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Cell differentiation lineage in the prostate

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Abstract Prostatic epithelium consists mainly of luminal and basal cells, which are presumed to differentiate from common progenitor/stem cells. We hypothesize that progenitor/stem cells are highly concentrated in the embryonic urogenital sinus epithelium from which prostatic epithelial buds develop. We further hypothesize that these epithelial progenitor/stem cells are also present within the basal compartment of adult prostatic epithelium and that the spectrum of differentiation markers of embryonic and adult progenitor/stem cells will be similar. The present study demonstrates that the majority of cells in embryonic urogenital sinus epithelium and developing prostatic epithelium (rat, mouse, and human) co-expressed luminal cytokeratins 8 and 18 (CK8, CK18), the basal cell cytokeratins (CK14, CK5), p63, and the so-called transitional or intermediate cell markers, cytokeratin 19 (CK19) and glutathione-S-transferase-pi (GSTpi). The majority of luminal cells in adult rodent and human prostates only expressed luminal markers (CK8, CK18), while the basal epithelial cell compartment contained several distinct subpopulations. In the adult prostate, the predominant basal epithelial subpopulation expressed the classical basal cell markers (CK5, CK14, p63) as well as CK19 and GSTpi. However, a small fraction of adult prostatic basal epithelial cells co-expressed the full spectrum of basal and luminal epithelial cell markers (CK5, CK14, CK8, CK18, CK19, p63, GSTpi). This adult prostatic basal epithelial cell subpopulation, thus, exhibited a cell differentiation marker profile similar to that expressed in embryonic urogenital sinus epithelium. These rare adult prostatic basal epithelial cells are proposed to be the progenitor/stem cell population.
Thus, we propose that at all stages (embryonic to adult) prostatic epithelial progenitor/stem cells maintain a differentiation marker profile similar to that of the original embryonic progenitor of the prostate, namely urogenital sinus epithelium. Adult progenitor/stem cells co-express both luminal cell, basal cell, and intermediate cell markers. These progenitor/stem cells differentiate into mature luminal cells by maintaining CK8 and CK18, and losing all other makers. Progenitor/stem cells also give rise to mature basal cells by maintaining CK5, CK14, p63, CK19, and GSTpi and losing K8 and K18. Thus, adult prostate basal and luminal cells are proposed to be derived from a common pleuripotent progenitor/stem cell in the basal compartment that maintains its embryonic profile of differentiation markers from embryonic to adult stages.

Key words differentiation · progenitor cells prostate · stem cells · urogenital sinus epithelium

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Introduction

Adult prostatic epithelium consists of basal, secretory, and neuroendocrine (NE) cells. In the basal compartment, a layer of cells is situated between the basement membrane and the overlying secretory cells. In the human prostate the secretory compartment consists of a luminal layer of tall columnar cells which express the androgen receptor (AR), CK 8 and 18, and prostatic specific antigen (PSA). The majority of basal cells can be distinguished from secretory cells, because they express p63, CK5 and CK14, and generally lack expression of luminal cell markers such as CK8 and CK18. Prostatic neuroendocrine cells expressing chromogranin A

are very rare and can be characterized by the lack of AR and PSA. In addition to typical basal, secretory, and NE cells, there are cells with intermediate phenotypes expressing a mixture of basal and luminal markers (CK5, CK8, CK14, CK18, and PSA) (Bonkhoff et al., 1994; Bonkhoff and Remberger, 1996; Xue et al., 1998; Hudson et al., 2000). Hudson et al. (2001) have recently demonstrated sub-populations of epithelial cells in the basal layer expressing CK15, CK17, and CK19 in various combinations. A subset of luminal cells were reported to express CK19 in addition to CK8 and CK18. Hudson et al. (2001) suggest that CK19-expressing cells represent a population of basal cells in the process of differentiating into luminal cells. This proposal is consistant with the generally held idea that progenitor/stem cells reside in the basal layer in the adult prostate (Bonkhoff and Remberger, 1996; Robinson et al., 1998).

While there is still debate regarding the location, phenotype, number, and nature of progenitor/stem cells in the adult prostate, the presence of progenitor/stem cells in the adult prostate is supported by several observations. In the rat ventral prostate, the majority of luminal cells undergo apoptosis after androgen deprivation. The remaining androgen-independent epithelial cells contain a high proportion of basal cells (Montpetit, 1988). After regression, the remaining epithelial population regenerates the prostate following androgen replacement. The cycle of prostatic involution and regeneration can be repeated many times, suggesting that the androgen-independent progenitor/stem cells that survive androgen deprivation have an extensive proliferative and regenerative capacity as well as pluripotency.

A feature characteristic of somatic stem cells is the enzyme, telomerase, which is responsible for maintenance of telomere length (Greider, 1996). Telomerase is undetectable in the prostate of intact males, whereas following castration, telomerase is detectable in the prostate (Meeker et al., 1996). This observation suggests that progenitor/stem cells are rare in the normal prostate, and because they are androgen-independent for survival, their numbers are proportionally increased as a result of castration. The presumed increase in the proportion of stem cells in the regressed prostate presumably allows detection of telomerase. Alternatively, telomerase in simply up-regulated in response to androgen deprivation.

Finally, we have demonstrated that a small ductal tip from adult prostate can be induced to form large amounts of new prostatic tissue by combining the ductal tip with urogenital sinus mesenchyme (UGM) (Norman et al., 1986; Hayashi et al., 1993). In these studies, an adult prostatic ductal tip containing about 5000 adult prostatic epithelial cells was able to regenerate several million prostatic epithelial cells when combined with UGM, suggesting that progenitor/stem epithelial cell populations are maintained in adult prostatic epithelia. Furthermore, when ductal tips from ventral prostate were associated with a dorsal-lateral-prostate-inducing mesenchyme, the resultant prostatic tissue expressed dorsal-lateral specific secretory proteins (Hayashi et al., 1993). This mesenchyme-induced change in functional differentiation is a manifestation of pleuripotency. Pleuripotency is a unique feature of the progenitor/stem cell. Taken together, these data support the interpretation that progenitor/stem cells reside in the adult prostate and are capable of extensive proliferation, pleuripotency and ability to regenerate the prostate.

