Proximal location of mouse prostate epithelial stem cells: a model of prostatic homeostasis

Akira Tsujimura,[1,5] Yasuhiro Koikawa,[1,6] Sarah Salm,[1] Tetsuya Takao,[1,5] Sandra Coetzee,[1] David Moscatelli,[1,4] Ellen Shapiro,[2,4] Herbert Lepor,[2,4] Tung-Tien Sun,[2,3,4] and E. Lynette Wilson[1,2,4]

[1]Department of Cell Biology, [2]Department of Urology, [3]Ronald O. Perelman Department of Dermatology and Department of Pharmacology, and [4]Kaplan Cancer Center, New York University School of Medicine, New York, NY 10016
[5]Department of Urology, Osaka University Medical School, Suita, Osaka 565-0871, Japan
[6]Department of Urology, Fukuoka City Medical Center of Sick Children and Infectious Diseases, Fukuoka 810-0063, Japan

Stem cells are believed to regulate normal prostatic homeostasis and to play a role in the etiology of prostate cancer and benign prostatic hyperplasia. We show here that the proximal region of mouse prostatic ducts is enriched in a subpopulation of epithelial cells that exhibit three important attributes of epithelial stem cells: they are slow cycling, possess a high in vitro proliferative potential, and can reconstitute highly branched glandular ductal structures in collagen gels. We propose a model of prostatic homeostasis in which mouse prostatic epithelial stem cells are concentrated in the proximal region of prostatic ducts while the transit-amplifying cells occupy the distal region of the ducts. This model can account for many biological differences between cells of the proximal and distal regions, and has implications for prostatic disease formation.

Introduction

The prostate is an androgen-dependent organ that undergoes involution after castration in experimental animals but can completely regenerate if androgen is resupplied. Because this involution–regeneration cycle can be repeated over 30 times, a population of long-lived, prostatic epithelial stem cells that can survive the involution and is responsible for the regeneration of the gland must exist (Isaacs, 1985). The identification and characterization of such stem cells is important, because they may represent a major target of carcinogenesis as well as a potential source of benign prostatic hyperplasia (BPH)* (De Marzo et al., 1998; Reya et al., 2001). An understanding of prostatic epithelial biology is also important because the evolution of androgen-independent prostate carcinoma, which happens frequently as the tumor progresses, may reflect a stem cell–like state of the tumor (Reya et al., 2001). Moreover, a better understanding of the location and properties of prostatic stem cells may help explain

some puzzling features of the gland. For example, in the human prostate, which can be divided anatomically into the central, transition, and peripheral zones, carcinoma arises mainly in the peripheral zone, whereas BPH develops largely in the transition zone (McNeal et al., 1988; De Marzo et al., 1998). Similar differences in disease patterns exist in the rodent prostates, which consist of the dorsolateral and ventral prostates. In this case, chemically induced carcinomas occur mainly in the dorsolateral prostate (Bosland and Prinsen, 1990; Hoover et al., 1990; Shirai et al., 1991). Whether these differences reflect the distribution of stem cells is currently unknown.

Although the overall organization of the rodent prostate differs from that of the human gland, the rodent prostate provides unique opportunities to study many important features of the prostate, including the involution–regeneration process as well as chemical and hormonal carcinogenesis. Each murine prostatic duct consists of a proximal region attached to the urethra, an intermediate region, and a distal tip (Sugimura et al., 1986a; Cunha et al., 1987; Lee et al., 1990; Hayashi et al., 1991). Proliferating cells are located at the tips of the ducts (Sugimura et al., 1986c; Cunha et al., 1987). Based on this finding, it has been suggested that prostatic stem cells reside in the distal region (Kinbara et al., 1996). However, studies on the stem cells of several other systems have indicated that the rapidly cycling cells are most likely the so-called transit-amplifying cells rather than stem cells. The location of prostatic epithelial stem cells is therefore currently unknown.
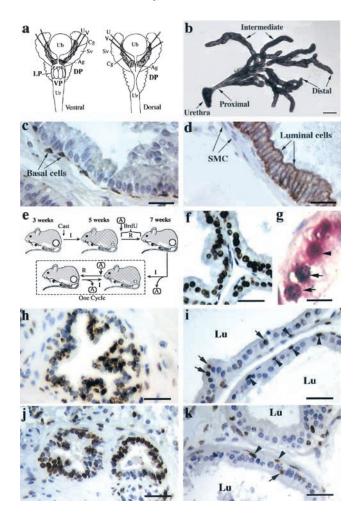
Figure 1. **The proximal region of mouse prostatic ducts contains a high density of slow-cycling stem cells.** (a) Schematic diagrams showing the ventral and dorsal views of the urogenital organs of a male mouse. Ag, ampullary gland; Cg, coagulating gland; DP, dorsal prostate; LP, lateral prostate; Sv, seminal vesicle; U, ureter; Ub, urinary bladder; Ur, urethra; V, vas deferens; VP, ventral prostate. (b) A segment from a microdissected mouse ventral prostate illustrating the distal, intermediate, and proximal regions of prostatic ducts. Bar, 1 mm. (c) A paraffin section of a prostatic duct showing basal cells (arrows) immunohistochemically stained using an antibody against K14 keratin. Bar, 125 μm. (d) A paraffin section of a prostatic duct showing luminal cells (arrows) immunohistochemically stained using an antibody against K8 keratin. SMC, smooth muscle cells. Bar, 125 μm. (e) A schematic diagram illustrating the BrdU labeling protocol used for the identification of the slow-cycling label-retaining cells. In this protocol, almost all prostatic epithelial cells are initially labeled with BrdU. The BrdU label of the more rapidly proliferating transit-amplifying cells is then diluted out during the subsequent involution–regeneration cycles so that the label is retained only by the slow-cycling stem cells that can thus be identified as the label-retaining cells. A, testosterone pellet; Cast, castrate; I, involute; R, regenerate. Each cycle comprised 10 d of regeneration and 7 d of involution. (f) A paraffin section of a prostatic duct indicating that almost all epithelial cells are BrdU labeled (black cells) after the labeling procedure depicted in Fig. 1 e. Bar, 50 μm. (g) A paraffin section of the proximal region of a prostatic duct from the dorsal prostate of a mouse that had been BrdU labeled as described in panel e, followed by androgen withdrawal to involute the prostate. 40 h after a pulse of androgen, animals were given ³H-thymidine and were killed 1 h later. Note that two of the red BrdU-labeled cells (arrow head) contain silver grains (arrows) due to ³H-thymidine incorporation, indicating that slow-cycling cells can divide after

A particularly powerful method for identifying epithelial stem cells takes advantage of the slow-cycling nature of such cells (Berardi et al., 1995; Thorgeirsson, 1996; Beauchamp et al., 2000; Lavker and Sun, 2000; Slack, 2000). In this procedure, a tissue is long-term labeled with ³H-thymidine or BrdU so that all cells, including the stem cells, are labeled. This is followed by a "chase" period during which the label is diluted out from all the rapidly dividing (transit amplifying) cells but is retained by the slow-cycling (stem) cells, which can thus be identified as the "label-retaining cells" (Cotsarelis et al., 1989; Tani et al., 2000; Taylor et al., 2000). Using this approach, corneal epithelial stem cells have been shown to reside in the peripheral cornea in the limbal region (Schermer et al., 1986; Cotsarelis et al., 1989) and this region has been used in limbal stem cell transplantation studies to reconstitute the corneal epithelium (Tsubota et al., 1999; Tsai et al., 2000). Using the same label-retaining cell approach, hair follicular epithelial stem cells have been shown to reside in the upper follicle in an area called the bulge (Cotsarelis et al., 1990; Morris and Potten, 1999; Lavker and Sun, 2000; Taylor et al., 2000; Oshima et al., 2001). Another important feature of stem cells is their high proliferative potential as reflected by their ability to yield large colonies in vitro (Barrandon and Green, 1987; Ogawa, 1993), as was shown to be the case for both the limbal zone of the corneal epithelium (Pellegrini et al., 1999) and the bulge area of the hair follicle (Oshima et al., 2001).

