Prostate progenitor cells proliferate in response to castration

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Abstract Androgen-deprivation is a mainstay of therapy for advanced prostate cancer but tumor regression is usually incomplete and temporary because of androgen-independent cells in the tumor. It has been speculated that these tumor cells resemble the stem/progenitor cells of the normal prostate. The purpose of this study was to examine the response of slow-cycling progenitor cells in the adult mouse prostate to castration. Proliferating cells in the E16 urogenital sinus were pulse labeled by BrdU administration or by doxycycline-controlled labeling of the histone-H2B GFP mouse. A small population of labeled epithelial cells in the adult prostate localized at the junction of the prostatic ducts and urethra. Fluorescence-activated cell sorting (FACS) showed that GFP label-retaining cells were enriched for cells co-expressing stem cell markers Sca-1, CD133, CD44 and CD117 (4-marker cells; 60-fold enrichment). FACS showed, additionally, that 4-marker cells were androgen receptor positive. Castration induced proliferation and dispersal of E16 labeled cells into more distal ductal segments. When naïve adult mice were administered BrdU daily for 2 weeks after castration, 16% of 4-marker cells exhibited BrdU label in contrast to only 6% of all epithelial cells (\(P < 0.01\)). In sham-castrated controls less than 4% of 4-marker cells were BrdU labeled (\(P < 0.01\)). The unexpected and admittedly counter-intuitive finding that castration induced progenitor cell proliferation suggests that androgen deprivation therapy in men with advanced prostate cancer could not only exert pleiotrophic effects on tumor sub-populations but may induce inadvertent expansion of tumor stem cells.

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Introduction

The mouse prostate develops from the urogenital sinus (UGS). Before embryonic day 16 (E16), the UGS is comprised of an outer layer of mesenchyme surrounding an inner epithelial layer from which outgrowth occurs to form the prostate ducts (Price, 1963; Wang et al., 2001). At E16.5 – 17.5 epithelial buds invade the surrounding mesenchyme and begin the process of ductal morphogenesis that generates the complex ductal structure of the adult prostate (Sugimura and Donjacour, 1982;...
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1986; Timms et al., 1994; Hayward et al., 1996). The adult mouse prostate has distinct anterior, dorsal–lateral and ventral lobes; each lobe is divided into proximal, intermediate and distal regions based on their relative location to the urethra (Salm et al., 2005; Cunha et al., 1987). Prostate development is androgen dependent and involves intimate signaling between epithelial and mesenchymal cells. Maintenance of the adult prostate is also androgen-dependent, and the prostate undergoes rapid involution following castration. This involves epithelial apoptosis concentrated in the distal duct segments, loss of androgen-dependent differentiation in the remaining epithelium and remodeling of the periductal stroma (Sugimura and Donjacour, 1986). This process is completely reversed by androgen supplement. The castration–regeneration cycle can repeat for many rounds without observable defects in the regenerated prostate (Sugimura and Donjacour, 1986). This observation suggested the presence of a progenitor cell population in the adult prostate capable of surviving androgen deprivation and sufficient to regenerate the ductal segments of the intact adult prostate.

Adult tissue progenitor cells possessing the ability for self-renewal and/or generation of lineage-committed cells are generally quiescent cells recruited into active proliferation during tissue regeneration and repair (Morrison and Spradling, 2008; Knoblich, 2008). The generally 'slow cycling' property of these cells has permitted localization by $^3$H thymidine, 5-bromo-2′-deoxyuridine (BrdU) and histone H2B-green fluorescent protein (GFP) labeling methods in a variety of tissues, such as mammary gland, hair follicles, small intestine, and cornea (Cotsarelis et al., 1989; Tani et al., 2000; Taylor et al., 2000; Berardi et al., 1995; Thorgeirsson, 1996; Tsujimura et al., 2002). The regenerative capacity of the prostate has been attributed to the existence of progenitor cells in the adult gland that survive castration-induced involution (Kinbara et al., 1996; Robinson et al., 1996; Hudson et al., 2000; Collins et al., 2001; Foster et al., 2002). Several lines of evidence suggest that these progenitor cells reside within the proximal region of the prostate ducts. When adolescent mice were BrdU labeled and then subjected to multiple rounds of castration and testosterone supplementation to ‘wash out’ the BrdU label in dividing cells, the ‘slow cycling’ label-retaining cells were concentrated in the proximal duct segments (Tsujimura et al., 2002). In another set of experiments it was demonstrated that cells from the proximal duct have higher tissue regenerative ability when grafted under the renal capsule of recipient host animals (Burger et al., 2005; Xin et al., 2005). The studies reported here build upon those previous observations. We localize a slow-cycling cell population enriched for stem cell markers to the proximal duct segments and show the surprising and clinically important observation that castration induces these cells to proliferate and migrate to more distal sites in the ductal network.