All theories on progenitor/stem cell biology and cell lineage relationships in the adult prostate are for the most part correlative with meager molecular evidence or functional tests of stem cell activity (Isaacs and Coffey, 1989; Bonkhoff and Remberger, 1996; Bui and Reiter, 1998; Hudson et al., 2001). Accordingly, cell lineage relationships are poorly understood in the prostate. We propose that an examination of cell lineage relationships during prostatic development from embryonic urogenital sinus epithelium may elucidate cell lineage relationships in the adult prostate. Because embryonic urogenital sinus epithelium is the progenitor of the prostate, we postulate that embryonic urogenital sinus epithelium should be rich in prostatic progenitor/stem cells. Several observations are consistent with this hypothesis: (1) Cells within the embryonic urogenital sinus epithelium have a vast proliferative capacity. (2) Embryonic urogenital sinus epithelium is capable of generating the entire prostate. Embryonic urogenital sinus epithelium is pleuripotent being able to give rise to urethra, urethral glands, prostate, and vagina (Cunha et al., 1987). Thus, pleuripotent progenitor/stem cells clearly exist in urogenital sinus epithelium, and prostatic progenitor/stem cells are likely to be particularly abundant in embryonic urogenital sinus epithelium. We propose that progenitor/stem cells in the adult prostatic epithelium should maintain a differentiation marker profile similar to the progenitor/stem cells present in urogenital sinus epithelium.

By using cytokeratin and other differentiation markers (p63 and GSTpi), we examined the differentiation profiles of embryonic urogenital sinus epithelium and compared the features of embryonic urogenital sinus epithelium with that of developing and adult prostate. We found that a small subpopulation of adult prostatic basal cells maintain a differentiation profile similar to that of embryonic urogenital sinus epithelium. The current findings provide a unified view of the cell lineage relationships in the prostate from embryonic to adult periods.

Methods

Rat and mouse urogenital sinus, developing and adult prostate

Sprague-Dawley rats and Balb/c mice (Charles River, Wilminton, MA) aged at 18 days of gestation (E18, rat), 16 days of gestation (E16, mouse), and postnatal days 1, 3, 5, 7, 10, 20, 30, and 60

(P1–60, rats and mice) were sacrificed by decapitation. Urogenital sinuses and prostates were harvested and fixed with 4% paraformadehyde on ice for 3 hours prior to processing.

Human urogenital sinus, developing and adult prostate

Urogenital sinus and prostatic rudiments from 12 human fetuses 9 to 22 weeks of gestational age were collected following surgical abortion performed for reasons unrelated to this investigation at the University of California, San Francisco (UCSF). Informed consent was obtained for all specimens used. Prostatic rudiments were identified and dissected aseptically. Ages of specimens were estimated by heel-toe length (Robboy et al., 1982) and based on the last menstrual period. Adult normal prostatic tissue was obtained from transurethral resection specimens of benign prostatic hyperplasia in which there was no evidence of prostate cancer. All specimens were fixed in 4% paraformadehyde on ice for 4 hours prior to processing.

Immunohistochemical staining

Immunohistochemical staining was carried out on rat, mouse and human prostatic tissue sections using a panel of mouse monoclonal and rabbit polyconal antibodies (Table 1). Tissue sections were deparaffinized in Histoclear (National Diagnostic, Atlanta, GA) and hydrated in graded alcoholic solutions and distilled water (DW). Antigen retrieval was carried out by microwave heating for 30 minutes in an antigen unmasking solution (Vector Laboratories, Foster City, CA). Endogenous peroxidase activity was blocked with 0.5% hydrogen peroxide in methanol for 30 minutes followed by washing in phosphate-buffered saline (PBS) pH 7.4. Normal goat serum was applied to the sections for 30 minutes to bind nonspecific sites. The sections were then incubated with the primary antibodies overnight at 4°C or with non-immune mouse or rabbit IgG. Sections were then washed and incubated with biotinylated goat anti-mouse or goat anti-rabbit immunoglobulin (Sigma) diluted with PBS at 1:200 for 30 minutes at room temperature (RT). After incubation with the secondary antibody, sections were washed in PBS (three, 10 minute washes) and then incubated with avidin-biotin complex (Vector laboratories, Foster City, CA) for 30 minutes at room temperature. Sections were then washed in PBS again (three 10 min. washes) before visualizing immunoreactivity using 3′, 3′-diaminobenzidine (DAB) in PBS and 0.03% H_2O_2. Sections were counterstained with hematoxylin and dehydrated in alcohol. Control sections were processed in parallel with mouse or rabbit non-immune IgG at the same concentration as the primary antibodies. For double staining with mouse monoclonal antibodies, a double staining kit (Dako, Carpinteria, CA) was used. For double staining with mouse and rabbit primary antibodies, flourescein-conjugated anti-mouse and rhodamine-conjugated anti-rabbit secondary antibodies (Dako, Carpinteria, CA) were used. To stain cell nuclei, sections were incubated with Hoechst dye 33258 (CalBiochem, La Jolla, CA). To assess the incidence of rare adult prostatic basal epithelial cells co-expressing both luminal and basal markers, sections of adult human prostate were stained for CK8 and HMW-CK (CK5/14) using rhodamine- and flourescein-labeled secondary antibodies. Images were captured using NIH Image in both the rhodamine and flourescein channels. When the two images were merged, cells co-expressing both CK8 and HMW-CK (CK5/14) were yellow. Multiple microscopic images were processed in this way. The number of basal cells co-expressing both CK8 and HMW-CK (CK5/14) was determined by direct cell counting from the images.

Results

Summary of prostatic development

In the human male embryo, prostatic buds emerge at week 10 of gestation (Kellokumpu-Lehtonen, 1980). Initially, 14 to 20 solid epithelial buds appear in 5 groups and grow into the surrounding mesoderm. During branching morphogenesis of the solid epithelial buds, ductal lumina appear, and definitive luminal and basal cells become recognizable for the first time. Solid buds canalize initially in the proximal ductal regions near the urethra. Canalization then progresses distally into the branching ducts. Therefore, both solid buds and canalized glandular structures can be observed in the same section of the developing prostate. By the end of the 15th week of gestation, secretory prostatic epithelial cells are functional and produce PSA. The basal cells underlying the luminal cells are well differentiated, and scattered neuroendocrine cells are also present. The cells of solid epithelial ducts remain undifferentiated in distal regions. Maturation of the gland continues during embryonic development when testosterone levels are high (Bentvelsen et al., 1995). After birth, the gland enters a quiescent state. The quiescent state persists until puberty, when testosterone levels again increase and the epithelium proliferates, giving rise to the complex ductal epithelium seen in the mature gland. The prostate increases in size during puberty. In adulthood, androgen receptors are expressed by the luminal epithelial cells, and the full secretory phenotype is established. In rats and mice, branching morphogenesis follows the same pattern as in

Table 1 Antibodies.