We describe here the identification of a subpopulation of mouse prostate epithelial cells, located in the proximal region of prostatic ducts, that are slow cycling and exhibit a high in vitro proliferative potential. Moreover, they are able to reconstitute complex glandular structures in collagen gels. These findings strongly suggest that these proximal cells are the stem cells. Cells located at the distal tips of prostatic ducts are rapidly proliferating, thus representing the transit-amplifying cells. Based on these findings, we propose a model in which the prostatic epithelial stem cells, which are maintained in a dormant state in the proximal region of prostatic ducts, give rise to the proliferating transit-amplifying cells that migrate distally to either maintain the normal prostate gland or repopulate the gland during androgen-induced regeneration.

Results

Localization of slow-cycling cells in the proximal region of prostatic ducts

To localize the stem cells of mouse prostate, we performed a series of cell kinetic experiments to identify the slow-cycling cells

androgen administration. Bar, 150 μm. (h) A paraffin section of the proximal region of a prostatic duct from the ventral prostate after 11 cycles of involution–regeneration. Note the high concentration of label-retaining cells in this region. Bar, 50 μm. (i) A paraffin section of the distal region of a prostatic duct from the ventral prostate after 11 cycles. Note the small number of label-retaining cells in this region. Arrowheads, labeled basal cells; arrows, labeled luminal cells. Lu, lumen of duct. Bar, 50 μm. (j) A paraffin section of the proximal region of prostatic ducts from the dorsal prostate after 11 cycles. Note the high concentration of label-retaining cells in this region. Bar, 50 μm. (k) A paraffin section of the distal region of prostatic ducts from the dorsal prostate after 11 cycles. Note the low number of label-retaining cells. Bar, 50 μm.

as the label-retaining cells. The mouse prostate can be divided into ventral (VP) and dorsolateral prostates, which can be further divided into the lateral (LP) and dorsal (DP) lobes (Fig. 1 a). These glands can be dissected to allow the visualization of individual ducts (Fig. 1 b), which consist of a small proximal region (adjacent to the urethra), an intermediate region that comprises the bulk of the duct, and a small distal region (Sugimura et al., 1986a; Cunha et al., 1987; Lee et al., 1990; Kinbara and Cunha, 1996). Previous studies have established that these three regions, although all containing basal epithelial cells (expressing K5/K14 keratins; Fig. 1 c) and luminal secretory cells (K8/K18; Fig. 1 d), are morphologically and kinetically distinct (Sugimura et al., 1986c; Rouleau et al., 1990; Hayashi et al., 1991; Sensibar et al., 1991; Nemeth and Lee, 1996). To label the entire population of prostatic epithelial cells, we castrated a group of 3-wk-old male mice to induce prostatic involution, followed 2 wk later by androgen replacement (by the subcutaneous implantation of testosterone pellets) to induce prostatic regeneration (Fig. 1 e). During androgen replacement, BrdU was administered continuously via a subcutaneously implanted osmotic pump. This resulted in the labeling of 97.5–99.8% of all prostatic epithelial cells (Fig. 1 f). To "chase" or deplete the label from the more rapidly proliferating transit-amplifying cells, we removed the androgen pellets to induce prostatic involution, followed by reimplantation of the androgen pellets to induce regeneration (one "cycle;" Fig. 1 e). Mice were killed after 1, 3, 4, 6, 7, 9, 11, 12, 14, and 16 cycles (corresponding to roughly 1, 7, 8, 15, 16, 21, 27, 29, 34, and 39 wk, respectively), and their prostates were examined histologically to identify the BrdU label–retaining cells. Our results indicate that the percentage of label-retaining cells in the dorsal prostate declined progressively over the course of 39 wk with repeated cycles of involution–regeneration, from almost 100 to 1% in the case of luminal cells located in the distal region (Fig. 2). Interestingly, we found that the highest number of label-retaining cells was located in the proximal region (Fig. 1 j and Fig. 2), with few located in the distal region (Fig. 1 k and Fig. 2). Similar results were obtained for both ventral (Fig. 1, h and i) and dorsal prostates (Fig. 1, j and k, and Fig. 2). Significantly higher percentages of both basal and luminal label-retaining cells were found in the proximal compared with the distal regions from the 7th to the 16th cycles ($P < 0.001$; Fig. 2). Although they divided infrequently, occasional label-retaining cells were observed that had incorporated ^{3}H-thymidine after the administration of androgen to an involuted prostate (Fig. 1 g). This indicates that although proximal cells are quiescent, as evidenced by their label-retaining property, they are able to proliferate in response to hormonal stimulation.

Although it has been suggested that prostatic basal cells serve as the progenitors for the secretory luminal cells (Bonkhoff et al., 1994a,b; Bonkhoff and Remberger, 1996; Robinson et al., 1998; Danielpour, 1999; Hayward et al., 1999), this concept is controversial (English et al., 1987; Evans and Chandler, 1987a,b; van der Kwast et al., 1998). We therefore counted the label-retaining basal and luminal cells separately (Fig. 2). In the proximal portion of the dorsal prostate, a high proportion (~25%) of both basal and luminal cells were found to retain the BrdU label, even after a chase period of 39 wk and a total of 16 cycles of involution–regeneration (Fig. 2). The results of the intermediate and distal regions were strikingly different, in
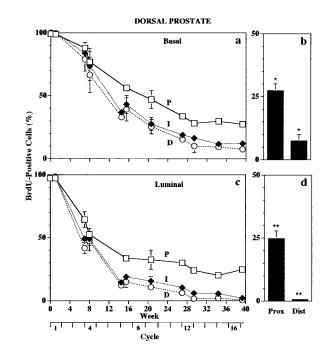
Figure 2. **Cell kinetic data showing that the proximal region of the mouse dorsal prostate contains the highest percentage of label-retaining cells.** (a) Quantification of BrdU-labeled basal cells in the distal (D), intermediate (I), and proximal (P) regions of ducts examined after various periods of chase. (b) Quantification of BrdU-labeled basal cells in the proximal (Prox) and distal (Dist) region after a 39-wk chase (16 cycles). *$P < 0.0001$. (c) Quantification of BrdU-labeled luminal cells in the distal (D), intermediate (I), and proximal (P) regions of ducts after various periods of chase. (d) Quantification of BrdU-labeled luminal cells in the proximal (Prox) and distal (Dist) region after a 39-wk chase (16 cycles). **$P < 0.0001$. The absence of error bars reflects an error range too small to be evident on the graph.

