American Journal of Pathology, Vol. 136, No. 4, April 1990

Basal Epithelial Cells of Human Prostate Gland Are Not Myoepithelial Cells

A Comparative Immunohistochemical and Ultrastructural Study with the Human Salivary Gland

John R. Srigley, Irving Dardick,*
R. Warren J. Hartwick, and Laurence Klotz†
From the Departments of Pathology, Sunnybrook Medical Centre, University of Toronto, Toronto General Hospital, and the Department of Urology, Sunnybrook Medical Centre, University of Toronto,† Toronto, Canada*

The hypothesis that basal epithelial cells of the human prostate are of myoepithelial origin was investigated using immunohistochemical and ultrastructural methodologies. The immunohistologic analyses show significant phenotypic differences between prostatic basal cells and myoepithelial cells of the salivary gland. Although both cell types stain intensely with the 312C8-1 monoclonal antibody, only true myoepithelial cells demonstrated significant amounts of muscle-specific actin as decorated by the HHF35 monoclonal antibody. Furthermore, using double-labeling experiments, the prostatic basal cells were strongly decorated with a fluorecein-tagged basal cell-specific keratin but were negative with the rhodamine-tagged phalloidin, a chemical that binds specifically to actin microfilaments. Ultrastructural studies also showed an absence of thin microfilament bundles, dense bodies, and micropinocytotic vesicles in the prostatic basal cells. The current investigations show that the prostatic acini do not have a basal myoepithelium. Although some authors have suggested a stem cell role for prostatic basal cells, the weight of experimental work argues against this hypothesis. The exact role of the basal epithelial cells of the prostate is not known, although they may serve endocrine, paracrine, or other regulatory functions and may be involved in modulating signals between prostatic stroma and epithelium. (Am J Pathol 1990, 136:957–966)

Proliferative diseases of the human prostate gland are a major cause of male morbidity and mortality. Despite this clinical relevance, relatively little is known about the fundamental cell biology of prostatic epithelium. The prostate acinus is composed of a luminal cell compartment that is surrounded by an attenuated basal cell layer. While it is generally accepted that the secretory cells are responsible for the production of the secretion and acid phosphatases, the role of the basal cells remains controversial.

A number of functional hypotheses have been put forth. Some authors have suggested that the basal cells are a stem cell compartment that continually renews the secretory layer.[1,2] Others have proposed that basal cells are reserve cells or so-called facultative stem cells responsible for secretory cell renewal in times of need, such as with androgen stimulation.[3,4] Still others believe that prostatic basal cells have a myoepithelial function analogous to the myoepithelial cells of the salivary gland or breast tissue.[5–8]

The current study was designed to investigate the myoepithelial hypothesis using modern ultrastructural, histochemical, and immunohistochemical methodologies.

Materials and Methods

For this comparative study, tissue was available from nine transurethral prostatectomies for benign hyperplasia and from portions of nine normal parotid salivary glands resected as superficial parotidectomies for pleomorphic adenomas.

Immunoperoxidase Microscopy

In all cases the tissue was fixed in modified methacarn (60% methanol, 30% chloroform, and 10% glacial acetic

Supported by the Moe Levin Family Foundation, Montréal, Québec, Canada.

Accepted for publication December 8, 1989.

Address reprint requests to Dr. John R. Srigley, Department of Pathology, Sunnybrook Medical Centre, 2075 Bayview Avenue, North York, Ontario, Canada M4N 3M5.

Table 1. *Monoclonal Antibodies and Reagents Used and Their Specificities*

Probe	Dilution	Source	Specificity		Reference
			Cytokeratin (CK) (Moll et al[19])	M_r *	
8.12	1:50	ICN ImmunoBiologicals, Lisle, IL	CK 13, 16	54, 48	Geiger et al 1987[16]
4.62	1:200	ICN ImmunoBiologicals, Lisle, IL	CK 19	40	Supplier
PKK1	1:150	LabSystems, Helsinki, Finland	CK 7, 8, 17, 18	54, 52.5, 46, 45	Supplier
312C8-1	1:1500	Supplied by Dr. S. H. Dairkee	CK 14	50	Dairkee et al[13,14]
RPN.1162	1:100	Amersham Canada, Ltd., Oakville, Ontario	CK 7	54	Supplier
HHF35	1:2000	Enzo Biochemical Inc., New York, NY	Muscle-specific actin	—	Tsukada et al[20,21]
Phalloidin	1:10	Molecular Probes, Junction City, OR	Filamentous actin	—	Wulf et al, 1979[15]
Ki-67	1:50	Dako Corp., Santa Barbara, CA	Cycling cells	—	Gerdes et al[17,18]
Anti- S-100	1:1000	Dako Corp., Santa Barbara, CA	S-100 protein	—	Supplier

* M_r, Molecular weight in kd.

acid) before paraffin embedding; this fixation provides superior immunohistochemical staining.[9]

The antibodies, their specificities, and sources used in this study are listed in Table 1. Sections were cut at 4 μm, floated onto Bondfast (Lepage, Bramalea, Ontario, Canada) glue-coated slides, and dried overnight at 37°C. They were deparaffinized in three washes of toluene followed by three washes in ethanol. Sections to be stained with mouse antibodies (PKK1, RPN.1162, 8.12, 312C8-1, 4.62, HHF35, and Ki-67) were blocked first with 3% hydrogen peroxide for 30 minutes followed by a 1:10 Tris buffer dilution of normal horse serum. The primary antibody was incubated for 30 minutes at 20°C. After two Tris buffer washes, the sections were developed with the Unistain detection kit (Becton Dickinson, Mountain View, CA), a goat anti-mouse immunoglobulin (Ig) peroxidase method. After washing, slides were incubated with 0.05% copper sulfate and finally counterstained with Mayer's hematoxylin.

