

Chapter 2

Isolation and Characterization of Prostate Stem Cells

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Abstract Based on the unique capacity of the rodent prostate to undergo seemingly endless rounds of androgen cycling in response to castration and androgen add-back, the prostate has been proposed to contain long-term self-renewing stem cells. However the prospective isolation and characterization of stem-like cells from rodent and human prostate tissue has only been described over the last 2 decades. Several models of epithelial homeostasis in the adult prostate have been proposed based on either the presence of a multipotent tissue stem cell that differentiates through a series of intermediate developmental stages or the coexistence of

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multiple unipotent lineage-restricted stem cells. The isolation of cells with stem and progenitor activity is an important first step to delineate the epithelial hierarchy of the prostate. In addition, isolation of stem cells allows characterization of their functional capacities and the molecular programs regulating their activity. These studies will enable detection or targeting of stem and progenitor cells during various stages of neoplastic transformation and tumor progression, including the lethal phase of the disease, castration-resistant prostate cancer.

2.1 Introduction

While the existence of stem cells in the prostate has long been postulated, their isolation or purification for functional testing has only been described in the last decade. Pioneering androgen cycling experiments by Isaacs and colleagues (Isaacs 1985), later repeated by Wilson and colleagues (Tsujimura et al. 2002), show that long-term castration-resistant stem cells in the rodent prostate are capable of almost indefinitely regenerating the gland after castration-mediated prostate involution and androgen add-back. The adult prostate gland predominantly comprises basal cells and luminal secretory cells with a very minor component of neuroendocrine cells (Abate-Shen and Shen 2000; Shen and Abate-Shen 2010). While basal cells in the rodent prostate predominantly remain after castration, a subset of luminal cells also survive and may participate in gland regeneration upon administration of androgen (English et al. 1987). Divergent models have been proposed to describe prostate homeostasis, from a bipotent prostate stem cell capable of regenerating all mature cell types in the gland to the coexistence of multiple unipotent or lineage-restricted stem cells (Lawson and Witte 2007; Shen et al. 2008). Based on differential keratin stains in both the mouse and human prostate, some have proposed a developmental model starting from a primitive stem cell that undergoes maturation and differentiation through several intermediate cell stages (Okada et al. 1992; Xue et al. 1998; van Leenders et al. 2000, 2003; Hudson 2004). However, functional evidence of such a hierarchical structure has been lacking until recently.

In order to accurately define the prostate epithelial hierarchy based on functional studies, isolation of prostate stem and progenitor cells is an essential first step. In addition to delineating hierarchical relationships, stem cell isolation enables both molecular and functional characterization to determine the capacity of isolated stem cells and define the pathways regulating stem-like behavior. A comprehensive analysis of stem cell properties may lead to targeted therapies of stem-like cells in various stages of disease including the lethal castration-resistant prostate cancer (Feldman and Feldman 2001; Shen and Abate-Shen 2010).

2.2 Methods of Stem Cell Isolation

A number of indirect approaches have been taken to investigate stem cells in the prostate, including the use of BrdU labeling to identify slow-cycling label-retaining cells (Tsujimura et al. 2002). While label retention is a property that some believe

to be associated with stem cells (Cotsarelis et al. 1989; Berardi et al. 1995; Thorgeirsson 1996; Beauchamp et al. 2000; Lavker and Sun 2000; Slack 2000), this method alone does not provide functional evidence for stem cell activity. Importantly, intestinal tissue stem cells and hair follicle stem cells marked by the Wnt target gene *Lgr5* are rapidly dividing (Barker et al. 2007; Jaks et al. 2008), indicating that quiescence is not a universal property of adult stem cells. In this chapter, we will describe the two general approaches that have been described to functionally define stem cell populations in the prostate: isolation of enriched cell preparations from dissociated mouse and human prostate tissues and lineage tracing in genetically engineered mouse models.

