Gazdar AF, Carney DN, Russel EK, Sims HL, Baylin SB, Bunn PAJ, Guccion JG, Minna JD: Establishment of continuous clonable cultures of small-cell carcinoma of the lung which have amine precursor uptake and decarboxylation cell properties. Cancer Res 40:3502–3507, 1980.
27. Carney DN, Gazdar AF, Bepler G, Guccion JG, Marangos PJ, Moody TW, Zweig MH, Minna JD: Establishment and identification of small cell lung cancer cell lines having classic and variant features. Cancer Res 45:2913–2923, 1985.
28. Hsu SM, Raine L, Fanger H: The use of avidin-biotin-peroxidase complex (ABC) and unlabelled antibody PAP procedures. J Histochem Cytochem 29:577–580, 1981.
29. Skalli O, Ropraz P, Treciak A, Benzonana G, Gillessen D, Gabbiani G: A monoclonal antibody against α-smooth muscle actin: A new probe for smooth muscle differentiation. J Cell Biol 103:2787–2796, 1986.
30. Schneider MD, Sellers JR, Vahey M, Preston YA, Adelstein RS: Localization and topography of antigenic domains within the heavy chain of smooth muscle myosin. J Cell Biol 101:66–72, 1985.
31. Longtine JA, Pinkus GS, Fuhiwara K, Corson JM: Immunohistochemical localization of smooth muscle myosin in normal human tissues. J Histochem Cytochem 33:179–184, 1985.
32. Altmannsberger M, Osborn M, Schauer A, Weber K: Antibodies to different intermediate filament proteins. Cell type-specific markers on paraffin-embedded human tissue. Lab Invest 45:427–434, 1981.
33. Bohn W, Wiegers W, Beuttenmüller M, Traub P: Species-specific recognition patterns of monoclonal antibodies directed against vimentin. Exp Cell Res 201:1–7, 1992.
34. Franke WW, Schmid E, Osborne M, Weber K: Different intermediate-sized filaments distinguished by immunofluorescence microscopy. Proc Natl Acad Sci USA 75:5034–5038, 1978.
35. Esterre P, Melin M, Serrar M, Grimaud JA: New specific markers of human and mouse fibroblasts. Cell Mol Biol 38:297–301, 1992.
36. Kooistra A, Elissen NMJ, König JJ, Vermey M, van der Kwast TH, Romijn JC, Schröder FH: Immunocytochemical characterization of explant cultures of human prostatic stromal cells. Prostate 27:42–49, 1995.
37. Ricciardelli C, Horsfall DJ, Skinner JM, Henderson DW, Marshall VR, Tilley WD: Development and characterization of primary cultures of smooth muscle cells from the fibromuscular stroma of the guinea pig prostate. In Vitro Cell Dev Biol 25:1016–1024, 1989.
38. Wu YX, Kawabe K: Primary culture of smooth muscle cells from benign prostatic hyperplasia. J Smooth Muscle Res 30:51–56, 1994.
39. Ricciardelli C, Horsfall DJ, Sykes PJ, Marshall VR, Tilley WD: Effects of oestradiol-17 beta and 5 alpha-dihydrotestosterone on guinea-pig prostate smooth muscle cell proliferation and steroid receptor expression in vitro. J Endocrinol 140:373–383, 1994.
40. Kimura N, Mizokami A, Oonuma T, Sasano H, Nagura H: Immunocytochemical localization of androgen receptor with polyclonal antibody in paraffin-embedded human tissues. J Histochem Cytochem 41:671–678, 1993.
41. Tilley WD, Horsfall DJ, Skinner JM, Henderson DW, Marshall VR: Effect of pubertal development on estrogen receptor levels and stromal morphology in the guinea pig prostate. Prostate 15:195–210, 1989.
42. West NB, Roselli CE, Resko JA, Greene GL, Brenner RM: Estrogen and progestin receptors and aromatase activity in rhesus monkey prostate. Endocrinology 123: 2312–2322, 1988.
43. Schulze H, Barrack ER: Immunocytochemical localization of estrogen receptors in the normal male and female canine urinary tract and prostate. Endocrinology 121:1773–1783, 1987.
44. Coffey DS, Isaacs JT: Control of prostate growth. J Urol 1:17–24, 1981.
45. Zhao GQ, Holterhus PM, Dammshauser I, Hoffbauer G, Aumuller G: Estrogen-induced morphological and immunohistochemical changes in stroma and epithelium of rat ventral prostate. Prostate 21:183–199, 1992.
46. Rohr HP, Naef HR, Holliger O, Oberholzer M, Ibach B, Weisbach L, Bartsch G: The effect of oestrogen on stromal growth of the dog prostate: A quantitative ultrastructural study. Urol Res 9:201–207, 1981.
47. Habenicht UF, el Etreby MF: Rationale for using aromatase inhibitors to manage benign prostatic hyperplasia. Experimental studies. J Androl 12:395–402, 1991.