Negative controls substituted phosphate-buffered saline for the monoclonal antibodies. Positive controls for the various cytokeratin antibodies used normal skin, breast, thyroid, esophageal tissues, and a variety of epithelial carcinomas. A detailed description of the cytokeratin immunostaining results obtained with methacarn-fixed parotid salivary gland has been reported.[10–12]

Fluorescence Microscopy

Tissue from four of the nine superficial parotidectomy specimens and all of the prostatectomy specimens was snap frozen in isopentane-cooled liquid nitrogen and stored at −70°C until required. For this part of the study, double fluorescent staining was performed on four parotidectomy and four prostatectomy samples. Sections 4 μm to 5 μm thick were mounted on glass slides, fixed for 10 minutes in cold absolute acetone, and air dried. After rehydration in phosphate-buffered saline (PBS), these sections were sequentially stained with the anti-cytokeratin monoclonal antibody 312C8-1[13,14] and rhodamine-conjugated phalloidin (Molecular Probes Inc., Junction City, OR), which detects filamentous actin in the cell cytoplasm.[15,16] A 1:700 dilution of antibody 312C8-1 in PBS was applied for 45 minutes, the sections washed with PBS, and a goat anti-mouse IgM conjugated to fluorescein isothiocyanate (1:100; Kirkegaard and Perry Lab. Inc., Gaithersburg, MA) applied for 45 minutes. After washing with PBS, rhodamine-phalloidin (supplied as a 3.3-mol/l solution in methanol), diluted 1:10 in PBS was applied to the sections for 20 minutes. After washing with PBS, the stained sections were coverslipped using a 50% aqueous glycerol-PBS solution to which was added p-phenylenediamine (0.1%) to retard fading of the fluorescence. A Zeiss epifluorescence microscope (Carl Zeiss, Inc., Oberkochen, West Germany) equipped with both an xenon and a mercury lamp and appropriate filters for both rhodamine and fluorescein was used for photography. Appropriate areas of the section were sequentially photographed Ilford XP1 400 film (Ilford Ltd., Mobberley, Cheshire, UK) using both flourescent markers.

Replicating Cell Assessment

To assess the replicative ability of prostatic glandular (particularly basal) cells, the monoclonal antibody Ki-67 (Table 1) was used. This antibody detects an antigen that is expressed only in the cycling cells of tissues.[17,18] Frozen sections, 4 μ to 5 μ thick, were cut from tissue blocks of all nine of the benign prostatic hyperplasia samples. Indirect immunoperoxidase staining was performed as described above. Frozen sections cut from two lymph node biopsies with reactive hyperplasia and prominent germinal centers were used as positive controls.

Transmission Electron Microscopy

Representative fragments of prostate and salivary gland tissue were immediately fixed in universal fixative, pro-

Table 2. *Principal Patterns of Filament Expression in Salivary and Prostate Glands*

Cell Type	8.12	4.62	PKK1	312C8-1	RPN.1162	HHF35	Anti- S-100
Salivary-duct luminal	+	+	++	−	+	−	−
Prostate-gland luminal	−	+	+	−	−	−	−
Salivary-acinar and intercalated duct myoepithelium	−	+	−	++	−	++	−
Salivary-duct striated and excretory basal cells	++	+	+	++	−	−	−
Prostate-gland basal cells	++	+	+	++	−	−	−
Cytokeratin (CK) and actin or S-100 identified	CK 13, 16	CK 19	CK 7, 8 17, 18	CK 14	CK 7	Muscle-specific actin	S-100 protein

Staining pattern: −, no staining; +, moderate staining; ++, strong staining.

cessed in the usual manner, and embedded in an epon-araldite mixture. One-micrometer thick sections were cut from an average of six blocks and viewed for representativeness. Appropriate areas from the blocks were cut on diamond knives, mounted on copper grids, and stained with both uranyl acetate and lead citrate. The grids were viewed on Zeiss model 10 or Philips 300 electron microscope and selected fields were photographed.

Results

Immunohistochemistry

Antibodies to single or combinations of the various cytokeratin intermediate filaments and to muscle-specific actin filaments (Table 1) were used to detect similarities and differences in the staining patterns of luminal and basal and/or myoepithelial cells in salivary and prostate glands; these results are summarized in Table 2.[19-21]

Luminal cells of salivary gland ducts and the glandular luminal cells of the prostate both reacted with antibodies 4.62 and PKK1 (detecting cytokeratins (CK) 19 and 7, 8, 17, and 18, respectively) and were negative with antibody 312C8-1 (anti-CK 14) and HHF35 (anti-muscle-specific actin) (Table 2). Unlike the prostatic gland luminal cells, duct luminal cells of salivary glands also expressed CKs 13 and 16 (antibody 8.12) and CK 7 (antibody RPN.1162) (Table 2).

Basal cells, whether major salivary ductal or prostatic glandular, had identical immunohistochemical staining patterns (Table 2). In the case of antibody 312C8-1, strongly stained basal cells were present as a discontinuous or continuous layer in the striated and excretory ducts of the salivary gland (Figure 1A) and a continuous layer in the glands of prostatic tissue (Figure. 1B). With antibody HHF35 (anti-muscle-specific actin), the basal cells of striated and excretory ducts of salivary gland and the basal cells of prostatic glands were all unstained (Figures 1C and D). Discontinuous basal cells in salivary ducts (Figure 1E) and a continuous layer of basal cells in prostatic glands (Figure 1F) were identified with monoclonal antibody 8.12.

Major distinctions were noted between the myoepithelial cells of acini and intercalated ducts of normal salivary gland and the basal cells of prostatic glands with benign hyperplasia (Table 2 and Figure 1). Like basal cells of both salivary ducts and prostatic glands, the myoepithelial cells of salivary glands were also stained by antibody 312C8-1 (anti-CK 14) (Figures 1A and B). However, in contrast to the basal cells of the glands in the prostate, myoepithelial cells of salivary glands were strongly positive for muscle-specific actin (antibody HHF35) (Figures 1C and D) and negative with antibodies 8.12 (Figures 1E and F) and PKK1 (Table 2).