Antigen	Species	Dilution	Source	Clone
CK8	Mouse	1:5	Gift, E.B.Lane	LE41
CK18	Mouse	1:5	Gift, E.B.Lane	LE61
CK14	Mouse	1:10	Gift, E.B.Lane	LL001
p63	Mouse	1:100	Santa Cruz Biotec	
CK19	Mouse	1:1	Gift, E.B. Lane	LP2K
GSTpi	Rabbit	1:600	Novocastra	
LMW-CK (CK8)	Mouse	1:5	Dako	35βH11 (IgM)
HMW-CK (CK1,5,10,14)	Mouse	1:30	Dako	34βE12 (IgG1)

human except that most ductal branching occurs neonatally (Sugimura et al., 1986; Hayashi et al., 1991).

Differentiation markers of the mouse, rat and human urogenital sinus epithelium

The male urogenital sinus was studied prior to the emergence the prostatic buds, which appear at 17 days of gestation in the mouse, day 19 in rats, and at week 10 in human fetuses (Cunha et al., 1991). In the male embryo, the first indication of formation of the prostate is the appearance of prostatic buds which emerge from the embryonic urogenital sinus epithelium below the bladder. Prior to the appearance of prostatic buds, luminal cell markers, CK8 (Fig. 1a) and CK18 (Fig. 1b), were strongly expressed uniformly in the mouse, rat, and human urogenital sinus epithelium. Likewise basal cell markers, CK14 (Fig. 1c) and p63 (Fig. 1d), were expressed in the majority of embryonic urogenital sinus epithelium except for some apical epithelial cells which were negative or weakly positive in all three species. Staining for the so-called transitional or intermediate cell marker, CK19 (Fig. 1e), was strong and uniform in urogenital sinus epithelium. GSTpi (Fig. 1f) was also expressed uniformly throughout the embryonic urogenital sinus epithelium (Table 2). This pattern of marker expression was seen in all 3 species (mouse and rat not illustrated).

Differentiation markers in the developing prostate

During ductal elongation and branching morphogenesis, there are two distinct kinds epithelial structures: solid cords and canalized ducts. In solid epithelial cords,

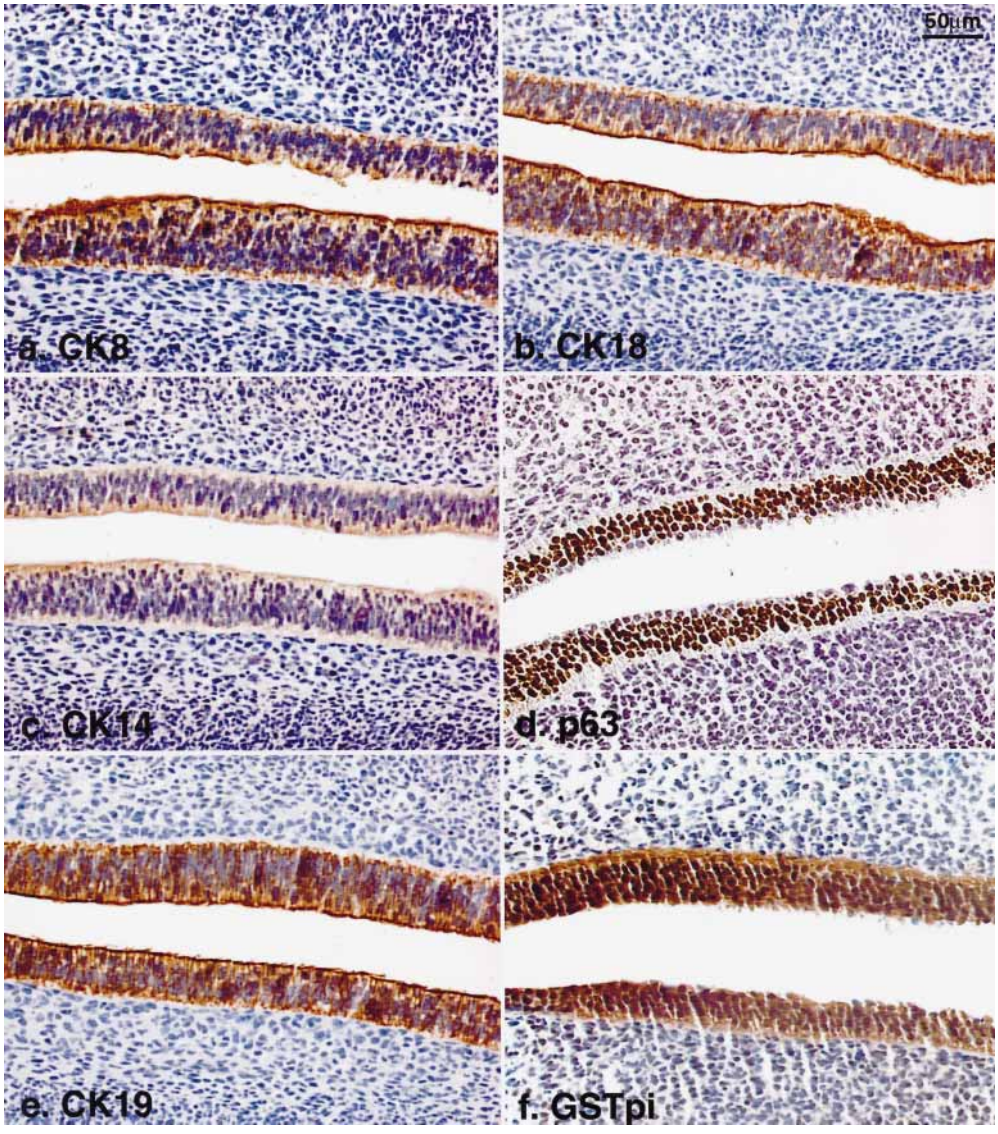

Fig. 1 Marker profiles in human urogenital sinus epithelium. Expression of luminal cell makers, CK8 (**a**) and CK18 (**b**). Expression of basal cell makers, CK14 (**c**), and p63 (**d**). Expression of other makers, CK19 (**e**) and GSTpi (**f**). All the above markers uniformly stained the urogenital sinus epithelium.

Table 2 Marker expression profiles in urogenital sinus epithelium, developing and adult prostate.