that after a chase of 39 wk, the basal cell layer contained a significant number of label-retaining cells (8% in distal region; Fig. 2, a and b), whereas only a few luminal cells retained the label (~1% in distal region; Fig. 2, c and d). Similar results were obtained in the ventral prostate (unpublished data). Although in the proximal region, ~25% of both basal and luminal cells retained their label, in the distal region, most of the label-retaining cells were found in the basal layer (8 vs. ~1% in the luminal cell layer; Fig. 2). Similar results were obtained from three independent experiments. These findings indicate that the label-retaining stem cells are concentrated in the proximal portion of the mouse prostate, and that both basal and luminal compartments contain slow-cycling cells. They also indicate that the basal cells in the intermediate and distal regions of the ducts retain their BrdU label longer than the luminal cells, suggesting that the transit-amplifying luminal cells replicate more rapidly than the basal cells (see below).

Heterogeneous distribution of the label-retaining cells

Although few label-retaining cells are present in the intermediate and distal regions of the ducts after a chase period of 39 wk (Fig. 2), ducts that had been chased for an intermediate period (9–12 cycles of involution and regeneration) revealed clusters of label-retaining cells (Fig. 3). These clustered, label-retaining cells were frequently associated with the "ridges" of epithelial
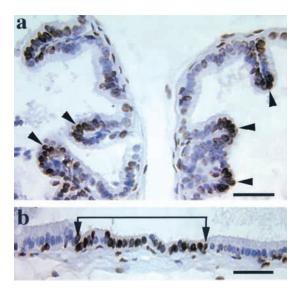
Figure 3. **Discrete clusters of (intermediate stage) label-retaining cells occur in the intermediate and distal regions of the ducts.** Paraffin sections of the ventral prostate after a 20-wk chase showing that clusters of label-retaining cells (a, arrowheads) are associated with ridges protruding into the lumen of a duct, and (b) occur along the ducts (between arrows). Bars, 50 μm.
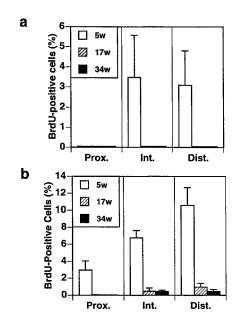

Figure 4. **Rapidly cycling cells are located in the distal regions of the prostatic ducts.** The percentage of BrdU-labeled basal (a) and luminal cells (b) in the dorsal prostate after a single pulse of BrdU. Distal (Dist), intermediate (Int), and proximal (Prox) regions of the ducts of 5-, 17-, and 34-wk-old mice.

folds projecting into the lumen of the duct, whereas the unlabeled cells were associated mainly with the "valleys" connecting the ridges (Fig. 3 a). Even in areas that were not folded, a similar clustering of the labeled cells was frequently noted (Fig. 3 b). Because most of these label-retaining cells eventually lost their label at the end of a 39-wk chase (16 cycles), they probably represent young transit-amplifying cells that had not yet divided sufficiently to dilute out the BrdU label.

Localization of the rapidly cycling transit-amplifying cells

To localize the rapidly proliferating transit-amplifying cells, we administered a pulse of BrdU to 5-, 17-, and 34-wk-old mice and killed them 24 h later. Consistent with the fact that the prostate is undergoing growth during adolescence, 1–10% of epithelial cells in the prostates of 5-wk-old mice were labeled, whereas almost no labeling was observed in adult prostates (17- and 34-wk-old mice; Fig. 4). In the dorsal prostate of 5-wk-old mice, more BrdU-incorporating cells were found in the distal and intermediate regions of the ducts than in the proximal region (Fig. 4), indicating that most cell proliferation occurred in the distal region (Sugimura et al., 1986c; Cunha

et al., 1987). In addition, we found that there were more DNA-synthesizing cells in the luminal compartment (Fig. 4 b) than in the basal compartment (Fig. 4 a); similar results were obtained with the ventral prostate (unpublished data). These results indicate that most of the rapidly proliferating transit-amplifying cells are located in the distal region of the ducts and that the transit-amplifying cells of the luminal compartment cycle faster than those of the basal compartment.

The proximal cells have a greater in vitro proliferative capacity than the distal cells

As slow-cycling stem cells have an extensive proliferative capacity (Barrandon and Green, 1987; Ogawa, 1993; Pellegrini et al., 1999; Oshima et al., 2001), we assessed the proliferative potential of cells isolated from proximal and distal ductal regions. Proximal cells from the dorsal prostatic ducts formed significantly more large colonies (16.7 ± 1.2 colonies that were >100 cells; Fig. 5 a) than distal cells (3.7 ± 0.3; $P < 0.001$; Fig. 5 b). Cell counting showed that the proximal cells of the dorsal prostate generated >200-fold more cells than the distal ones (Fig. 5 c). Cells from the prox-

Figure 5. **The proliferative capacity of proximal cells is greater than that of distal cells.** Cells isolated from the proximal (a) and distal (b) regions of the dorsal prostate were seeded at 4×10^3 cells/well in duplicate wells of eight-well chamber slides. Colonies were fixed and stained 5 d later. Bars, 175 μm. Cells isolated from the proximal (P) and distal (D) regions of the dorsal prostate (DP) (c) were seeded at 2×10^3 cells/well in duplicate wells in 96-well plates. The total cell output from 2×10^3 cells from each region was determined at the end of the serial culture when their growth capacity was exhausted (see Materials and methods). *$P < 0.0001$.

imal region of the ventral prostate also had a greater proliferative capacity than those from the distal region (unpublished data). These differences in proximal/distal ratios were noted in animals that were 5, 10, and 34 wk old. These findings clearly indicate that cells of the proximal region have a higher proliferative potential than those of the distal region.

The proximal cells form large glandular structures in collagen gels

As prostatic cells have been shown to form three-dimensional acinus-like structures in collagen gels, we isolated cells from the proximal and distal regions of the ducts and suspended these cells in collagen gels (Elsdale and Bard, 1972; Turner et al., 1990; Ma et al., 1997). Cells from the proximal region gave rise to numerous, large-branched ducts (444 ± 9) (Fig. 6, a–f) that contained both basal and luminal cells (Fig. 6 e) and produced prostatic secretory products (Fig. 6, j and k). Cells from the distal region formed far fewer such structures (46 ± 6; $P < 0.001$). In addition, the sizes of the glandular structures derived from the proximal cells were ninefold larger (Fig. 6 b; $P < 0.001$) and contained eightfold more branch points (Fig. 6 c; $P < 0.001$) than those derived from the distal cells. Similar results were obtained with cells from the ventral prostate. Wells containing single proximal cells gave rise to branching ductal structures containing both basal and luminal cells, indicating that these structures can be obtained from a single cell (Fig. 6 l). These results indicate that the proximal region is enriched in cells capable of reconstituting large, branched glandular structures that can produce prostatic secretory products.