In the methacarn-fixed tissue, neither salivary gland nor prostatic gland tissue stained positive for S-100 protein. Internal nerve tissue served as a positive control. In particular, the myoepithelial cells of the salivary gland were uniformly negative.

Immunofluorescence Microscopy

Indirect double immunofluorescence labeling of frozen sections of normal salivary glands and benign prostatic hyperplasia was carried out to determine if basal cells of prostate synthesized both cytokeratin and actin filaments. In normal salivary glands, the myoepithelial cells encompassing both acini and intercalated ducts coexpress cytokeratin 14 (Figure 2A) (detected by monoclonal antibody 312C8-1) and actin (Figure 2B) (visualized by the rhodamine-phalloidin probe) with a similar and uniform distribution throughout the cell and its many processes. When monoclonal antibody 312C8-1 was applied to prostatic tissue, the basal cells of the glandular epithelium were exclusively labeled (Figure 2C). However, although the smooth muscle cells of the prostatic stromal tissue were intensely stained by rhodamine-phalloidin, the CK 14-containing basal cells did not contain filamentous actin (Figure 2D), at least in the amounts detectable in myoepithelial cells of the salivary gland (Figure 2B). Very weak diffuse background fluorescence was noted in the cytoplasm of most ductal, secretory acinar, and basal epithelial cells. This phenomenon was thought to be caused by

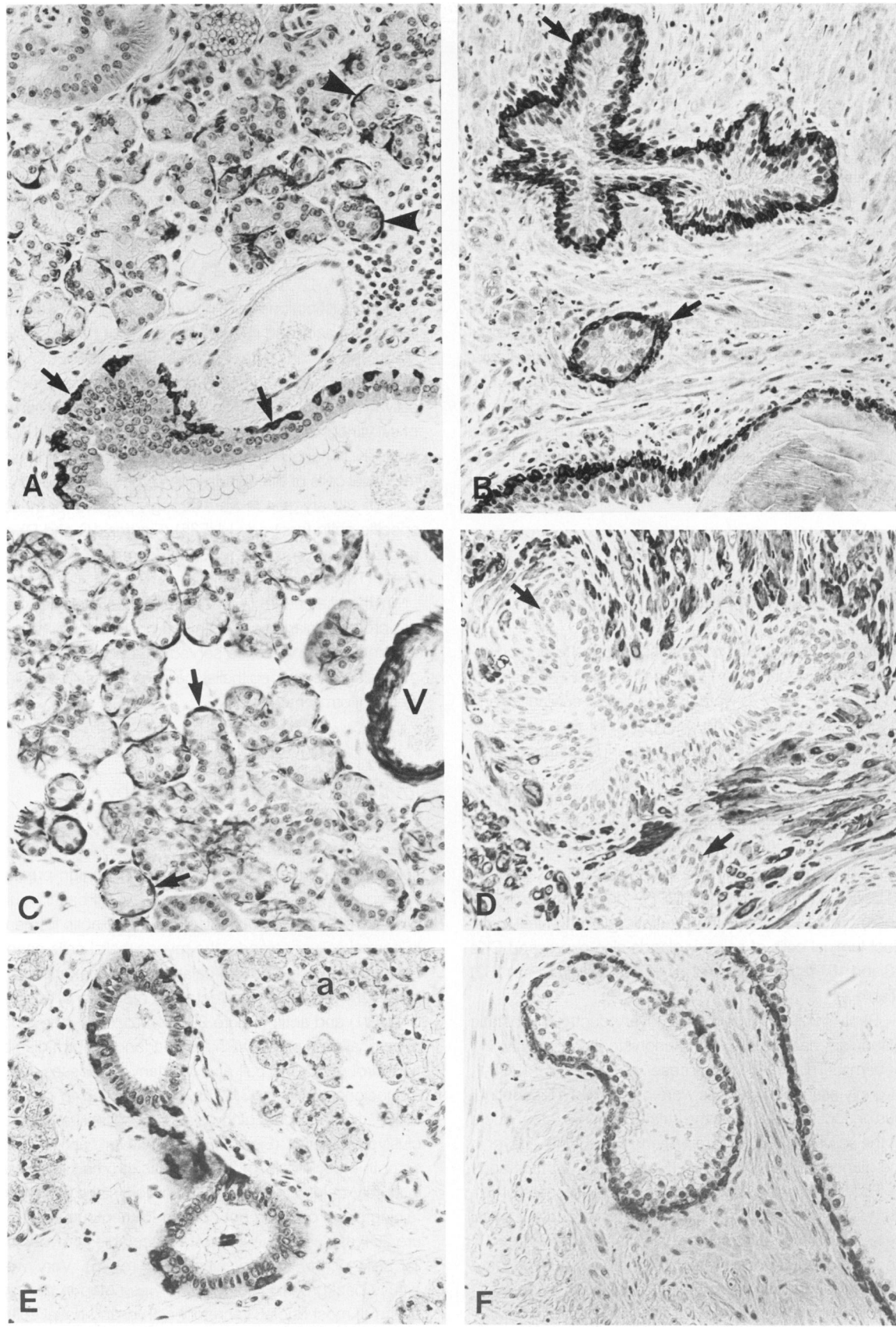
A
B
C
V
D
a
E
F

Figure 1. *Indirect immunoperoxidase staining of salivary* (A, C, E) *and prostate* (B, D, F) *glands using monoclonal antibody (MAb) 312C8-1 for CK* (A, B), *MAb HHF35 for muscle-specific actin* (C, D), *and MAb 8.12 for CKs 13 and 16* (E, F). *MAb 312C8-1 stains both the myoepithelial cells (arrowheads) of acini and the basal cells (arrows) of ducts in salivary gland* (A) *and the basal cells of prostate* (B); *in both of these tissues, duct luminal and acinar cells are unstained. Using MAb HHF35, along with the smooth muscle cells of blood vessels (V), myoepithelial cells (arrows) of salivary gland are stained* (C), *but although the smooth muscle of the prostatic stroma is strongly stained, the basal cells (arrows) are unstained* (D); *compare the CK 14-positive basal cells of prostate in* B *to the muscle-specific actin-negative basal cell in the same glands in an adjacent section in* D. *MAb 8.12 strongly labels basal cells of both salivary* (E) *and prostatic* (F) *glands, but the myoepithelial cells of acini (a) and intercalated ducts are negative in salivary gland* (E). *Hematoxylin counterstain. Magnification, ×225.*

phalloidin staining of the ubiquitous network of cytoplasmic actin.