Markers	UGE	Developing Prostate				Adult Prostate			
		Solid Ducts	Canalized Ducts			Mature Luminal	Intermediate Luminal	Mature Basal	Progenitor basal
			Early	Middle	Late				
p63	+	+	+	+/−	+/−	−	−	+	+
CK5	+	+	+	+/−	+/−	−	−	+	+
CK14	+	+	+	+/−	+/−	−	−	+	+
CK8	+	+	+	+	+/−	+	+	−	+
CK18	+	+	+	+	+/−	+	+	−	+
CK19	+	+	+	+	+/−	−	−/+	+	+
GSTpi	+	+	+	+	+/−	−	−/+	+	+

the differentiation marker profile is similar to that seen in embryonic urogenital sinus epithelium. Canalized "ducts" can be generally classified into two stages: (a) early canalization and (b) advanced canalization. In the early phase of canalization, lumina are just beginning to form, and the differentiation marker profile is similar to that seen in solid prostatic buds and embryonic urogenital sinus epithelium. Using CK8 and HMW-CK in dual staining procedures, almost all epithelial cells co-express the luminal marker, CK8, (Fig. 2a) and basal cell marker, HMW-CK (Fig. 2b). Thus, almost all epithelial cells appear yellow when viewed simultaneously in the flourescein and rhodamine channels. Only a small minority of cells express CK8 or HMW-CK only (Fig. 2c). In the advanced stage of canalization, a large well defined lumen is present lined with luminal cells stained strongly and uniformly for CK8 (Fig. 3a) and CK18 (Fig. 3b). Basal cell markers, CK14 (Fig. 3c), HMW-CK (not shown) and p63 (Fig. 3d), were expressed in basal epithelial layers, but not in the centrally or apically located epithelial cells of canalizing ducts. CK19 (Fig. 3e) and GSTpi (Fig. 3f) were strongly and uniformly expressed throughout luminal and basal epithelial layers. Thus, within developing prostate ducts undergoing canalization, three epithelial populations are present. One is a partially differentiated luminal epithelial cell population mainly expressing CK8, CK18, CK19 and GSTpi located apically or centrally within the duct. The second is an apically located epithelial cell population expressing CK8 and CK18 only, and thus appears to be a fully differentiated luminal cell population. Both of these cell populations are negative for basal cell markers (CK14, HMW-CK, p63). The third epithelial cell population in the developing prostate is located within the basal compartment and expresses the full spectrum of luminal and basal markers, CK8, CK18, CK14, CK5, p63, CK19, and GSTpi (Table 2). As above, the pattern of immunostaining was virtually identical for all three species.

Expression profile of adult mouse, rat and human prostate

In adult human prostate, the epithelium contains basal and secretory epithelial compartments and the rare neuroendocrine cells. The luminal secretory cell compartment consisted of a layer of tall columnar secretory cells, which express mainly CK8 (Fig. 4a) and CK18. A few luminal cells also were positive for CK19 and GSTpi, but all luminal cells were negative for CK14, HMW-CK, and p63. Luminal cells also expressed AR and PSA (data not shown). In the basal compartment, the ma-

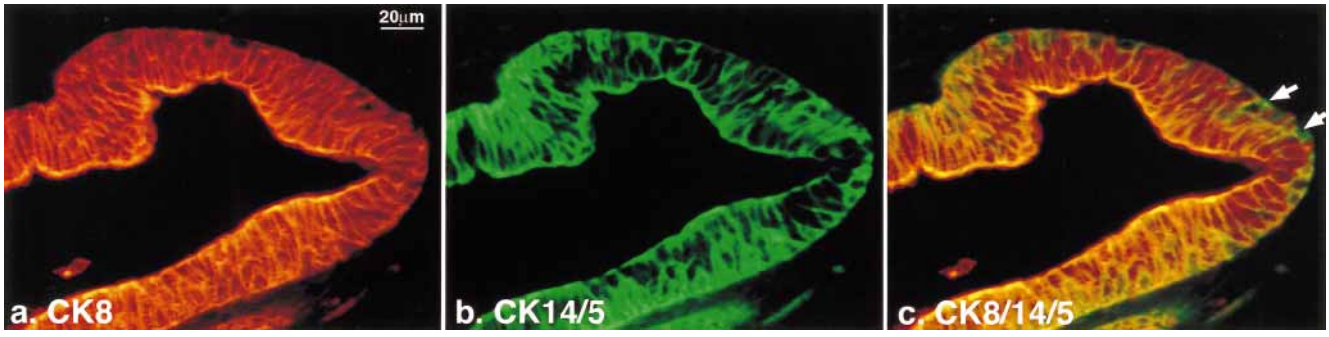

Fig. 2 Double staining of luminal cell makers, CK8 and basal cell makers, HMW-CK (CK14/5) in the early stage of canalization of developing human prostate. A few basal cells predomintly express CK14/5 *(arrows)*.