Discussion

Prostatic stem cells are located in the proximal region of the ducts

We have shown that the proximal cells of mouse prostatic ducts are slow cycling (Fig. 1, h and j, and Fig. 2), have a high proliferative potential (Fig. 5), and give rise to large, branched glandular structures that produce prostatic secretory products (Fig. 6). These data strongly suggest that prostatic epithelial stem cells are concentrated in the proximal region of the ducts of intact animals. The proximal stem cell location is consistent with the fact that the proximal region is least affected by castration (Sugimura et al., 1986b; Lee et al., 1990, 1999; Rouleau et al., 1990). In addition, the proximal region of the ducts has the highest level of telomerase (Banerjee et al., 1998), which is associated with the germinative compartments of many self-renewing tissues (Kim et al., 1994; Wright et al., 1996). It has also been shown that telomerase activity increases in rat prostate after castration (Meeker et al., 1996); this can be explained by the selective loss of the nonstem cells. Taken together, these results strongly support the idea that the prostatic epithelial stem cells are concentrated in the proximal region of the ducts.

Our data indicate that the distal region of the intact gland contains a population of actively proliferating cells (Fig. 4; also see Sugimura et al., 1986c; Cunha et al., 1987). The distal tips of the dorsal and lateral rat prostates undergo significant growth when combined with embryonic urogenital sinus mesenchyme and implanted under the renal capsule (Kinbara et al., 1996). These findings were interpreted to mean that the distal region of prostatic ducts contains the stem cells. However, because the implant studies did not include tissues from the proximal region for comparison, one cannot exclude the
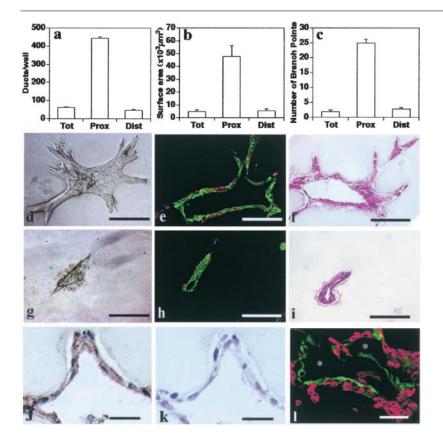

Figure 6.   **Proximal cells form large, branched glandular structures in collagen gels.** Proximal (Prox) and distal (Dist) cells (8×10^3 cells) from the dorsal prostate as well as cells from the entire dorsal prostate (Tot) were seeded in collagen gels and cultured for 6 d. The numbers of ductal structures (a), surface area of structures (b), and branch points/structure (c) in each population were measured. (d and g) Phase contrast microscopy showing the morphology of ductal structures from the proximal (d) and distal (g) regions of ducts. Bars, 150 μm. (e and h) Paraffin sections of proximal (e) and distal (h) ductal structures showing immunofluorescence of basal (PE, red fluorescence) and luminal (FITC, green fluorescence) cells stained using antibodies against K14 (basal) and K8 (luminal) keratins. Bars, 150 μm. (f and i) Paraffin sections of proximal (f) and distal (i) ductal structures stained with hematoxylin and eosin. Bars, 150 μm. (j and k) Paraffin sections of proximal ducts immunohistochemically stained with antibodies specific for prostatic secretory products (j, brown stain). Control (k) section stained with nonspecific antibodies. Sections were counterstained with hematoxylin. Bars, 50 μm. (l) Paraffin section of a branched ductal structure obtained from a single proximal cell stained with antibodies against K14 (basal) and K8 (luminal) keratins showing that it is comprised of both basal (PE, red immunofluorescence) and luminal (FITC, green immunofluorescence) cells. An asterisk indicates the lumen. Bar, 150 μm.

possibility that under identical experimental conditions, cells from the proximal region may grow even better than the distal cells (compare Figs. 5 and 6). The observed growth of the distal region may therefore be explained by the presence of some (young) transit-amplifying cells (Fig. 3; also see below). There are many recent examples, including skin (Taylor et al., 2000; Oshima et al., 2001), bone marrow (Williams, 1993; Berardi et al., 1995), and liver (Fausto, 2000; Strain and Crosby, 2000), showing that a post-stem cell population of young transit-amplifying cells can undergo significant growth.

Clusters of young transit-amplifying cells

Another interesting finding we made is that some of the intermediate stage label-retaining cells, which eventually lose their label, form clusters that are interspersed by groups of nonlabeled cells (Fig. 3). A related, earlier observation is that the castration-induced apoptotic cells were also found to form clusters (Rauch et al., 1997). These two observations can be explained if one assumes that the clusters of intermediate stage label-retaining cells (Fig. 3) represent young transit-amplifying cells that have not yet diluted out all their BrdU label via cell division, and that such cells are more resistant to the apoptotic effects of androgen depletion than the older transit-amplifying and terminally differentiated cells. Having groups of young transit-amplifying cells capable of active proliferation placed strategically along the intermediate and distal ducts may allow the gland to respond more rapidly to signals for increased growth or regeneration without having to recruit cells from the distant proximal stem cell region.

Such a dispersion of young transit-amplifying cells may facilitate prostatic gland regeneration, which occurs regularly in some species. For example, male squirrel monkeys, woodchucks, and the vizcacha (a South American rodent) are known to undergo seasonal variations in testosterone levels that are accompanied by pronounced changes in testicular volume (Baldwin et al., 1985; Pasqualini et al., 1986; Fuentes et al., 1993). Although measurements of prostatic size have not been done, it is likely that prostatic involution occurs concomitantly with testicular regression. Thus, the prostate of these animals may undergo physiological involution–regeneration and the strategically located, young, transit-amplifying cells within the ducts may facilitate the rapid regeneration of the gland.

Basal and luminal cells may serve as prostatic epithelial progenitor cells

There are conflicting data on the nature of the epithelial compartment in which prostatic stem cells are located. The existence of a population of cells that possess markers for both the basal (keratins K5 and K14) and luminal cells (K8 and K18) suggests that these represent an intermediate stage between the precursor basal and the mature luminal cells (Bonkhoff et al., 1994a; Bonkhoff and Remberger, 1996; Robinson et al., 1998). In addition, a prostatic epithelial basal cell line has been shown to give rise to luminal cells both in vitro (Danielpour, 1999) and in vivo (Hayward et al., 1999). These data suggest that basal cells are the precursors of luminal cells. On the other hand, kinetic data showing that luminal cells divide before basal cells after an androgen pulse (Evans and Chandler, 1987a,b), and that the luminal cells divide faster than the basal cells in individuals on a long-term androgen block (van der Kwast et al., 1998) have been interpreted to indicate that luminal cells are self-renewing and are thus independent from the basal compartment. However, the more rapid response of luminal cells to androgens could also result from transit-amplifying luminal cells being more responsive to androgens than basal cells.

Our data indicate that label-retaining cells are concentrated in the proximal region of prostatic ducts and, importantly, are present in both the basal and luminal compartments (Figs. 1 and 2). The presence of label-retaining cells in the luminal compartment is unexpected and raises the interesting possibility that luminal cells are self-sustaining. On the other hand, our finding does not exclude the possibility that basal cells can give rise to luminal cells (see below), or that these label-retaining luminal cells represent the youngest transit-amplifying cells, which require a longer period of chase to dilute out their label.