Replicating Cell Assessment

No staining of either prostatic basal, secretory, or stromal cells was noted with the Ki-67 marker. The follicular center cells in the reactive lymph node control slides stained intensely with the Ki-67 antibody.

Electron Microscopic Studies

The prostatic acini were composed of two distinct cellular compartments—secretory and basal. The secretory cell layer was a continuous layer that lined luminal spaces and was composed of cuboidal to columnar cells with apical cytoplasm containing many secretory granules and lysosomes. Golgi structures and mitochondria were easily identified. The nuclei were round to oval and contained a moderate amount of peripheralized heterochromatin. The lateral aspects of the secretory cells were joined at the apices by tight junctional complexes. Desmosomes were readily seen between secretory cells as well as between secretory and basal cells.

The prostatic basal cells formed a nearly continuous layer, although small areas of discontinuity were noted. The basal cells had a distinctly different ultramorphology from secretory cells. Intermediate forms between secretory and basal cells were not seen. The basal cells were generally oval or spindle in shape and stretched over the anteluminal aspect of several secretory cells (Figure 3A). In some areas, the basal cells had a more cuboidal appearance (Figure 3B). In one case that showed focal basal cell hyperplasia, multilayering of oval to polygonal basal cells showing striking nuclear irregularity was seen (Figure 3B). The basal cells sat on a delicate and continuous basement membrane. Small but well-formed desmosomes were seen between basal cells and with the overlying secretory cells. The nuclei often had a dispersed chromatin pattern with less heterochromatin than the secretory cells. Small nucleoli were sometimes noted. The cytoplasm was more simplified than that of secretory cells. Free ribosomes were plentiful and there were scattered strands of rough endoplasmic reticulum, mitochondria, and primary lysosomes. In some cases, small tonofilamentlike collections of intermediate filaments were seen (Figure 4), but significant accumulations of microfilaments were not visualized.

In contrast, the cytoplasm of true myoepithelial cells, which surrounded either acinar or intercalated duct structures, contained definite collections of microfilaments as well as dense bodies and subplasmalemmal attachment plaques (Figure 3C and D). Tonofilamentlike collections of intermediate filaments were sometimes noted. The salivary myoepithelial cells tended to have elongated cytoplasmic extensions that wrapped in and around acinar cells (Figure 3C). This cytoplasmic architectural complexity was not seen in the prostatic basal cells.

Discussion

Myoepithelial cells are generally seen in ectodermally derived glandular tissues such as breast, salivary, lacrimal, or sweat glands.[22,23] They have a hybrid phenotype, sharing both epithelial and mesenchymal features. Myoepithelial cells are interposed between luminal cells and the basement membrane and are believed to have a contractile function that aids in the expulsion of secretion from acini. The ultrastructural and immunophenotypic features of salivary gland myoepithelial cells have been studied by a number of authors.[10–12,24–26]

Based on early light microscopic studies, it was suggested that certain cells located beneath the basement membrane of the prostatic acinus in rats and humans were of myoepithelial origin.[27] Subsequently, Rowlatt and Franks described the ultrastructural features of fusiform or stellate cells lying between the basement membrane and the secretory cell layer of the prostatic acinus.[5] Filaments resembling myofilaments arranged parallel to the long axis of these cells were noted and the authors concluded that the cells were myoepithelial because of structural similarities with salivary myoepithelial cells. They also noted the presence of similar myoepithelial cells in the human prostate.[5] This myoepithelial appelation has been applied to basal cells of the prostate by a number of authors.[6–8] Arguing from the teleologic perspective, the prostatic acini do not seem to need specialized myoepithelial cells to aid in the expulsion of acinar contents because of the abundant fibromuscular stroma. In contrast,