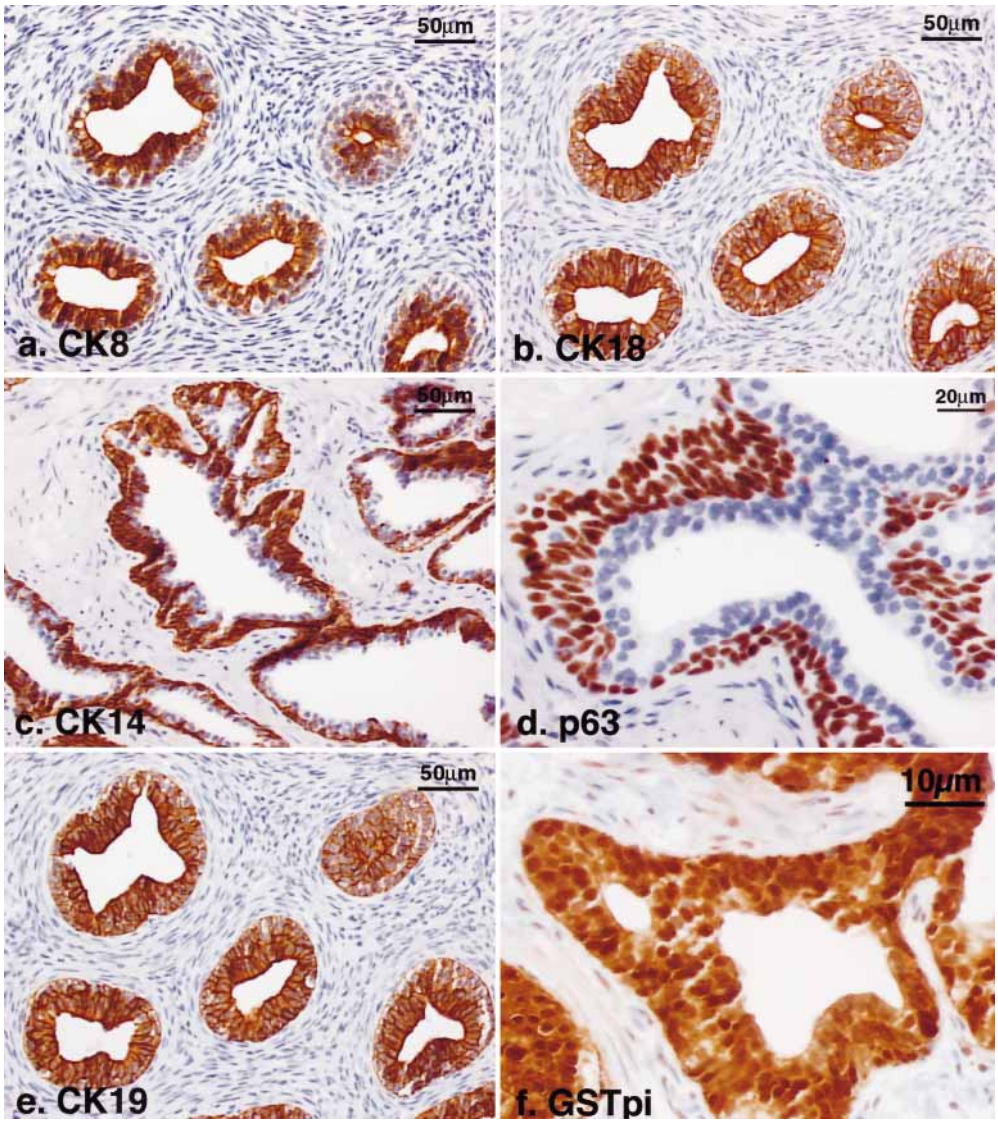

Fig. 3 Marker profiles in the middle stage of canalization of developing human prostatic epithelium. Expression of luminal makers, CK8 (**a**) and CK18 (**b**); basal cell makers, CK14 (**c**) and p63 (**d**); CK19 (**e**) and GSTpi (**f**).

jority of basal cells expressed HMW-CK (CK5/14) (Fig. 4b), CK14 (Fig. 4c), p63 (Fig. 4d), CK19 (Fig. 4e) and GSTpi (Fig. 4f), but were negative for CK8, 18. A small proportion of cells in basal compartment co-expressed the full complement of luminal and basal cell markers (Fig. 5c). Cells co-expressing CK8 (Fig. 5a) and CK5/14 (Fig. 5b) were stained yellow (Fig. 5c) in sections of adult prostate double stained with CK8 using a rhodamine-labeled secondary antibody and with CK5/14 using a flourescein-labeled secondary antibody (Table 2). HMW-CK expression always overlaps with CK14, p63, CK19, and GSTpi. Thus, embryonic urogenital sinus epithelium-like cells, co-expressing CK8, CK18, CK14, HMW-CK, p63, CK19, and GSTpi remain present in small numbers in the basal compartment of the adult prostatic epithelia. As above, similar patterns of staining were obtained for mouse, rat, and human prostate. To assess the incidence of these rare adult prostatic basal epithelial cells, cell counting was performed in the adult human prostate. Based upon the analysis of 17 microscopic fields of adult human prostate, only 7/1183 (0.59%) basal cells co-expressed both luminal and basal cytokeratins (CK8 and HMW-CK [CK5/14]).

Discussion

Patterns of cytokeratin expression serve as useful markers for identification of epithelial sub-populations, and the use of cytokeratin antibodies is one of the most frequently applied markers for identifying different epithelial cell types within the prostate (Brawer et al., 1985; Wernert et al., 1986; Verhagen et al., 1988; Okada et al., 1992; Peehl et al., 1996). Normal adult prostatic epithelium is composed of two major cell types, which express distinct cytokeratins. The luminal compartment consists of a layer of tall columnar secretory cells that mainly express CK8 and CK18, although a few luminal epi-

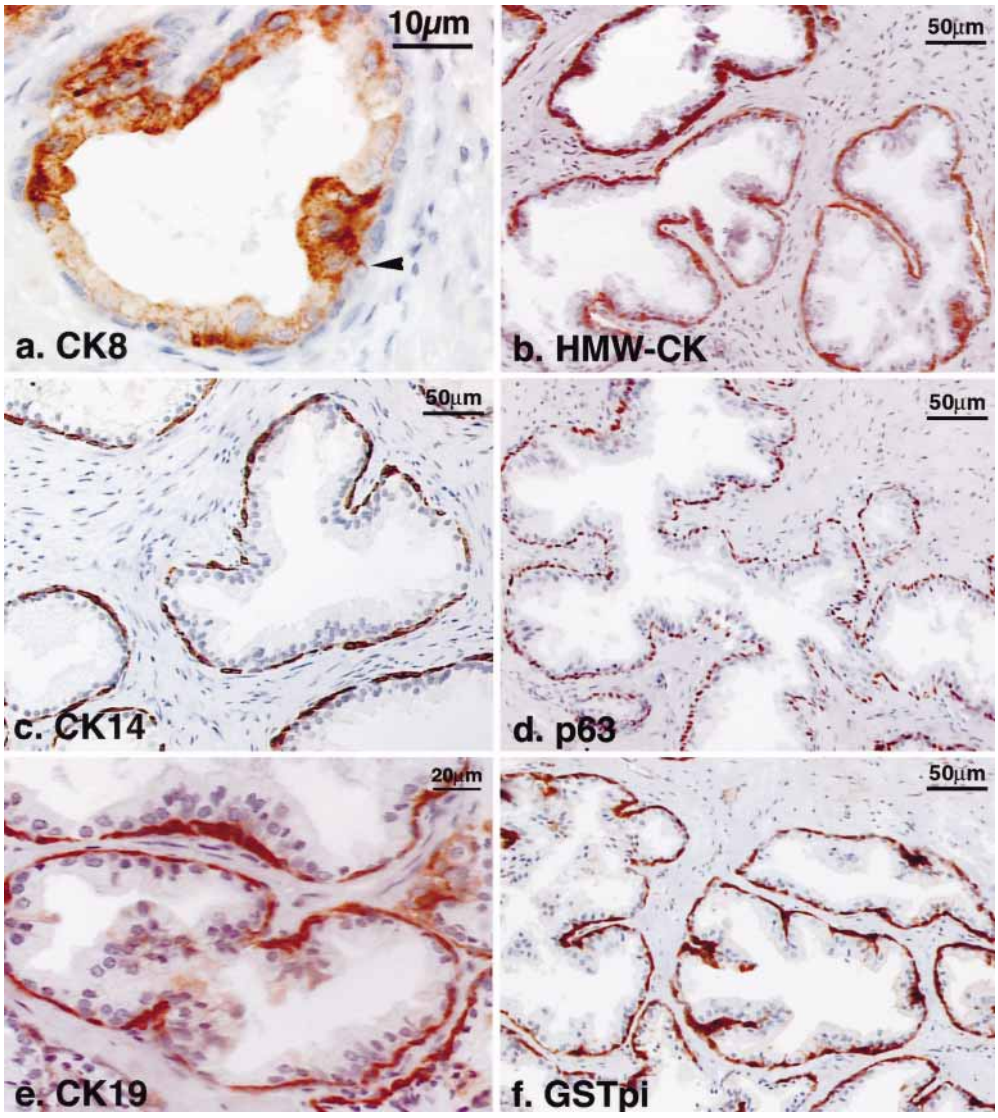

Fig. 4 Marker profiles in adult human prostatic epithelium. Expression of luminal cell makers, CK8 (**a**). Expression of basal cell makers, HMW-CK (CK5/14) (**b**), CK14 (**c**), and p63 (**d**). Expression of other makers, CK19 (**e**), and GSTpi (**f**).