We have also shown that in the distal and intermediate regions of the prostatic ducts, the luminal cells retain the BrdU label for a shorter period of time than the basal cells (Fig. 2). Because we know that the transit-amplifying luminal cells replicate more rapidly than the basal cells (Fig. 4), it is not surprising that the BrdU label is diluted out more efficiently from the luminal cells.

The prostatic stem cell niche and its possible regulation: a hypothesis

Stem cells are usually located in a niche where they are maintained in a quiescent state (Potten and Loeffler, 1990; Lavker and Sun, 2000; Slack, 2000). Because the proximal region of the prostatic ducts is enveloped by a thick band of smooth muscle cells (Nemeth and Lee, 1996) that are known to produce a high level of TGF-β (Nemeth et al., 1997), it is possible that this smooth muscle–produced TGF-β plays a role in inhibiting the proliferation of prostatic epithelial stem cells. Consistent with this possibility, it has recently been shown that the expression of a dominant-negative form of the TGF-β receptor in the prostate induced an excessive proliferation of the prostatic epithelial cells in the proximal region (Kundu et al., 2000). Moreover, there is a gradient of TGF-β expression in the stroma along the ductal axis; it is highest in the stroma surrounding the proximal epithelium and lowest in the distal region, which is the site of cell proliferation (Nemeth et al., 1997). A similar inhibitory role of TGF-β is known to be responsible for maintaining hematopoietic stem cells in a quiescent state (Cashman et al., 1990; Hatzfeld et al., 1991; Fortunel et al., 2000).

A model for prostatic epithelial stem cells

Based on the above considerations, we propose a model of prostatic epithelial homeostasis in which the stem cells reside in the proximal region of the ducts and are maintained in a quiescent state by the high local levels of TGF-β (Fig. 7). During prostatic regeneration, either a reduced level of active TGF-β or the production of an overriding, positive signal (Fig. 7, GF) stimulates the stem cells to divide, giving rise to a cadre of transit-amplifying cells ($TA_1 \rightarrow TA_2 \rightarrow TA_3$ . . . etc.) that migrate distally. These transit-amplifying cells function as an immediate source of replacement cells along the ductal axis. Upon cell division and distal migration, transit-amplifying cells progressively lose their proliferative potential. During involution, the oldest transit-amplifying
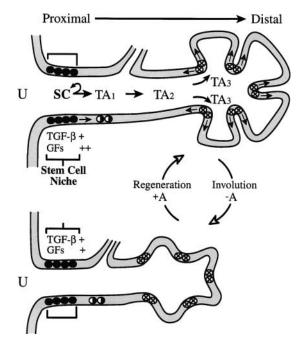
Figure 7.   **A model of prostatic stem cell homeostasis.** A schematic diagram showing the location of prostatic epithelial stem cells (SC) in the proximal region of the prostatic duct. The stem cell "niche" is encircled by a thick band of smooth muscle cells that express a high level of TGF-β. It is hypothesized that TGF-β maintains the stem cells in a quiescent state that can be overcome by an androgen-induced increase in the production of growth factors (GFs). The stem cells give rise to young transit-amplifying cells (TA$_1$) that in turn give rise to further generations of transit-amplifying cells (TA$_2$, TA$_3$, etc.) with progressively diminished proliferative potential. Young transit-amplifying cells are positioned in clusters within the ducts where they are available to respond rapidly to androgen and to repopulate the ducts after a surge in the androgen (A) level. U, urethra.

cells are preferentially lost through apoptosis; conversely, during regeneration, the surviving (young) transit-amplifying cells, which are clustered at multiple sites along the ducts, serve as the immediate source of cell replication, thus rapidly supplying new cells to form a regenerated gland.

This model can account for many interesting features of the prostate gland. (a) It explains our current findings that after a 39-wk chase and 16 cycles of involution and regeneration, the slow-cycling (label retaining) cells (Fig. 1, h and j, and Fig. 2) are concentrated in the proximal region. (b) It explains why cells with a high in vitro proliferative potential (Fig. 5) and the ability to reconstitute complex glandular structures (Fig. 6) are preferentially located in the proximal region. (c) It can account for our observation that young transit-amplifying cells are distributed in clusters along the prostatic ducts (Fig. 3). (d) It is consistent with the fact that the proximal region is least affected by castration (Sugimura et al., 1986b; Lee et al., 1990; Rouleau et al., 1990). (e) It can account for the observation that the proximal region has the highest telomerase activity (Banerjee et al., 1998). (f) It provides a possible functional explanation for the observation that the proximal region has the highest levels of TGF-β (Nemeth et al., 1997), and that proximal cells undergo hyperproliferation when TGF-β activity is abolished in vivo (Kundu et al., 2000).

An alternative interpretation of our results may be that the low numbers of label-retaining cells that we observe in the intermediate and distal regions of ducts may also represent stem cells rather than transit-amplifying cells. These cells would survive involution, resulting in a greater proportion of stem cells in these regions in a regressed gland. During regeneration, these cells may give rise to transit-amplifying cells that migrate in two directions, namely toward the distal as well as the proximal regions. However, our results indicate that even if low numbers of stem cells are located in the intermediate and distal regions, they are concentrated in the proximal region in a regenerated prostate.

Our finding that prostatic stem cells are concentrated in the proximal region of ducts will facilitate their isolation and characterization and help to elucidate the processes involved in prostatic homeostasis and the etiology of prostatic diseases. As both stem cells and androgen-independent prostate cancer cells have extensive self-renewal abilities and do not depend on androgen for their survival, the isolation and characterization of prostatic stem cells will be of major importance for understanding the mechanisms that result in the generation of androgen-independent prostatic carcinoma.

Materials and methods

Animals
C57Bl/6 mice were housed in a climate-controlled facility and all animal care and procedures were performed in compliance with the New York University institutional review board requirements.

Detection of quiescent, slow-cycling (label retaining) cells
To locate the cells in the prostatic epithelium that turned over at the slowest rate, we developed a method for labeling the entire epithelial compartment of the prostate with BrdU (Sigma-Aldrich). This was followed by chasing the BrdU from the cells using multiple cycles of androgen withdrawal and replacement as depicted in Fig. 1 e. Young animals (3 wk old) were required to ensure labeling in all prostatic epithelial cells. Mice were castrated and 2 wk later, prostate regeneration and labeling were promoted by the simultaneous subcutaneous implantation of testosterone pellets (Innovative Research of America) and mini-osmotic pumps (model 2002; Alza Corp.) containing 5 mg BrdU in 0.25 ml Ringer's solution (Eldridge et al., 1990; Lehrer et al., 1998). After 2 wk, three mice were killed to ascertain that 98–99% of the basal and luminal compartments of the ventral and dorsal prostates were labeled with BrdU. As prostatic epithelium turns over at a slow rate, the process of chasing out the BrdU label was hastened by cycling. The ventral and dorsal prostates were analyzed for BrdU-containing cells at various times up to 39 wk (16 cycles of androgen withdrawal and supplementation) after chasing out the label.