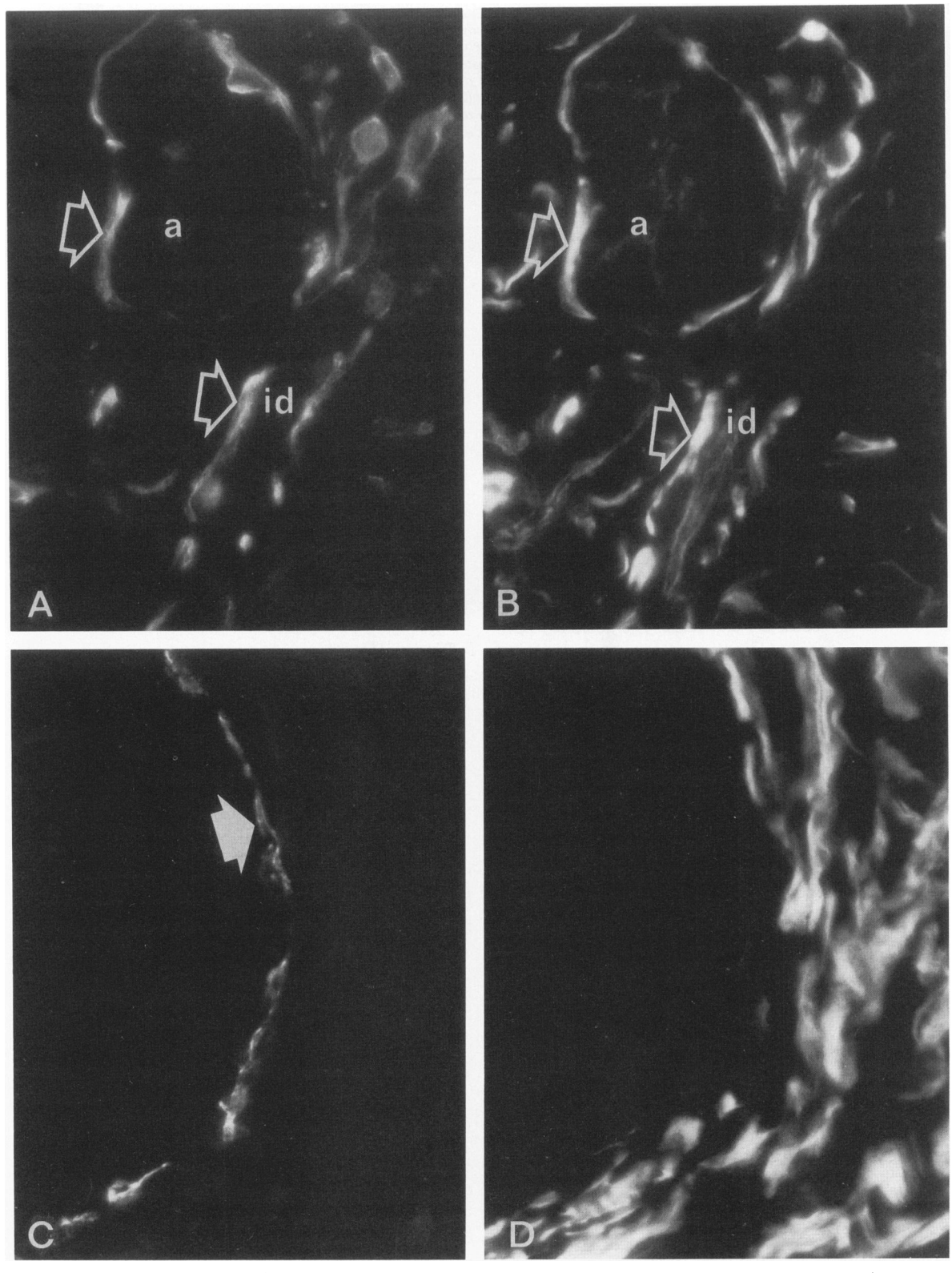

Figure 2. *Indirect double immunofluorescence labeling of normal salivary* (A, B) *and prostate* (C, D) *using MAb 312C8-1 for cytokeratin 14* (A, C) *and rhodamine-phalloidin for filamentous actin* (B, D). *Salivary gland myoepithelial cells (open arrows) of an acinus (a) and intercalated ducts (id) are identically labeled by MAb 312C8-1* (A) *and rhodamine-phalloidin* (B). *Basal cells (solid arrow) of a prostatic gland are also labeled by MAb 312C8-1* (C), *but are unlabeled by rhodamine-phalloidin that does decorate the smooth muscle cells of the prostatic stroma on the right* (D). *Magnification;* A *and* B, ×*1000;* C *and* D, ×*600.*