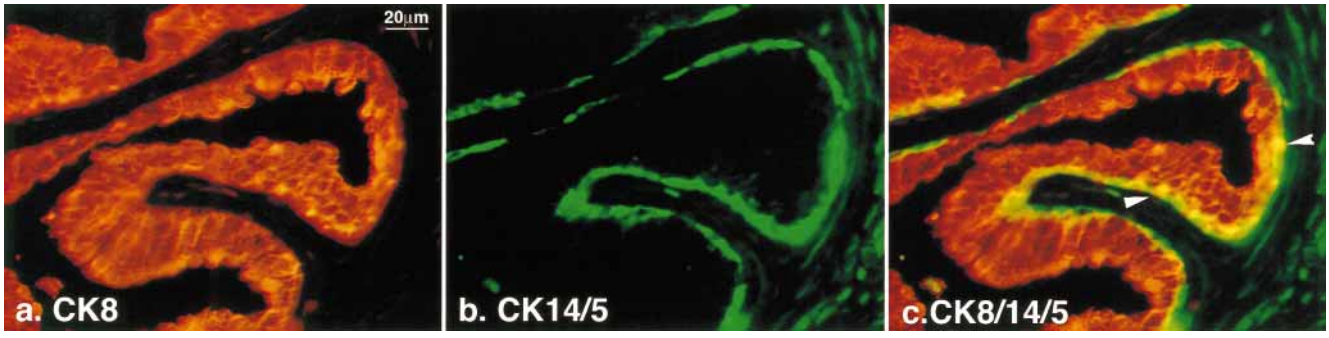

Fig. 5 Co-expression of luminal makers in adult human prostate, CK8 (**a**) and basal cell makers, HMW-CK (CK5/14) (**b**) in adult human prostate. (**c**) Double expored for flourescein and rhodamine showing yellow cells in basal compartment co-expressing basal and luminal markers.

thelial cells also express CK19 and GSTpi. The majority of adult prostatic basal epithelial cells express CK14, CK5, p63, CK19, and GSTpi and are negative for CK8, CK18. Cytokeratin 5, CK14 and p63 (Signoretti et al., 2000) are always expressed in basal cells and can be considered to be basal cell specific in the adult prostate. GSTpi is highly specific for adult prostatic basal cells but is also expressed in a percentage of luminal cells. CK19 is a protein mainly expressed in adult prostatic basal cells but is also expressed in a subset of luminal cells. Interestingly, there is a very small population of cells in the basal compartment of the adult prostate that co-express the full range of luminal (CK8 and CK18) and basal cell markers (CK14, CK5, p63) in addition to CK19, CK17, and GSTpi (Bonkhoff et al., 1994; Bonkhoff and Remberger, 1996; Xue et al., 1998; Hudson et al., 2000). These rare adult prostatic basal cells comprise only about 0.59% of the basal cells. These rare prostatic basal cells and other prostatic epithelial cells co-expressing a spectrum of luminal and basal cell markers were originally interpreted to be basal cells in transition differentiating into luminal cells (Hudson et al., 2001).

Interpreting these intermediate cell types from a developmental perspective raises other possible cell lineage relationships. Progenitor cells of the embryonic prostate, the urogenital sinus epithelium, co-express the full range of luminal and basal epithelial differentiation markers (CK8, CK18, CK14, CK5, p63, CK19, GSTpi). Fully differentiated basal cells expressing only CK14, CK5, CK19, p63, and GSTpi are not present in fetal urogenital sinus epithelium. Likewise, fully differentiated luminal cells expressing only CK8, CK18 are not present in fetal urogenital sinus epithelium. Instead all embryonic urogenital sinus epithelium cells co-express the full spectrum of basal and luminal cell markers. Based on these observations our interpretation of prostatic epithelial cell lineage differs in four major ways with previous stem cell and cell lineage models. (1) If the definitive fully differentiated basal cell expressing only basal cell markers (CK14, CK5, p63, CK19, and GSTpi) is the progenitor/stem cell for the prostate, then these cells should be present in the embryonic urogenital sinus epithelium because embryonic urogenital sinus epithelium is indisputably the prostatic epithelial precursor. However, epithelial cells expressing only the basal cell markers were not observed in embryonic urogenital sinus epithelium. (2) Likewise, epithelial cells expressing only basal markers (CK14, CK5, p63, CK19, and GSTpi) were not observed in developing prostatic buds and elongating solid epithelial cords. Instead, during ductal elongation and branching morphogenesis the developing prostate contains three general epithelial cell populations: (a) cells co-expressing the full range of luminal and basal differentiation markers (CK8, CK18, CK14, HMW-CK, p63, CK19, GSTpi), (b) partially differentiated luminal epithelial cells expressing only CK8, CK18, CK19, and GSTpi, and (c) differentiated luminal cells expressing only CK8 and CK18. (3) Definitive basal cells expressing only basal cell markers were only observed in fully differentiated adult prostate. (4) Finally, the designation of CK19 as a marker of intermediate cells (basal cells in the process of differentiating into luminal cells) is untenable with its uniform expression in the embryonic urogenital sinus epithelium and developing prostate. Clearly, definitive basal and luminal cells expressing their distinctive markers do not exist in the fetal urogenital sinus epithelium. Expression of CK19 throughout the embryonic urogenital sinus epithelium would, according to previous theories, suggest that all embryonic urogenital sinus epithelium cells are "intermediate cells", which is also clearly not the case.