Detection of rapidly cycling cells
To compare the location of slow-cycling cells with that of rapidly cycling cells and to determine the turnover within the epithelial compartment in 5-wk-old mice (the age at the start of the chase) with older animals, we also administered BrdU as a pulse and determined the incidence and location of labeled epithelial cells within the three regions (proximal, intermediate, and distal) of the prostatic ducts. Mice 5, 17, and 34 wk old (the start, midpoint, and end of the long-term labeling experiment) were injected intraperitoneally with BrdU at 50 μg/g body weight, killed 24 h later, and the ventral and dorsal prostates were removed. To identify slow-cycling cells that were stimulated to divide after an androgen pulse, we injected castrated BrdU-labeled animals intraperitoneally with testosterone propionate (4 μg/g body wt) followed 40 h later with ³H-thymidine (8.1 μCi/g body wt). Animals were killed 1 h later and BrdU was detected by immunohistochemistry and ³H-thymidine by autoradiography (Lehrer et al., 1998; Taylor et al., 2000).

Tissue preparation
Animals were killed by cervical dislocation and the urogenital tract was removed en block and transferred into PBS, pH 7.6. The ventral and dorsal prostates were dissected in PBS under a dissecting microscope, using 25-gauge needles (Sugimura et al., 1986a). The tissue was fixed in 70% ethyl alcohol in 50 mM glycine, dehydrated, and embedded in paraffin.

Immunohistochemistry

Tissue sections (3 μm) were deparaffinized and endogenous peroxidase was blocked by immersing sections in 0.3% H_2O_2 for 20 min. After incubation in 1.5 N HCl for 15 min at 37°C, slides were washed in 0.1 M borax buffer, pH 8.5, for 10 min followed by three washes in PBS. Nonspecific binding sites were blocked by incubation in PBS containing 10% FCS and the slides were then incubated with peroxidase-conjugated anti-BrdU antibody (Boehringer) for 1 h at room temperature. The antibody binding sites were visualized using the substrate DAB (Sigma-Aldrich). Slides were lightly counterstained with hematoxylin. The presence of basal and luminal cells was determined (Salm et al., 2000a) using antibodies to basal keratins 5 and 14 (BL18 and LL001) and luminal keratins 8 and 18 (LE41 and LE61), which were a gift of E.B. Lane (University of Dundee, Dundee, Scotland). The presence of prostatic secretory material was detected using specific antibodies to prostatic secretory products, which were a gift of C. Abate-Shen (Robert Wood Johnson Medical School, Piscataway, NJ; M. Kim, M.M. Shen, and C. Abate-Shen, personal communication), and secondary HRP-conjugated antibodies with DAB as the substrate.

Enumeration of BrdU-labeled cells

The morphology of the rodent prostatic ductal system has been well described (Sugimura et al., 1986a, Cunha et al., 1987; Lee et al., 1990; Rouleau et al., 1990; Hayashi et al., 1991; Sensibar et al., 1991; Kinbara and Cunha, 1996; Nemeth and Lee, 1996). It can be divided into three regions, proximal, intermediate, and distal (Fig. 1 b). At each time point, three mice were killed and two sections from each prostate (ventral and dorsal) were examined immunohistochemically for evidence of labeled basal and luminal cells within each region. At least 1,000 luminal cells and 100–200 basal cells were counted in the distal and intermediate regions of each section, making a total of 6,000 luminal and 600–1,200 basal cells scored for each time point. Basal cells are less abundant than luminal cells, so fewer basal cells could be enumerated. The proximal region comprises a small area and hence fewer basal and luminal cells could be scored in this region than in the intermediate and distal regions. At least 100–200 luminal and 20–40 basal cells were counted in the proximal region of each section, making totals of 600–1,200 luminal and 120–240 basal cells scored for each time point.

Statistical analyses

The percentages of the BrdU-containing cells were determined by dividing the number of labeled cells by the total number of cells scored in each region. Each time point had three animals and two sections were scored from each ventral and dorsal prostate. For the detection of rapidly cycling cells in animals of different age groups, three mice were used per age group and two sections were scored from each ventral and dorsal prostate. The results are depicted as the means and standard deviations at each time point and comparisons between groups were made using the t test.

Cellular proliferation

The ventral and dorsal prostates of 6-wk-old mice were removed and dissected under a dissecting microscope in the presence of collagenase (type II from *Clostridium histolyticum*; Sigma-Aldrich) (Sugimura et al., 1986a). The proximal and distal regions were excised and incubated in 0.5% collagenase for 30 min at 37°C, followed by digestion in 0.25% trypsin (BD Biosciences) for 5 min at 37°C. Cells were resuspended in a mixture of basal and luminal epithelial growth media (Salm et al., 2000a,b), with conditioned medium from a prostatic smooth muscle cell line, PSMC1 (Salm et al., 2000b) (40:40:20, vol/vol/vol). Cells from both regions were seeded at 2×10^3 cells/well in collagen-coated (Cohesion) 96-well plates. Cells were harvested, counted, and reseeded at 2×10^3 cells/well every 4–5 d. The total cell output from 2×10^3 cells of each region was determined at the end of the serial culture when their growth capacity was exhausted. The ability of cells from proximal and distal regions of ducts to form colonies was determined by seeding cells at 4×10^3 cells/well in collagen-coated eight-well chamber slides. Colonies were fixed after 5 d in 70% ethanol and stained with hematoxylin and eosin.

Duct formation in collagen gels

Cells isolated from the proximal and distal regions of ducts were suspended in collagen (Elsdale and Bard, 1972) at 8×10^3 cells/100 μl type 1 collagen (BD Biosciences) in 96-well dishes. After gelation, the collagen was overlaid with 100 μl medium comprising a mixture of basal and luminal growth media (Salm et al., 2000a,b) with conditioned medium as described above. Medium was replaced daily. The collagen gels were removed after 6 d, and the numbers of ducts and branch points/duct were scored. Collagen gels were also processed for immunohistochemistry (see above). The area of ducts was measured using an NIH image analysis program (http://rsb.info.nih.gov/nih-image). Clonal analysis was done by seeding cells at limiting dilution in 96-well microtitre dishes and verifying microscopically those wells that contained single cells.

References

Baldwin, B.H., B.C. Tennant, T.J. Reimers, R.G. Cowan, and P.W. Concannon. 1985. Circannual changes in serum testosterone concentrations of adult and yearling woodchucks (*Marmota monax*). *Biol. Reprod.* 32:804–812.

Banerjee, P.P., S. Banerjee, B.R. Zirkin, and T.R. Brown. 1998. Telomerase activity in normal adult Brown Norway rat seminal vesicle: regional distribution and age-dependent changes. *Endocrinology.* 139:1075–1081.

Barrandon, Y., and H. Green. 1987. Three clonal types of keratinocyte with different capacities for multiplication. *Proc. Natl. Acad. Sci. USA.* 84:2302–2306.

Beauchamp, J.R., L. Heslop, D.S. Yu, S. Tajbakhsh, R.G. Kelly, A. Wernig, M.E. Buckingham, T.A. Partridge, and P.S. Zammit. 2000. Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. *J. Cell Biol.* 151:1221–1234.

Berardi, A.C., A. Wang, J.D. Levine, P. Lopez, and D.T. Scadden. 1995. Functional isolation and characterization of human hematopoietic stem cells. *Science.* 267:104–108.