Materials and methods

Animal maintenance and tissue recovery

Wild-type and nude CD-1 mice were purchased from Charles River Laboratories (Wilmington, MA). The transgenic mouse line expressing the histone H2B-green fluorescent protein (H2B-GFP) under the control of tetracycline-responsive element (Tumbar et al., 2004) was from Jackson Laboratories (Bar Harbor, ME); the mouse line expressing tetracycline responsive element under the control of CK5 promoter (tTA) was a generous gift from Adam B. Glick lab (Diamond et al., 2000). At the appropriate time prostates were harvested and analyzed for the presence of label retaining cells. The isolated prostate tissue was either enzymatic digested to single cells for FACS analysis or fixed in 10% neutral buffered formalin for embedding and sectioning.

All animal procedures were performed in accordance with institutional guidelines and approval by the Animal Care and Use Committee at the University of Wisconsin-Madison.

BrdU label retaining cells

To produce BrdU label-retaining cells labeled at E16, we injected (IP) pregnant dams at E16 with 0.5 ml of 50 mM BrdU in PBS. We found that a single injection of BrdU was sufficient to mark cells in the developing prostate, while at 10 weeks of age still retaining a small fraction of those cells.

GFP label retaining cells

To control for expression of GFP in the H2B-GFP pulse-chase experiments, the pregnant dam was fed a diet containing doxycycline from before conception until E15.5 at which time the diet was changed to a standard feed without doxycycline for 24 h after which the diet was maintained on the doxycycline containing feed until the animals were harvested. This 24-hour period of GFP expression was sufficient to label many cells and provide a population of label retaining cells in the adult animals at 10 weeks of age.

Castration

To determine the fate of the label retaining cells following androgen withdrawal we castrated animals, under an approved protocol, at 8 weeks of age and allowed them to recover for up to 14 days at which time the animals were sacrificed and prostates were harvested.

Renal capsule graft

Rat urogenital sinus mesenchyme (rUGM) was isolated from 1% trypsin digested E18 rat urogenital sinus. A single cell suspension of UGM was collected after 0.5% collagenase digestion. FACS sorted 4-marker + or 4-marker – cells (1000 each) were mixed with 25,000 UGM cells and resuspended in 15 µl of type I collagen (BD biosciences, Bedford, MA), and implanted under the renal capsule of athymic nude mice as described (Tsujimura et al., 2002).

Histology

Dissected prostate tissues or renal grafts were fixed in 10% formaldehyde for 12 h at 4 °C, appropriately orientated and embedded in 4% agarose. The samples were then sectioned at
200 μm in thickness with a vibratome. The vibratome sections were processed, paraffin embedded and sectioned. The 5 μm paraffin sections were dewaxed, and stained with Mayer’s hematoxylin and eosin (H&E) for histological evaluation.

Indirect immunofluorescent staining

Dewaxed sections were used for antigen retrieval which was performed in the citrate buffer and heated in the microwave oven for 30 to 50 min at 95% power. The tissue sections were blocked with 3% BSA in PBS plus 10% normal donkey serum. The primary antibodies used were: rabbit anti-androgen receptor (Santa Cruz, Dallas, Texas); rat anti-Ki67 (Dako North America, Carpinteria, CA); and mouse anti-BrdU (Roche USA, Nutley, NJ). The secondary antibodies were from Molecular Probes (Eugene, Oregon). Following primary antibody incubation, the sections were washed with PBS, and the secondary antibody was applied at 1:200 at room temperature for 1 h. Then sections were washed with PBS and counter stained with 4′,6-diamidino-2-phenylindole (DAPI). Fluorescent images were captured using an Olympus BX51 fluorescent microscope (Olympus, Center Valley, PA) with a digital camera and SPOT advanced software or BD Pathway bioimaging systems (BD Biosciences, San Jose, CA).