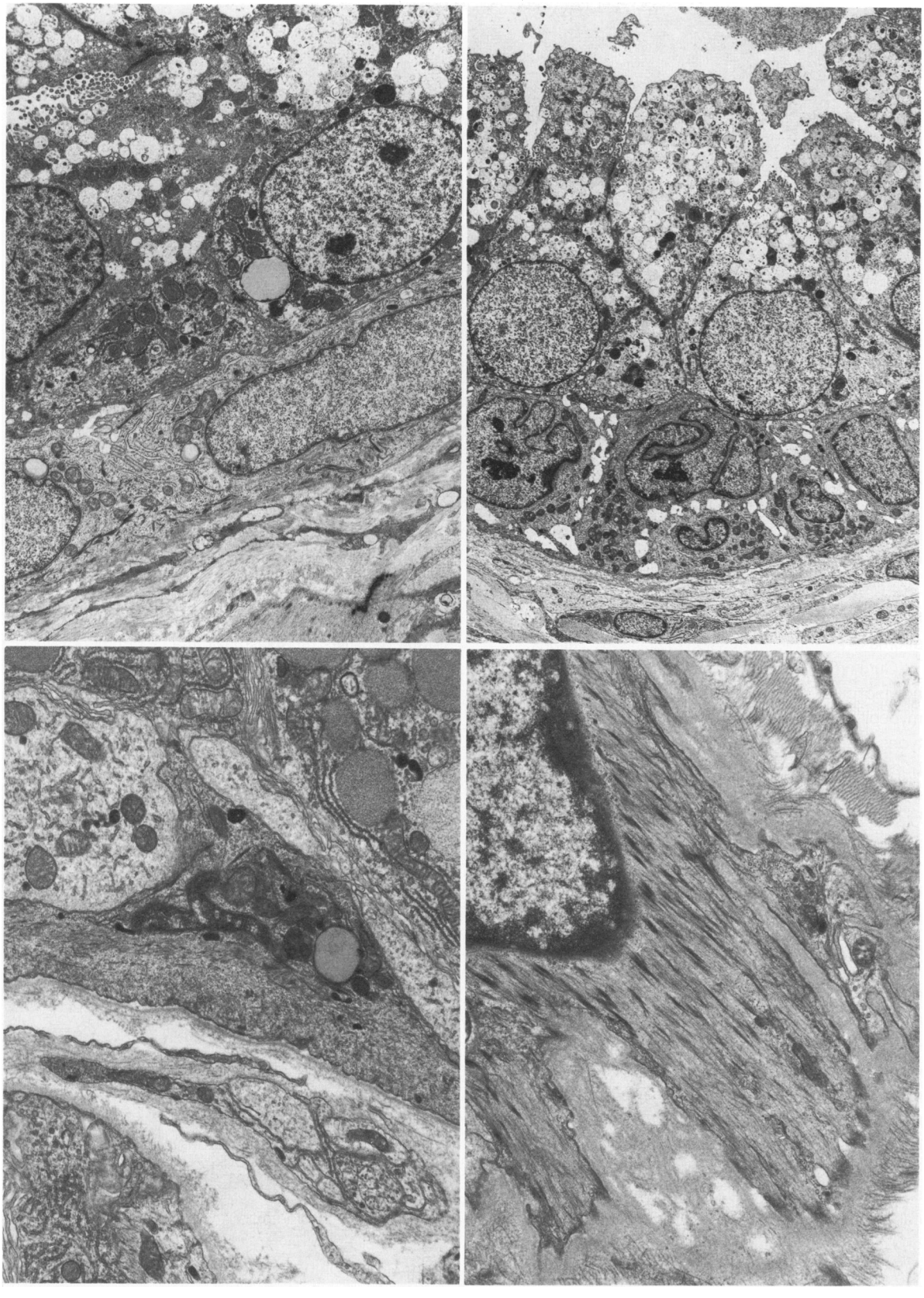

Figure 3. *Ultrastructural comparison between prostatic basal cells and salivary gland myoepithelium.* **A:** *Oval-shaped basal cells rest on a delicate basal lamina bridging several overlying secretory cells. Note dispersed nuclear chromatin and paucity of cytoplasmic granules in comparison with the secretory cells. Small desmosomes are seen between basal cells and secretory cells (×5000).* **B:** *Note proliferation of round to polygonal basal cells showing irregular nuclear configuration and complex intercellular canaliculi (×5000).* **C:** *A triangular portion of a myoepithelial cell resting on a delicate basal lamina insinuates between adjacent acinar cells (×10,000).* **D:** *Myoepithelial cells of striated salivary duct. Note abundance of microfilaments with associated dense bodies and subplasmalemmal attachment plaques. Micropinocytotic vesicles are also noted (×15,000).*

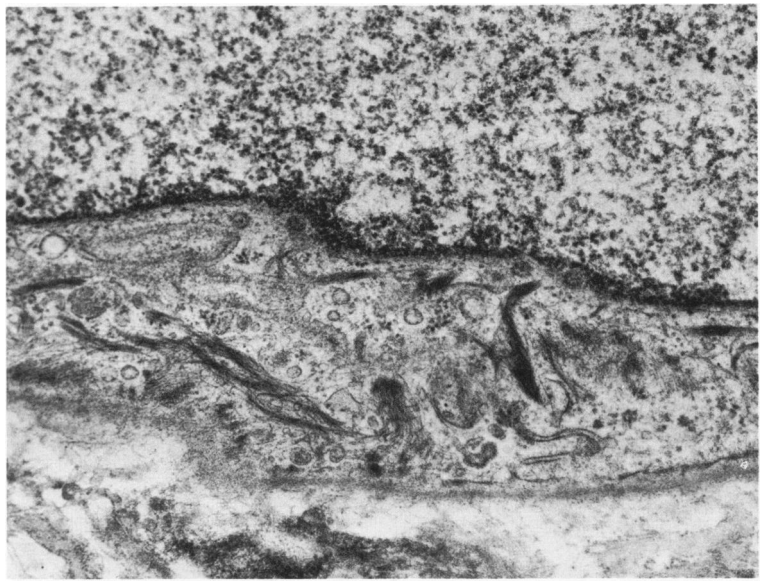

Figure 4. *Prostatic basal cell showing tonofilamentlike collections of intermediate filaments (×31,500).*

the breast and salivary glands have very little contractile stroma to aid in the expression of acinar secretions.

Our immunohistologic analyses show significant phenotypic differences between prostatic basal cells and myoepithelial cells of salivary glands. Although both cell types stain intensely with the 312C8-1 monoclonal antibody, only true myoepithelial cells demonstrate a significant amount of muscle-specific actin as decorated by the HHF35 monoclonal antibody. This observation is further supported by the double labeling experiments in which the same cells were labeled with a fluorescein-tagged basal cell-specific keratin and the rhodamine-tagged phalloidin, a chemical that binds specifically to actin microfilaments.[15]

Such results indicate that the basal cells of prostatic glands are not the direct counterpart of the myoepithelium associated with salivary gland acini and intercalated ducts. However, the immunohistochemical and fluorescence microscopy suggest that most of the basal cells of other salivary ducts have cytoplasmic filament complements like the basal cells of prostatic glands. Based on ultrastructural and immunohistochemical investigations of the cell types in salivary gland ducts, it is evident that a small number of myoepithelial and myoepithelial-like cells can be associated with other basal cells of the striated ducts in some parotid glands.[10,11] Basal cell populations of salivary ducts therefore have certain similarities to the basal cells of prostatic epithelium, but the former are distinctly more heterogenous than the latter. These features indicate fundamental differences between the two glands.

The ultrastructural observations also support the conclusion that prostatic basal cells normally do not have a myoepithelial nature. Myoepithelial cells surrounding acini or ductal elements of salivary gland tissue contain many 6- to 8-nm microfilaments with associated intrafilamentous densities, subplasmalemmal attachment densities, and micropinocytotic vesicles. Prostatic basal cells often contain collections of intermediate filaments sometimes arranged in tonofilamentlike collections, but microfilaments are inconspicuous.

Furthermore, the extensive cytoplasmic processes that are characteristic of salivary gland myoepithelial cells are not a component of the more simply organized basal cells of prostatic glands.

In a recent paper,[28] S-100 protein was found to be negative in prostatic glandular epithelium when studied by immunohistochemistry. Myoepithelial cells of salivary and sweat glands and of the breast have been noted to stain positively for the S-100 marker.[29] Interestingly, in our methacarn-fixed material, no S-100 staining of either salivary myoepithelium, or prostatic basal cells was noted. These results require further study to determine whether the myoepithelial positivity for S-100 might be related to the mode of fixation.