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

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

Bonkhoff, H., U. Stein, and K. Remberger. 1994b. The proliferative function of basal cells in the normal and hyperplastic human prostate. *Prostate.* 24:114–118.

Bosland, M.C., and M.K. Prinsen. 1990. Induction of dorsolateral prostate adenocarcinomas and other accessory sex gland lesions in male Wistar rats by a single administration of N-methyl-N-nitrosourea, 7,12-dimethylbenz(a)anthracene, and 3,2'-dimethyl-4-aminobiphenyl after sequential treatment with cyproterone acetate and testosterone propionate. *Cancer Res.* 50:691–699.

Cashman, J.D., A.C. Eaves, E.W. Raines, R. Ross, and C.J. Eaves. 1990. Mechanisms that regulate the cell cycle status of very primitive hematopoietic cells in long-term human marrow cultures. I. Stimulatory role of a variety of mesenchymal cell activators and inhibitory role of TGF-β. *Blood.* 75:96–101.

Cotsarelis, G., S.Z. Cheng, G. Dong, T.T. Sun, and R.M. Lavker. 1989. Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. *Cell.* 57:201–209.

Cotsarelis, G., T.T. Sun, and R.M. Lavker. 1990. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell.* 61:1329–1337.

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

Danielpour, D. 1999. Transdifferentiation of NRP-152 rat prostatic basal epithelial cells toward a luminal phenotype: regulation by glucocorticoid, insulin-like growth factor-I and transforming growth factor-beta. *J. Cell Sci.* 112:169–179.

De Marzo, A.M., W.G. Nelson, A.K. Meeker, and D.S. Coffey. 1998. Stem cell features of benign and malignant prostate epithelial cells. *J. Urol.* 160:2381–2392.

Eldridge, S.R., L.F. Tilbury, T.L. Goldsworthy, and B.E. Butterworth. 1990. Measurement of chemically induced cell proliferation in rodent liver and kidney: a comparison of 5-bromo-2'-deoxyuridine and [^{3}H]thymidine administered by injection or osmotic pump. *Carcinogenesis.* 11:2245–2251.

Elsdale, T., and J. Bard. 1972. Collagen substrata for studies on cell behavior. *J. Cell Biol.* 54:626–637.

English, H.F., R.J. Santen, and J.T. Isaacs. 1987. Response of glandular versus basal rat ventral prostatic epithelial cells to androgen withdrawal and replacement. *Prostate.* 11:229–242.

Evans, G.S., and J.A. Chandler. 1987a. Cell proliferation studies in rat prostate. I. The proliferative role of basal and secretory epithelial cells during normal growth. *Prostate.* 10:163–178.

Evans, G.S., and J.A. Chandler. 1987b. Cell proliferation studies in the rat prostate. II. The effects of castration and androgen-induced regeneration upon basal and secretory cell proliferation. *Prostate.* 11:339–351.

Fausto, N. 2000. Liver regeneration. *J. Hepatol.* 32:19–31.

Fortunel, N., J. Hatzfeld, S. Kisselev, M.N. Monier, K. Ducos, A. Cardoso, P. Batard, and A. Hatzfeld. 2000. Release from quiescence of primitive human hematopoietic stem/progenitor cells by blocking their cell-surface TGF-β type II receptor in a short-term in vitro assay. *Stem Cells.* 18:102–111.

Fuentes, L.B., J.C. Calvo, E.H. Charreau, and J.A. Guzman. 1993. Seasonal variations in testicular LH, FSH, and PRL receptors; in vitro testosterone production; and serum testosterone concentration in adult male vizcacha (Lagostomus maximus maximus). *Gen. Comp. Endocrinol.* 90:133–141.

Hatzfeld, J., M.L. Li, E.L. Brown, H. Sookdeo, J.P. Levesque, T. O'Toole, C. Gurney, S.C. Clark, and A. Hatzfeld. 1991. Release of early human hematopoietic progenitors from quiescence by antisense transforming growth factor beta 1 or Rb oligonucleotides. *J. Exp. Med.* 174:925–929.

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

Hayward, S.W., P.C. Haughney, E.S. Lopes, D. Danielpour, and G.R. Cunha. 1999. The rat prostatic epithelial cell line NRP-152 can differentiate in vivo in response to its stromal environment. *Prostate.* 39:205–212.

Hoover, D.M., K.L. Best, B.K. McKenney, R.N. Tamura, and B.L. Neubauer. 1990. Experimental induction of neoplasia in the accessory sex organs of male Lobund-Wistar rats. *Cancer Res.* 50:142–146.

Isaacs, J.T. 1985. Control of cell proliferation and cell death in the normal and neoplastic prostate: a stem cell model. *In* Benign Prostatic Hyperplasia. Vol. 87-2881. C.H. Rodgers, D.S. Coffey, G. Cunha, J.T. Grayshack, R. Henman, Jr., and R. Horton, editors. Department of Health and Human Services, NIH, Washington DC. 85–94.

Kim, N.W., M.A. Piatyszek, K.R. Prowse, C.B. Harley, M.D. West, P.L. Ho, G.M. Coviello, W.E. Wright, S.L. Weinrich, and J.W. Shay. 1994. Specific association of human telomerase activity with immortal cells and cancer. *Science.* 266:2011–2015.

Kinbara, H., and G.R. Cunha. 1996. Ductal heterogeneity in rat dorsal-lateral prostate. *Prostate.* 28:58–64.

Kinbara, H., G.R. Cunha, E. Boutin, N. Hayashi, and J. Kawamura. 1996. Evidence of stem cells in the adult prostatic epithelium based upon responsiveness to mesenchymal inductors. *Prostate.* 29:107–116.

Kundu, S.D., I.Y. Kim, T. Yang, L. Doglio, S. Lang, X. Zhang, R. Buttyan, S.J. Kim, J. Chang, X. Cai, et al. 2000. Absence of proximal duct apoptosis in the ventral prostate of transgenic mice carrying the C3(1)-TGF-beta type II dominant negative receptor. *Prostate.* 43:118–124.

Lavker, R.M., and T.T. Sun. 2000. Epidermal stem cells: properties, markers, and location. *Proc. Natl. Acad. Sci. USA.* 97:13473–13475.

Lee, C., J.A. Sensibar, S.M. Dudek, R.A. Hiipakka, and S.T. Liao. 1990. Prostatic ductal system in rats: regional variation in morphological and functional activities. *Biol. Reprod.* 43:1079–1086.

Lee, C., S.M. Sintich, E.P. Mathews, A.H. Shah, S.D. Kundu, K.T. Perry, J.S. Cho, K.Y. Ilio, M.V. Cronauer, L. Janulis, and J.A. Sensibar. 1999. Transforming growth factor-beta in benign and malignant prostate. *Prostate.* 39:285–290.

Lehrer, M.S., T.T. Sun, and R.M. Lavker. 1998. Strategies of epithelial repair: modulation of stem cell and transit amplifying cell proliferation. *J. Cell Sci.* 111:2867–2875.