In the first approach, cells are purified and isolated from preparations of dissociated prostate tissue. Isolated cells, separated or fractionated into populations enriched for stem cells and those depleted for stem cells, are placed into various *in vitro* and *in vivo* functional assays to determine their inherent proliferative, clonogenic, and regenerative capacities. This strategy enables parallel identification of cell populations from naïve rodent (primarily mouse) and human tissues taken from surgical specimens (Lukacs et al. 2010a; Goldstein et al. 2011). Importantly, cell preparations are easily collected for analysis of DNA, RNA, or protein to determine what molecular characteristics distinguish stem cells from their non-stem cell counterparts. An alternative strategy described to define prostate stem cells is lineage tracing in genetically engineered mice. In this approach, genetic marking is performed in a stem cell leading to expression of a reporter protein in the stem cell and all of its progeny (Wang et al. 2009; Choi et al. 2012). This strategy (described in greater detail in Sect. 2.2.2.2) allows for demonstration of hierarchical relationships within the intact gland, maintaining important interactions with neighboring epithelial cells, non-epithelial stromal and immune cell populations, and other components of the local microenvironment.

2.2.1 *In Vitro Assays to Measure Function of Isolated Stem/Progenitor Cells*

When taken out of their native site and grown in tissue culture, primitive cells should possess the capacity to extensively proliferate and self-renew under the appropriate conditions including growth factors and adhesive substratum (Barrandon and Green 1987; Ogawa 1993; Hudson et al. 2000; Uzgare et al. 2004; Lukacs et al. 2008). In contrast, more mature or differentiated cell populations, particularly those that are postmitotic, would be less likely to grow or persist long term. For this reason, clonogenic assays have been particularly useful in identifying cell subsets enriched for long-term immature cell activity (Ploemacher and Brons 1989; Reynolds and Weiss 1996; Dontu et al. 2003; Shackleton et al. 2006; Lim et al. 2009; Rock et al. 2009). Isolated cell populations can be tested using clonogenic *in vitro* assays that provide a quantitative measure of their proliferative and self-renewal activity. The two most commonly used assays are the colony-forming assay, measuring clonogenic and proliferative potential, and the sphere-forming assay, measuring both clonogenicity and self-renewal *in vitro* in a quantitative fashion (Fig. 2.1).

In vitro clonogenic assays

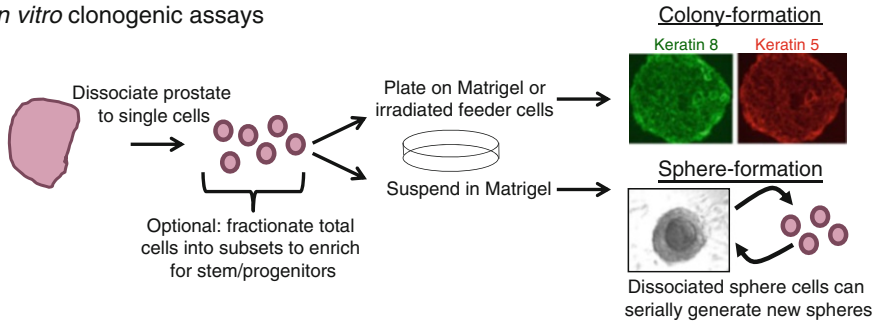


Fig. 2.1 *In vitro* clonogenic assays. Mouse or human prostate tissues are dissociated to single cells. Subsets of epithelium can be fractionated from dissociated preparations to compare functional activity of enriched or depleted cell populations. Cells that are plated in a two-dimensional environment on irradiated feeder cells or Matrigel will form colonies, the majority of which express both luminal (keratin 8) and basal (keratin 5) epithelial keratins. Alternatively, cells that are suspended in a three-dimensional environment of Matrigel will form spheres that can be dissociated to single cells and serially replated to measure self-renewal activity