Our general observations related to keratin staining of prostatic epithelial cells is similar to those in the literature. Both panepithelial and basal cell-specific keratin staining has been previously reported.[30–33]

A number of authors have suggested that prostatic basal cells may have a stem cell role and be responsible for renewal of the luminal secretory cells either on an ongoing basis or in times of stimulation such as with androgen administration. Descriptive morphologic observations of human and animal tissue and experimental data have been used to support this conclusion.[1–4] More recently, authors have used immunophenotypic studies of keratin subtypes in a rat prostate model to support a lineage relationship between prostatic basal and luminal cells.[34] After testosterone deprivation brought on by cas-

tration, there was a rapid loss of cells almost entirely from the luminal compartment. In the process of androgen-induced regeneration, a heterogeneous population of cells having luminal morphology but having a keratin phenotype intermediate between basal and luminal cells patterns was seen. We have not seen cells with an intermediate keratin phenotype in the current study, which essentially observed a kinetically static state as witnessed by the negativity of staining for the Ki-67 replicating cell marker. We are not aware of detailed human studies of keratin expression after castration or during the administration of exogenous androgens.

Many studies have been conducted on explant tissue cultures of human normal and hyperplastic prostate glands.[1,2,35,36] Initial light microscope and ultrastructural observations of these cultures suggested that the luminal aspect of cultured explants was derived from the prostatic basal cells.[1,35] These authors also initially suggested that the basal cells had a key role in the carcinogenesis of the human prostate. More recently, the concept of direct neoplastic transformation of columnar secretory cells has been endorsed by Heatfield.[37] This latter observation is also supported by studies of putative preneoplastic lesions in the human prostate in cases of early invasive carcinoma.[38] In the human prostatic explant studies, it was observed that a stratified metaplastic epithelium was regenerated by basal cells with a complete absence of columnar secretory component.[37] The basal cell regeneration was hormonally independent and appeared to maintain integrity of the epithelium very similar to the metaplastic response of basal cells adjacent to areas of prostatic infarction.[37]

In some recently reported cell biologic studies of rat prostate during normal growth and after castration and androgen-induced regeneration, the authors found no support for a stem cell role for prostatic basal cells.[38,39] Interestingly, these authors refuted earlier experiments that described a compensatory hyperplasia of basal cells after castration.[4] The apparent hyperplasia of basal cells was shown to represent a macrophage infiltration of glands in response to apoptosis of epithelial cells.[39] The weight of evidence now suggests that both basal and luminal compartments of the human prostate may have their own stem cells responsible for renewal of their respective cell populations. There is little convincing support for a lineage relationship between basal and luminal cells.

The physiologic function of prostatic basal cells remains elusive. The role of basal cells in the development of benign prostatic hyperplasia and prostatic adenocarcinoma is even more speculative. The presence of endocrine or paracrine cells in a basal location has been noted with appropriate immunohistochemical and ultrastructural studies.[40–42] It is possible that the prostatic basal cells may have a role in the endocrine or neuroendocrine regulation of the prostatic environment. The location of the prostatic basal cells between stromal and luminal compartments suggests that they may play a strategic role in communication between the two compartments and possibly in the regulation of stromally induced epithelial hyperplasia, which is seen in nodular hyperplasia. Additional experimental work is needed to explore some of these novel hypotheses.

References

1. Heatfield BM, Sanefugi H, Trump BF: Studies on carcinogenesis of human prostate. III. Human explant culture of normal prostate and benign prostatic hyperplasia: Transmission and scanning electron microscopy. J Natl Cancer Inst 1982, 69:757–766
2. Merchant DJ, Clarke SM, Ives K, Harris S: Primary explant culture: An *in vitro* model of human prostate. The Prostate 1983, 4:523–542
3. Aumuller G: Morphologic and endocrine aspects of prostatic function. The Prostate 1983, 4:195–214
4. Secchi J, Bonne C: Mitoses des cellules basales de l'epithelium prostatique survanant apreś la castration ches le rat pubère. Cr Soc Biol 1973, 167:1331–1334
5. Rowlatt C, Franks LM: Myoepithelium in mouse prostate. Nature 1964, 202:707–708
6. Barwick KW, Mardi JA: An immunohistochemical study of the myoepithelial cell in prostatic hyperplasia and neoplasia. Lab Invest 1983, 48:7A
7. Koss LG, Woyke S, Olszewski W: Aspiration Biopsy. Cytologic Intrepretation and Histologic Bases. New York, Igaku-Schoin, 1984. p. 223.
8. Kline TS: Handbook of Fine Needle Aspiration Biopsy Cytology, Second Edition. New York, Churchill-Livingstone, 1988. p. 365
9. Mitchell V, Ibrahim S, Gusterson BA: Improved immunohistochemical localization of tissue antigens using modified methacarn fixation. J Histochem Cytochem 1985, 33:491–495
10. Dardick I, Rippstein P, Skimming L, Boivin M, Parks WR, Dairkee SH: Immunohistochemistry and ultrastructure of myoepithelium and modified myoepithelium of the ducts of human major salivary glands: Histogenetic implications for salivary gland tumors. Oral Surg, Oral Med, Oral Pathol 1987, 64:703–715
11. Dardick I, Parks WR, Little J, Brown DL: Characterization of cytoskeletal proteins in basal cells of human parotid salivary gland ducts. Virchows Archiv (A) Pathol Anat Histopathol 1988, 412:525–532
12. Burns BF, Dardick I, Parks WR: Intermediate filament expression in normal parotid glands and pleomorphic adenoma. Virchows Archiv (A) 1988, 413:103–112
13. Dairkee SH, Blayney-Moore CM, Smith HS, Hackett AJ: Monoclonal antibody that identifies human myoepithelium. Proc Natl Acad Sci USA 1985, 82:7409–7413
14. Diarkee SH, Blayney-Moore CM, Smith HS, Hackett AJ: Concurrent expression of basal and luminal epithelial markers in cultures of normal human breast analyzed using monoclonal antibodies. Differentiation 1986, 32:93–100