FACS analysis of H2B-GFP label retaining cells for the stem cell markers (Sca-1, CD133, CD44 and CD117)

H2B-GFP mice were mated with K5-Tet (tTA, Tet off) mice and genotyped. Mice that were positive for both GFP and K5-Tet were used for experiments and double negative littersmates were used as control. The prostate tissue was dissected from 10-week old mice in cold PBS under a dissecting microscope. The tissue was minced into 2-3 mm pieces and digested by collagenase and hyaluronidase (Stemcell Technologies, Inc., Vancouver, BC, Canada; Cat. No. 07912) at 37 °C for 3 h. This digestion was followed by a second digestion in 0.25% trypsin/EDTA in 2% BSA on ice for 30 min according to instructions (Stemcell Technologies, Inc., Vancouver, BC, Canada). We found that digestion with 0.25% trypsin on ice for 30 min increased yield of single cells from prostate tissue without affecting FACS analysis of stem cell markers. The digested cells were passed through a 40 μm cell strainer to isolate single cells with an aliquot of cells used to determine cell concentration and checked for viability by trypan blue staining. Single cells were treated with a mouse lineage depletion kit according to instructions (Miltenyi Biotec Inc., Auburn, CA). The lineage depleted cells (Lin−) then suspended in DMEM (Mediatech, Manassas, VA) containing 5% FBS followed by addition of primary antibodies directly coupled to specific fluorochromes (Sca-1, CD133, CD44 and CD117 as previously reported (Leong et al., 2008) for 30 min on ice. DAPI was added to the cell suspension prior to FACS analysis to select viable cells. Single stem cell maker stained cells and isotype matched IgG were used to identify positive and negative signal. Fluorescence-minus-one (FMO) controls were used to determine gating in multicolor flow cytometry. FACS analysis was performed on a BD LSR-II flow cytometry (BD Biosciences, San Jose, CA). The cells were gated for single events and viability. The flow cytometry data was analyzed using FlowJo8.6 software. The relationship between GFP positive label retaining cells and stem cell marker expression was analyzed statistically.

FACS analysis of progenitor cell proliferation after castration by correlating of BrdU labeling and stem cell marker expression

Adult (8 week old) CD-1 mice were divided into 4 groups: Sham-BrdU, Sham-PBS, castration-BrdU and castration-PBS. 500 μl of 10 mM BrdU (Sigma, MO) was given by IP injection to castrated or sham mice daily for 14 days starting at the time of castration and 500 μl PBS was injected as control. On day 14 the prostate was processed and stained for the four stem cell markers as described above. After staining with antibodies to stem cell markers, the cells were fixed and stained with a BrdU Flow Kit (BD Bioscience, San Jose) as instructed. FACS analysis was performed as described above for the 4-markers and BrdU after gating for single events.

Quantitative and statistical analysis of the relationship between label retaining cells and other cell markers

The percentage of BrdU positive cells were determined by BrdU positive cells over the total cells of interest. Each group has 3–5 animals, and the paraffin embedded prostate tissue from each animal was completely sectioned, and 5 sections were used for analysis. At least 10 of 200× high power field (HPF) images from each section were included in the analysis. Statistical analysis was conducted using one-way analysis of variance (ANOVA) and values were presented as mean ± SEM derived from at least three independent experiments, unless otherwise stated. The ANOVA was followed by Tukey’s post hoc test. P value less than 0.05 was regarded as statistically significant. Statistical analysis of IHC data was performed with assistance from the UW Madison Department of Surgery’s Statistical Core.

Results

Prenatal BrdU labeling yields label-retaining cells in the proximal duct segments

Previous studies have identified slow-cycling epithelial cells in the adult prostate by BrdU labeling during adolescence followed by multiple rounds of castration-induced involution and testosterone-induced re-growth. This approach yields a relatively abundant population of labeled cells preferentially localized to the proximal duct segments (Tsujimura et al., 2002). We speculated that initiation of prostate ductal budding (E16.5–17.5) would entail a robust expansion of prostate tissue-specific stem cells, primitive progenitor cells or both, and that pulse labeling with BrdU at E16 would yield a rarified population of label-retaining cells in the adult prostate that had remained mitotically quiescent since the earliest stages of prostate development. Pregnant dams were given a single dose of BrdU by intraperitoneal injection at E16 and the male offspring sacrificed at 10 weeks of age. The lower genitourinary tract was removed en bloc. The
Prostate and urethra were fixed, serially sectioned and immunostained for BrdU. We reproducibly observed a small number of labeled epithelial cells located at the junction of the prostatic main ducts and the urethra and in the proximal ducts (Fig. 1). Epithelial labeling was confirmed by co-staining with epithelial markers. Labeled epithelial cells were rarely observed in intermediate and distal ducts. Note was made of scattered BrdU-labeled stromal cells that were most abundant in the region of the proximal ducts. Co-staining for BrdU and Ki67 or Caspase 3 revealed a very low proliferative index and rare instances of apoptosis among the labeled cells. We conclude that pulse BrdU labeling at E16 yields a population of slow-cycling labeled cells in the proximal duct segments of the adult prostate.