During human prostatic development from 10 to 18 weeks of gestation or during the first 7 days postnatal in the rat and mouse, solid prostatic buds emerge from the embryonic urogenital sinus epithelium (Figs. 6a, 6b), elongate, and branch. During early stages of prostatic development, the ducts are solid distally and canalized proximally. In solid epithelial buds, most cells maintain a differentiation marker profile identical to embryonic urogenital sinus epithelium and express the full range of differentiation markers. In partially canalized ducts, apical or superfical cells express CK8 and CK18 or CK8, CK18 and CK19. The basal cell layers during ductal canalization initially continue to co-express the full range of both luminal and basal epithelial differentiation markers, and thus immature basal cells maintain a differentiation marker profile similar to embryonic urogenital sinus epithelium (Fig. 6b). After 18 weeks of gestation in human and day 10 in rodent, definitive luminal and basal cell types appear, expressing their unique differentiation marker profiles. As prostatic epithelium matures, luminal cells express only the CK8/CK18 pair, and most basal cells express CK14, CK5, CK19, GSTpi, and p63. The embryonic urogenital sinus epithelium-like population of basal cells expressing the full range of differentiation markers (CK8, CK18, CK14, CK5, CK19, GSTpi, and p63) decreases progressively during development. By adulthood, basal epithelial cells expressing the full embryonic profile of markers are extremely rare and constitute only about 0.59% (7/1183) of the epithelial cells (Fig. 6c). From a developmental perspective, it is clear that definitive luminal and basal cells arise from urogenital sinus epithelium-like cells co-expressing the full range of luminal and basal differentiation markers rather from fully differentiated basal cells expressing the CK14/5 pair only. We propose that the lineage relationship during development also applies to basal epithelial cells in adult prostate. Embryonic-like basal calls co-expressing the full range of luminal and basal differentiation markers are present in adult prostate in small numbers and are suggested to be progenitor/stem cells in adult prostate. These cells are the likely source of definitive basal and luminal cells. The rarity of these embryonic-like basal

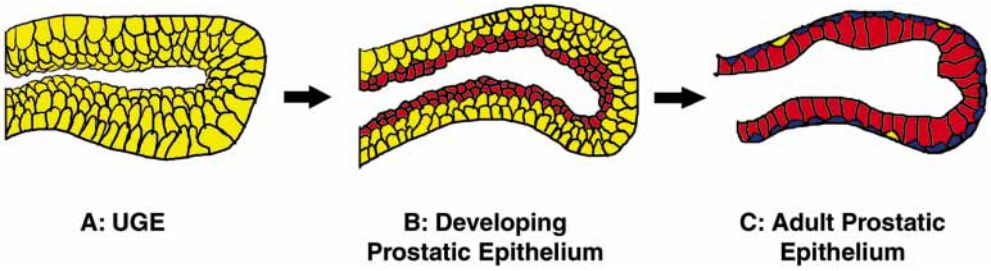

Fig. 6 Cell lineage relationships in adult prostate: Definitive basal cells (CK5/14/p63/CK19/GSTpi positive/CK8/18 negative) are *blue*; definitive luminal cells (CK8/18 positive/ CK5/14/p63 negative) are *red*; embryonic-like cells co-expressing both luminal and basal cell markers (CK8/18/14/5/p63/CK19/GSTpi positive) are in *yellow*. In urogenital sinus epithelium, the epithelial cells co-express all markers (yellow cells), and definitive basal and luminal cells are not seen (no red and blue cells). The developing prostate contains embryonic-like cells (yellow) and some definitive luminal cells (red), but no definitive basal cells. The adult prostate contains definitive luminal cells (red) and definitive basal cells (blue) and the extremely rare embryonic-like progenitor/stem cells (yellow). Appearance of the definitive basal cells is a late event.

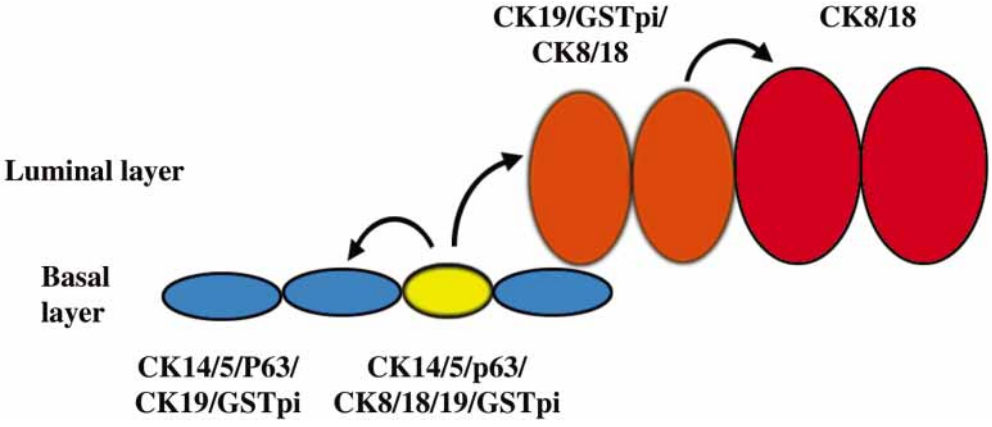

Fig. 7 Hypothetical cell differentiation pathway model in adult prostate. Cells co-expressing CK5/14/p63/CK8/18/CK19/GSTpi give rise to definitive basal cells (CK5/14/p63/CK19/GSTpi positive only) and luminal cells (CK8/18 positive only).

cells in the adult prostate is also consistent with their identification as progenitor/stem cells, however a functional test is needed to confirm this hypothesis.

Our data suggest the following model of prostatic epithelial cell lineage (Fig. 7). Prostatic epithelial progenitor cells within embryonic urogenital sinus epithelium maintain their embryonic differentiation marker profile and stem cell characteristics into adulthood, even though the proportional size of this progenitor/stem cell population is dramatically reduced in adulthood. These rare adult prostatic progenitor/stem cells co-expressing the full range of luminal and basal differentiation markers are proposed to be telomerase positive, androgen-independent for survival, but androgen responsive and are present at all stages (embryonic to adult). These pleuripotent progenitor/stem cells give rise to differentiated luminal cells by maintaining CK8 and CK18, losing CK14, CK5, and p63, and gaining the AR and PSA. Progenitor/stem cells also give rise to definitive basal cells by maintaining CK14, CK5, p63, CK19, and/or GSTpi, while losing CK8 and CK18. In adult prostate, the embryonic-like progenitor/stem cell population is located in basal layer of prostate epithelium resting on the basement membrane. Thus, the basal compartment contains cells with a combination of differentiation markers. CK14, CK5, p63 are co-expressed by both definitive basal cells and progenitor/stem cells. Other basal cells express CK14, CK5, p63, CK19, and GSTpi appear to be mature basal cells. Only progenitor/stem cells express the full spectrum of both luminal and basal differentiation markers (CK8, CK18, CK14, CK5, p63, CK19, and GSTpi). The current hypothesis may also be important to test the progenitor/stem cell model in prostatic carcinogenesis by examining changes in the expression of basal and luminal cell markers during the development of prostate cancer, especially in the early dysplastic lesions.