15. Wulf E, Debobon A, Bautz FA, Faulstich H, Wieland T: Fluorescent phaliotoxin, a tool for the visualization of cellular actin. Proc Natl Acad Sci USA 1979, 76:4498–4502
16. Geiger S, Geiger B, Leitner O, Marshak G: Cytokeratin polypeptides expression in different epitheliomas in human salivary gland. Virchows Archiv (A) 1987, 410:403–414
17. Gerdes J, Schwab U, Lemke H, Stein H: Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 1983, 81:13–20
18. Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H: Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 1984, 133:1710–1715
19. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R: The catalogue of human cytokeratin polypeptides: Patterns of expression of cytokeratins in normal epithelia, tumors and culture cells. Cell 1982, 31:11–24
20. Tsudada T, Tippens D, Gordon D, Ross R, Gown AM: HHF35, muscle actin-specific monoclonal antibody: I. Immunocytochemical and biochemical characterization. Am J Pathol 1987, 126:51–60
21. Tsudada T, McMutt AM, Ross R, Gowan AM: HHF35, muscle actin-specific monoclonal antibody: II. Reactivity in normal, reactive and human neoplastic tissues. Am J Pathol 1987, 127:384–402
22. Cutler LS, Chaudhry AP: Differentiation of the myoepithelial cells of the rat submandibular gland *in vivo* and *in vitro*: An ultrastructural study. J Morphol 1973, 140:343–354
23. Palmer RN: The identification of myoepithelial cells in human salivary glands. A review and comparison of light microscopical methods. J Oral Pathol 1986, 15:221–229
24. Mori M, Sumitomo S, Iwai Y, Meenagham MA: Immunolocalization of keratins in salivary gland pleomorphic adenomas using monoclonal antibodies. Oral Surg, Oral Med, Oral Pathol 1986, 61:611–616
25. Caselitz J, Osborn M, Seifert G, Weber K: Intermediate-sized filament proteins (prekeratin, vimentin, desmin) in the normal parotid gland and parotid gland tumors. Immunofluorescent study. Virchows Archive (Pathol) Anat 1981, 393:273–286
26. Franke WW, Schmidt E, Freudenstein C, Appelhans B, Osborn M, Weber K, Keenan TW: Intermediate-sized filaments of the prekeratin type in myoepithelial cells. J Cell Biol 1980, 84:633
27. Franks LM, Barton A: The effects of testosterone on the ultrastructure of prostate *in vivo* and in organ cultures. Exp Cell Res 1960, 19:35–50
28. Howat AJ, Mills PM, Lyons TJ, Stephenson TJ: Absence of S-100 protein in prostatic glands. Histopathol 1988, 13:468–470
29. Egan MJ, Newman J, Crocker J, Collard M: Immunohistochemical localization of S-100 protein in benign and malignant conditions of the breast. Arch Pathol Lab Med 1987, 111:28–31
30. Brawer MK, Peehl DM, Stamey TA, Bostwick DG: Keratin immunoreactivity in the benign and neoplastic human prostate. Cancer Res 1985, 35:3663–3667
31. Nagel RB, Ahmann FR, McDaniel KM, Paquin ML, Clark VA, Cetniker A: Cytokeratin characterization of human prostatic carcinoma and its derived cell lines. Cancer Res 1987, 47: 281–286
32. Purnell DM, Heatfield BM, Anthony RL, Trump BF: Immunohistochemistry of the cytoskeleton of human epithelium. Evidence for disturbed organization in neoplasia. Am J Pathol 1987, 126:384–395
33. Leong AS-Y, Gilham P, Milios J: Cytokeratin and vimentin intermediate filament proteins in benign and neoplastic prostatic epithelium. Histopathol 1988, 13:435–442
34. Verhagen APM, Aalders TW, Ramaekers FCS, Debruyne FM, Shalken JA: Differential expression of keratins in the basal and luminal compartments of rat prostatic epithelium during degeneration and regeneration. The Prostate 1988, 13:25–38
35. Sanefuji H, Heatfield BM, Trump BF, Yound JD: Studies on carcinogenesis of human prostate. II. Long-term explant cultures of normal prostate and benign prostatic hyperplasia: Light microscopy. J Natl Cancer Inst 1982, 69: 751–756
36. Dermer JB: Basal cell proliferation of benign prostatic hyperplasia. Cancer 1978, 41:1857–1862
37. Heatfield BM: In vitro model: Organ explant culture of normal and neoplastic human prostate. Prog Clin Biol Res 1987, 239:391–475
38. Evans GS, Chandler JA: Cell proliferation studies in rat prostate: I. The proliferative role of basal and secretory epithelial cells during normal growth. The Prostate 1987, 10:163–178
39. Evans GS, Chandler JA: Cell proliferation studies in the rat prostate: II. The effects of castration in androgen-induced regeneration upon basal and secretory cell proliferation. The Prostate 1987, 11:339–351
40. Capella C, Usellini L, Buffa R, Frigerio B, Solcia E: The endocrine component of prostatic carcinomas, mixed adenocarcinoma-carcinoid tumors and non-tumor prostate. Histochemical and ultrastructural identification of the endocrine cells. Histopathol 1981, 5:175–192
41. Di Sant'Agnese PA, De Mesy Jansen KL: Endocrine-paracrine cells of the prostate and prostatic urethra. An ultrastructural study. Hum Pathol 1984, 15:1034–1041
42. Di Sant'Agnese PA, De Mesy Jansen KL, Churukian CJ, Agarall MM: Human prostatic endocrine-paracrine (APUD) cells. Distributional analysis with a comparison of serotonin and neuron-specific enolase immunoreactivity and silver stains. Arch Pathol Lab Med 1985, 119:607–612