Ma, Y.L., C. Fujiyama, Z. Masaki, and H. Sugihara. 1997. Reconstruction of prostatic acinus-like structure from ventral and dorsolateral prostatic epithelial cells of the rat in three-dimensional collagen gel matrix culture. *J. Urol.* 157:1025–1031.

McNeal, J.E., E.A. Redwine, F.S. Freiha, and T.A. Stamey. 1988. Zonal distribution of prostatic adenocarcinoma. Correlation with histologic pattern and direction of spread. *Am. J. Surg. Pathol.* 12:897–906.

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

Morris, R.J., and C.S. Potten. 1999. Highly persistent label-retaining cells in the hair follicles of mice and their fate following induction of anagen. *J. Invest. Dermatol.* 112:470–475.

Nemeth, J.A., and C. Lee. 1996. Prostatic ductal system in rats: regional variation in stromal organization. *Prostate.* 28:124–128.

Nemeth, J.A., J.A. Sensibar, R.R. White, D.J. Zelner, I.Y. Kim, and C. Lee. 1997. Prostatic ductal system in rats: tissue-specific expression and regional variation in stromal distribution of transforming growth factor-beta 1. *Prostate.* 33:64–71.

Ogawa, M. 1993. Differentiation and proliferation of hematopoietic stem cells. *Blood.* 81:2844–2853.

Oshima, H., A. Rochat, C. Kedzia, K. Kobayashi, and Y. Barrandon. 2001. Morphogenesis and renewal of hair follicles from adult multipotent stem cells. *Cell.* 104:233–245.

Pasqualini, T., O. Colillas, and M.A. Rivarola. 1986. Testicular and serum testosterone variations in squirrel monkeys during seasonal cyclicity. *J. Androl.* 7:298–302.

Pellegrini, G., O. Golisano, P. Paterna, A. Lambiase, S. Bonini, P. Rama, and M. De Luca. 1999. Location and clonal analysis of stem cells and their differentiated progeny in the human ocular surface. *J. Cell Biol.* 145:769–782.

Potten, C.S., and M. Loeffler. 1990. Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. *Development.* 110:1001–1020.

Rauch, F., B. Polzar, H. Stephan, S. Zanotti, R. Paddenberg, and H.G. Mannherz. 1997. Androgen ablation leads to an upregulation and intranuclear accumulation of deoxyribonuclease I in rat prostate epithelial cells paralleling their apoptotic elimination. *J. Cell Biol.* 137:909–923.

Reya, T., S.J. Morrison, M.F. Clarke, and I.L. Weissman. 2001. Stem cells, cancer, and cancer stem cells. *Nature.* 414:105–111.

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

Rouleau, M., J. Leger, and M. Tenniswood. 1990. Ductal heterogeneity of cytokeratins, gene expression, and cell death in the rat ventral prostate. *Mol. Endocrinol.* 4:2003–2013.

Salm, S.N., Y. Koikawa, V. Ogilvie, A. Tsujimura, S. Coetzee, D. Moscatelli, E. Moore, H. Lepor, E. Shapiro, T.T. Sun, and E.L. Wilson. 2000a. Generation of active TGF-beta by prostatic cell cocultures using novel basal and luminal prostatic epithelial cell lines. *J. Cell. Physiol.* 184:70–79.

Salm, S.N., Y. Koikawa, V. Ogilvie, A. Tsujimura, S. Coetzee, D. Moscatelli, E. Moore, H. Lepor, E. Shapiro, T.T. Sun, and E.L. Wilson. 2000b. Transforming growth factor-beta is an autocrine mitogen for a novel androgen-responsive murine prostatic smooth muscle cell line, PSMC1. *J. Cell. Physiol.* 185:416–424.

Schermer, A., S. Galvin, and T.T. Sun. 1986. Differentiation-related expression of a major 64K corneal keratin in vivo and in culture suggests limbal location of corneal epithelial stem cells. *J. Cell Biol.* 103:49–62.

Sensibar, J.A., M.D. Griswold, S.R. Sylvester, R. Buttyan, C.W. Bardin, C.Y. Cheng, S. Dudek, and C. Lee. 1991. Prostatic ductal system in rats: regional variation in localization of an androgen-repressed gene product, sulfated glycoprotein-2. *Endocrinology.* 128:2091–2102.

Shirai, T., S. Tamano, T. Kato, S. Iwasaki, S. Takahashi, and N. Ito. 1991. Induction of invasive carcinomas in the accessory sex organs other than the ventral prostate of rats given 3,2′-dimethyl-4-aminobiphenyl and testosterone propionate. *Cancer Res.* 51:1264–1269.

Slack, J.M. 2000. Stem cells in epithelial tissues. *Science.* 287:1431–1433.

Strain, A.J., and H.A. Crosby. 2000. Hepatic stem cells. *Gut.* 46:743–745.

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

Sugimura, Y., G.R. Cunha, and A.A. Donjacour. 1986b. Morphological and histological study of castration-induced degeneration and androgen-induced regeneration in the mouse prostate. *Biol. Reprod.* 34:973–983.

Sugimura, Y., G.R. Cunha, A.A. Donjacour, R.M. Bigsby, and J.R. Brody. 1986c. Whole-mount autoradiography study of DNA synthetic activity during postnatal development and androgen-induced regeneration in the mouse prostate. *Biol. Reprod.* 34:985–995.

Tani, H., R.J. Morris, and P. Kaur. 2000. Enrichment for murine keratinocyte stem cells based on cell surface phenotype. *Proc. Natl. Acad. Sci. USA.* 97:10960–10965.

Taylor, G., M.S. Lehrer, P.J. Jensen, T.T. Sun, and R.M. Lavker. 2000. Involvement of follicular stem cells in forming not only the follicle but also the epidermis. *Cell.* 102:451–461.

Thorgeirsson, S.S. 1996. Hepatic stem cells in liver regeneration. *FASEB J.* 10:1249–1256.

Tsai, R.J., L.M. Li, and J.K. Chen. 2000. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. *N. Engl. J. Med.* 343:86–93.

Tsubota, K., Y. Satake, M. Kaido, N. Shinozaki, S. Shimmura, H. Bissen-Miyajima, and J. Shimazaki. 1999. Treatment of severe ocular-surface disorders with corneal epithelial stem-cell transplantation. *N. Engl. J. Med.* 340:1697–1703.

Turner, T., H.A. Bern, P. Young, and G.R. Cunha. 1990. Serum-free culture of enriched mouse anterior and ventral prostatic epithelial cells in collagen gel. *In Vitro Cell. Dev. Biol.* 26:722–730.

van der Kwast, T.H., B. Tetu, E.R. Suburu, J. Gomez, M. Lemay, and F. Labrie. 1998. Cycling activity of benign prostatic epithelial cells during long-term androgen blockade: evidence for self-renewal of luminal cells. *J. Pathol.* 186:406–409.

Williams, D.A. 1993. Ex vivo expansion of hematopoietic stem and progenitor cells—robbing Peter to pay Paul? *Blood.* 81:3169–3172.

Wright, W.E., M.A. Piatyszek, W.E. Rainey, W. Byrd, and J.W. Shay. 1996. Telomerase activity in human germline and embryonic tissues and cells. *Dev. Genet.* 18:173–179.