To further investigate prostatic epithelial cell lineage, clones of various prostate epithelial cells must be established and characterized with respect to differentiation marker expression and developmental potential both in vitro and in vivo. Clonally derived cell colonies expressing luminal cell markers only should be incapable of giving rise to basal cells. Clonally derived cells expressing basal cell markers only may be incapable of giving rise to prostatic ducts containing luminal cells. Putative progenitor/stem cells co-expressing both luminal and basal cell markers should be capable of giving rise to prostatic ducts containing both definitive luminal and basal cells. Determination of the true development potential of luminal, basal and progenitor prostatic epithelial cells will be facilitated by combining clonally derived epithelial

cells with rat urogenital sinus mesenchyme (UGM) and grafting cell-cell recombinants into nude mouse hosts. Rat UGM + prostatic epithelium tissue recombinants composed of relatively small numbers (5,000–20,000) of rodent or human prostatic epithelial cells will form well differentiated prostate tissue when UGM + prostatic epithelium recombinants are grown in vivo (Hayward et al., 1998).

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References

Bentvelsen, F.M., Brinkmann, A.O., van der Schoot, P., van der Linden, J.E., van der Kwast, T.H., Boersma, W.J., Schroder, F.H. and Nijman, J.M. (1995) Developmental pattern and regulation by androgens of androgen receptor expression in the urogenital tract of the rat. Mol Cell Endocrinol 113:245–253.

Bonkhoff, H. and Remberger, K. (1996) Differentiation pathways and histogenetic aspects of normal and abnormal prostatic growth: a stem cell model. Prostate 28:98–106.

Bonkhoff, H., Stein, U. and Remberger, K. (1994) Multidirectional differentiation in the normal, hyperplastic, and neoplastic human prostate: simultaneous demonstration of cell-specific epithelial markers. Hum Pathol 25:42–46.

Brawer, M.K., Peehl, D.M., Stamey, T.A. and Bostwick, D.G. (1985) Keratin immunoreactivity in the benign and neoplastic human prostate. Cancer Res 45:3663–3667.

Bui, M. and Reiter, R.E. (1998) Stem cell genes in androgen-independent prostate cancer. Cancer Metastasis Rev 17:391–399.

Cunha, G.R., Cooke, P.S., Bigsby, R. and Brody, J.R. (1991) Ontogeny of Sex Steroid Receptors in Mammals. In: Parker, M.G. (ed.) The Structure and Function of Nuclear Hormone Receptors. Academic Press, New York, pp 235–268.

Cunha, G.R., Donjacour, A.A., Cooke, P.S., Mee, S., Bigsby, R.M., Higgins, S.J. and Sugimura, Y. (1987) The endocrinology and developmental biology of the prostate. Endocrine Rev 8:338–362.

Greider, C.W. (1996) Telomere length regulation. Annu Rev Biochem 65:337–365.

Hayashi, N., Cunha, G.R. and Parker, M. (1993) Permissive and instructive induction of adult rodent prostatic epithelium by heterotypic urogenital sinus mesenchyme. Epithelial Cell Biol 2:66–78.

Hayashi, N., Sugimura, Y., Kawamura, J., Donjacour, A.A. and Cunha, G.R. (1991) Morphological and functional heterogeneity in the rat prostatic gland. Biol Reprod 45:308–321.

Hayward, S.W., Haughney, P.C., Rosen, M.A., Greulich, K.M., Weier, H.U., Dahiya, R. and Cunha, G.R. (1998) Interactions between adult human prostatic epithelium and rat urogenital sinus mesenchyme in a tissue recombination model. Differentiation 63:131–140.

Hudson, D.L., Guy, A.T., Fry, P., O'Hare, M.J., Watt, F.M. and Masters, J.R. (2001) Epithelial Cell Differentiation Pathways in the Human Prostate. Identification of intermediate phenotypes by keratin expression. J Histochem Cytochem 49:271–278.

Hudson, D.L., O'Hare, M., Watt, F.M. and Masters, J.R. (2000) Proliferative heterogeneity in the human prostate: evidence for epithelial stem cells. Lab Invest 80:1243–1250.

Isaacs, J.T. and Coffey, D.S. (1989) Etiology and disease process of benign prostatic hyperplasia. Prostate Suppl 2:33–50.

Kellokumpu-Lehtonen, P. (1980) Embryonic development of the human prostate. University of Turku, Location??.

Meeker, A.K., Sommerfeld, H.J. and Coffey, D.S. (1996) Telomerase is activated in the prostate and seminal vesicles of the castrated rat. Endocrinology 137:5743–5746.

Montpetit, M., Abrahams, P., Clark, A.F. and Tenniswood, M. (1988) Androgen-independent epithelial cells of the rat ventral prostate. Prostate 12:13–28.

Norman, J.T., Cunha, G.R. and Sugimura, Y. (1986) The induction of new ductal growth in adult prostatic epithelium in response to an embryonic prostatic inductor. Prostate 8:209–220.

Okada, H., Tsubura, A., Okamura, A., Senzaki, H., Naka, Y., Komatz, Y. and Morii, S. (1992) Keratin profiles in normal/hyperplastic prostate and prostate carcinoma. Virchows Archiv A Pathol Anat 421:157–161.

Peehl, D.M., Sellers, R.G. and McNeal, J.E. (1996) Keratin 19 in the adult human prostate: tissue and cell culture studies. Cell Tissue Res 285:171–176.

Robboy, S.J., Taguchi, O. and Cunha, G.R. (1982) Normal development of the human female reproductive tract and alterations resulting from experimental exposure to diethylstilbestrol. Human Pathol 13:190–198.

Robinson, E.J., Neal, D.E. and Collins, A.T. (1998) Basal cells are progenitors of luminal cells in primary cultures of differentiating human prostatic epithelium. Prostate 37:149–160.

Signoretti, S., Waltregny, D., Dilks, J., Isaac, B., Lin, D., Garraway, L., Yang, A., Montironi, R., McKeon, F. and Loda, M. (2000) p63 is a prostate basal cell marker and is required for prostate development. Am J Pathol 157:1769–1775.

Sugimura, Y., Cunha, G.R. and Donjacour, A.A. (1986) Morphogenesis of ductal networks in the mouse prostate. Biol Reprod 34:961–971.

Verhagen, A.P.M., Aalders, T.W., Ramaekers, F.C.S., Debruyne, F.M.J. and Schalken, J.A. (1988) Differential expression of keratins in the basal and luminal compartments of rat prostatic epithelium during degeneration and regeneration. Prostate 13:25–38.

Wernert, N., Seitz, G., Goebbels, R. and Dhom, G. (1986) Immunohistochemical demonstration of cytokeratins in the human prostate. Pathol Res Pract 181:668–674.

Xue, Y., Smedts, F., Debruyne, F.M., de la Rosette, J.J. and Schalken, J.A. (1998) Identification of intermediate cell types by keratin expression in the developing human prostate. Prostate 34:292–301.