Prenatal CK-5/GFP labeling yields labeled cells in the proximal duct segments

To corroborate this observation and facilitate FACS analysis of stem cell marker expression, cytokeratin 5 (CK-5) expressing cells were selectively pulse labeled at E16 using a doxycycline-controlled histone-H2B GFP mouse (Tumbar et al., 2004). Histone labeling by this technique is effectively an equivalent way of introducing label into proliferating cells that is “washed out” by subsequent rounds of replication. However, it has the advantage that the histone-H2B GFP labeling is amenable to FACS analysis of living cells. The labeling technique differs from BrdU labeling in that only proliferating cells expressing CK5 are labeled with GFP. The male offspring were sacrificed at 10 weeks of age and the prostate and urethra were serially sectioned and imaged by immunofluorescence microscopy. We again observed (GFP) labeled epithelial cells localized to the proximal duct segments and their urethral junction (Fig. 1F). Sca-1, CD133, CD44 and CD117 have been implicated as prostate stem cell markers since a single cell co-expressing these 4-markers can generate fully differentiated prostate tissue when combined with urogenital mesenchyme (Leong et al., 2008). FACS analysis showed 6% of the GFP labeled cells in the 10 week adult prostate co-expressed Sca-1, CD133, CD44 and CD117. This contrasted with co-expression of these stem cell markers in only ~0.1% of the total epithelial cell population (Fig. 2). Further analysis showed that 26% of cells co-expressing Sca-1, CD133, CD44 and CD117 were GFP positive even though GFP label was present in only 1.5% of the total epithelial cell population (Fig. 2). These data show that pulse GFP labeling CK-5 expressing cells at E16 yields a population of label retaining cells located in the proximal duct segments that are significantly enriched for co-expression of prostate stem cell markers and, indeed, account for over a quarter of all co-expressing cells in the adult prostate.

It is intuitively obvious and commonly accepted that prostate progenitor cells that participate in testosterone-
induced regeneration of the castrated mouse prostate are not dependent on androgen for survival. However, it has not been established whether or not these cells express the androgen receptor. We performed staining for androgen receptor (AR) in BrdU label retaining cells. Mice were BrdU pulse labeled at E16 as described above and sacrificed at 10 weeks of age. Co-staining for BrdU and androgen receptor (AR) revealed approximately 75% of BrdU-labeled cells to be AR+ (Fig. 3A–B). This is less than the...
expression of AR in 95% of the total epithelial cell population, but
the preponderance of AR expression in this population was
unexpected and prompted us to examine the expression of AR in
cells co-expressing Sca-1, CD133, CD44 and CD117. FACS analysis
was performed on intact adult mice: we observed that 99.7%
cells co-expressing Sca-1, CD133, CD44 and CD117 also express
AR (Fig. 3C).

Label retaining cells proliferate after castration

Castration of the adult mouse induces widespread epithelial
apoptosis and glandular involution; however, several studies
have shown that a low level of epithelial proliferation persists even while glandular involution is occurring (Alison et al., 1976; Evans and Chandler, 1987). To examine the
response of slow-cycling cells in the adult prostate to
castration, E16-BrdU labeled mice were castrated or sham
operated at 8 weeks of age and sacrificed 2 weeks later.
In sham-operated mice, we observed label retaining cells
located exclusively in the proximal duct segments and their
junction with the urethra (Fig. 1A–D). The labeled cells were
usually solitary; they were occasionally paired. In castrated
mice there was an increase in the total number of label
retaining cells and dispersal of labeled cells into the
non-proximal duct segments (Fig. 4). Most labeled cells in

Figure 3  The majority of prostate 4-marker cells stain positive for androgen receptor. CD-1 mice were BrdU labeled at E16 and
sacrificed at 10 weeks. Co-staining was performed for androgen receptor (AR) and BrdU. (A) A representative section of co-stained
prostate epithelium. (B) Quantitative analysis of co-staining for AR and BrdU in the prostate epithelium (n = 5). (C) Ten week old CD-1
mice were sacrificed and the epithelium was subjected to FACS analysis for AR, Sca-1, CD133, CD44 and CD117. Quantitative analysis
shows that most 4-marker cells are androgen receptor positive (n = 3).

Figure 4  BrdU labeled cells are dispersed post-castration. CD-1 mice BrdU labeled at E16 were castrated at 8 weeks and sacrificed
2 weeks after castration. (A) Immunostaining for BrdU labeled cells (Montage image of the sagittal sections of prostate). (B) Quantitative
analysis for the location of BrdU labeled cells (n = 5). (C and D) Boxed regions in A. (E and F) Colocalization of BrdU label-retaining cells
(red) with CK5 (green) in non-proximal and proximal ducts.
the castrated mice were solitary; however, we frequently observed clusters of labeled cells. Clusters were a unique feature of the castrated mice (Fig. 5A). From this finding, we inferred that label-retaining cells proliferate following castration. This inference was confirmed by Ki67 staining (Fig. 5B and C).

Castration induces a selective proliferation of cells co-expressing stem cell markers

The observation that slow-cycling cells in the adult prostate proliferate in response to castration was surprising and prompted us to examine the behavior of cells co-expressing the stem cell markers Sca-1, CD133, CD44 and CD117. Adult males were castrated or sham operated and then administered BrdU daily by injection for 2 weeks. The prostates from sham-operated and castrated mice were harvested, enzymatically digested to single cells and subjected to FACS analysis to determine the proportion of BrdU labeling in cells co-expressing the stem cell markers Sca-1, CD133, CD44 and CD117. This analysis revealed a selective expansion of 4-marker cells following castration. We observed a significantly higher percentage of BrdU+ 4-marker cells in the castrated versus sham operated mice (16% versus 3.7%; Fig. 6A). This finding indicates that castration increases proliferation of the 4-marker positive cell population. To distinguish a selective

Figure 5  Castration induces proliferation of BrdU labeled cells. (A) Clusters of BrdU labeled cells (arrows) were observed in mice labeled and castrated as described in Fig. 4. Similar clusters were never observed in the intact controls. Co-staining for BrdU and CK5 identified the cluster of labeled cells as basal cells. (B and C) Staining for Ki67 in mice labeled and castrated as described in Fig. 4 revealed increased proliferation of BrdU labeled cells. (B) Immunofluorescence staining for BrdU (red), Ki67 (green) and DAPI (blue) shows co-localization of BrdU with Ki67. This is quite uncommon in intact controls. Quantitative analysis of co-staining for Ki67 and BrdU confirms that co-staining is significantly increased in castrated animals (C) (n = 5).

Figure 6  Castration induces selective proliferation of 4-marker progenitor cells. Eight week old mice were sham operated or castrated. BrdU was injected daily after surgery to label the proliferating cells, and mice were sacrificed 2 weeks after castration. (A) FACS analysis showed significantly higher labeling of 4-marker cells in the castrated versus sham operated mice (16% versus 3.7%; 3 independent experiments; Fig. 6A). These data signify increased proliferation of 4-marker cells in the castrated mouse prostate. (B) Proliferation was greater in 4-marker progenitor cells than that in non-4-marker positive cells in the castrated prostate group (n = 3). Taken together, these data indicate that castration induces preferential proliferation of 4-marker cells.
Discussion

Epithelial proliferation is concentrated at the tips of the developing prostate ducts and early studies focused on the duct tip as the probable site of progenitor/stem cells (Kinbara et al., 1996). Subsequent studies examining label retention and regenerative capacity implicated the proximal duct as the reservoir of stem/progenitor cells in the adult prostate (Tsujimura et al., 2002; Kinbara et al., 1996; Burger et al., 2005; Xin et al., 2005). Further studies sorting for putative stem cell markers and testing for regenerative potential confirmed a relative abundance of stem cells in the proximal duct as compared to the intermediate and distal duct segments (Evans and Chandler, 1987; Lawson et al., 2007; Burger et al., 2005; Xin et al., 2005). Labeling with either BrdU or GFP at the onset of ductal budding yielded a small population of labeled cells in the adult prostate concentrated in the proximal ducts near their urethral origin. In our study, we also observed label-retaining stromal cells localized to the stromal compartment surrounding the proximal ducts. This could be coincidence, but it does suggest the possibility that slow-cycling epithelial and stromal cells are co-localized within a specific niche in the adult gland and share regulatory signaling mechanisms (Goto et al., 2006; Potten and Loeffler, 1990; Lavker and Sun, 2000). The most striking findings were robust proliferation of these cells at E16, the preponderance of AR gene expression and the proliferative response to castration.

The ducts of the adult mouse prostate are lined by a pseudostratified epithelium composed of basal, luminal cells and rare neuroendocrine cells. The identity and location of stem cells within this epithelial layer are still a matter of debate. At one time basal cells were widely believed to contain progenitor cells capable of differentiating into basal, luminal and neuroendocrine cells (Collins et al., 2001; Wang et al., 2001). This view has been challenged by recent observations suggesting that stem cells may also reside in the luminal cell layer (Slack, 2000). Single cells co-expressing the markers used in our studies (Lin<sup>−</sup>Sca-1<sup>−</sup>CD133<sup>−</sup>CD44<sup>−</sup>CD117<sup>+</sup>) have been shown capable of regenerating a fully differentiated prostate epithelium (Leong et al., 2008). We found that GFP label retaining cells were enriched for co-expression of Lin<sup>−</sup>Sca-1<sup>−</sup>CD133<sup>−</sup>CD44<sup>−</sup>CD117<sup>+</sup>. While only a fraction of label retaining cells co-expressed the four stem cell markers, they accounted for approximately a quarter of all the cells co-expressing these markers. This was unexpected and suggests that initiation of prostate ductal budding is associated with a uniquely robust proliferation of epithelial stem/progenitor cells. This is to our knowledge the first evidence that initiation of prostate organogenesis is accompanied by a burst of proliferation among cells that will become a tissue specific stem or progenitor cells in the adult organ. We speculate that this proliferative burst potentially creates a window of vulnerability of these reserve cells to mutation or imprinting changes that could predispose to neoplasia in the adult. Whether a similar burst of proliferation among stem/progenitor cells in other developing organs remains to be determined.

AR is present in the nucleus of most epithelial and stromal cells of the intact adult prostate. Luminal cells are predominantly, if not exclusively, androgen positive whereas only half of all basal cells are (Wang et al., 2009). AR in the stroma mediates paracrine stimulation of epithelial proliferation while AR in epithelial cells stimulates luminal cell differentiation and protein synthesis. It has been assumed that prostate stem cells lack AR (Mirosevich et al., 1999); however, there is some evidence to challenge this view as it applies to the human prostate (Oldridge et al., 2012) and the mouse prostate (Slack, 2000). We found that most BrdU label retaining epithelial cells in the adult prostate were AR positive. More striking was the observation that four-marker positive cells are nearly all AR positive. Insofar as previous studies showed that 14 in 94 seven-marker positive cells exhibit the regenerative capacity bone fide stem cells in a tissue recombination assay (Leong et al., 2008), our data suggests that at least some, if not all, stem cells are AR positive.

AR has been shown to exert a growth inhibitory effect in luminal cells. In transgenic mice lacking epithelial AR, epithelial cells are less differentiated and hyper-proliferative (Heer, 2011). It is possible that selective proliferation of slow-cycling and 4-marker cells after castration reflects a release of androgen-mediated inhibition of proliferation of cells that do not require androgen for survival. There is precedent for this in the breast where estrogen suppresses stem cell proliferation (Wu et al., 2007). An alternative explanation for proliferation and migration of LRCs and 4-marker cells following castration would be the response to injury. Progenitor cells play a primary role in the regenerative response to injury in a variety of adult tissues (Simões et al., 2011; Amcheslavsky et al., 2009; Imitola et al., 2004; Brunt et al., 2010; Zhang et al., 2000) and it has been postulated that factors released by injured tissues stimulate stem cell proliferation and attract stem cells to the sites of injury (Crosnier et al., 2006; Inui and Sakaguchi, 1992). The effects of castration on the adult prostate are a combination of ischemic injury due to vascular disruption, epithelial apoptosis and acute inflammation (Bodine, 1995; Hayek et al., 1999; Kerr and Searle, 1973). Whether a direct response to decreased testosterone levels or in response to castration-induced injury, our observation may be relevant to the behavior of tumor stem cells in prostate cancer. To the extent that tumor stem cells phenocopy the features of normal adult stem/progenitor cells, our findings suggest that treatment of human prostate cancer with androgen deprivation could inadvertently produce an expansion of tumor stem cells.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2014.04.005.
References


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