2.2.1.1 Colony-Forming Assay

In the colony-forming assay, isolated cells are plated either directly onto a tissue culture dish, cocultured with irradiated feeder cells (such as adult fibroblasts, 3T3 fibroblasts, mouse embryonic fibroblasts), or on a matrix substratum (collagen or Matrigel) (Collins et al. 2001; Lawson et al. 2007; Lukacs et al. 2010a). Interestingly, colonies from mouse prostate co-express both basal (K5) and luminal (K8) keratins, representing an intermediate or putative transit-amplifying phenotype rarely observed in normal glands. When dissociated to single cells and serially replated, colonies exhibit limited self-renewal activity which is at least partially due to a Rho-kinase (ROCK)-mediated response, as inhibition of ROCK promotes colony self-renewal *in vitro* (Zhang et al. 2011). Alternatively, cultures of human prostate epithelial cells (PrECs) grown in low-calcium conditions demonstrate stem cell-like colony-forming activity (Litvinov et al. 2006).

While primary cells that form colonies maintain expression of some markers of cells in the gland, such as epithelial keratin expression, these cells lose the glandular structure characteristic of their native environment. Tsujimura et al. (2002) described a clonogenic assay where primary mouse prostate cells are suspended in collagen and grown *in vitro* to form ductal structures (Tsujimura et al. 2002). Cells from the proximal region are enriched for this activity and can generate glands containing distinct keratin 14+ basal and keratin 8+ luminal cells. Although a range of conditions can be used to measure colony-forming activity of naïve primary mouse and human prostate cells, each assay has been effectively utilized to compare the growth of distinct epithelial subpopulations (described further in Sect. 2.3). Recently, methods have been developed for long-term culture of mouse prostate stem cells that retain multi-lineage differentiation and self-renewal *in vitro* and *in vivo* (Barclay et al. 2008). These methods are discussed in more detail elsewhere in this book (Chaps. 9 and 10).

2.2.1.2 Sphere-Forming Assay

To quantitatively measure self-renewal activity, primary cells grown within a three-dimensional matrix of Matrigel can be expanded *in vitro* (Lawson et al. 2007; Shi et al. 2007; Xin et al. 2007). Matrigel is rich in extracellular matrix proteins including laminin, collagen, and fibronectin (Emonard et al. 1987a, b) which are found in the basement membrane structures surrounding benign prostate glands in their native microenvironment (Bonkhoff et al. 1991; Fong et al. 1991). Dissociated single cells can be isolated from primary spheres and replated in Matrigel to generate secondary spheres, demonstrating self-renewal activity (Lawson et al. 2007; Xin et al. 2007). This activity can be repeated numerous times during serial replating or passaging to demonstrate the presence of long-term self-renewing cells (Lawson et al. 2007; Shi et al. 2007; Xin et al. 2007; Goldstein et al. 2008; Garraway et al. 2010).

The outer layer of cells in mouse prostate spheres comprises p63+ cells, analogous to the outer layer of p63+ basal cells in the gland, that are proliferating based on positive stains for Ki67. Proliferating p63+ cells appear to spontaneously differentiate toward the center or luminal space, which is filled with p63- cells undergoing apoptosis as marked by TUNEL staining (Xin et al. 2007). Sphere cells of both mouse and human origin retain *in vivo* stem-like activity to reconstitute glandular structures containing both basal and luminal epithelial cells when transplanted into mice (Shi et al. 2007; Xin et al. 2007; Garraway et al. 2009). Both spheres and colonies are clonally derived indicating that they arise from a single stem or progenitor cell (Lawson et al. 2007; Garraway et al. 2010). These assays allow for identification of markers to enrich for functional stem/progenitor cell subsets that can grow *in vitro*. They also provide quantitative measures of progenitor function that can be used to determine pathways and factors regulating stem and progenitor cell activity (Mulholland et al. 2009; Lukacs et al. 2010b; Shahi et al. 2011).

2.2.2 *In Vivo* Assays to Measure Prostate Stem Cells

While *in vitro* assays have been utilized to quantify the functional activity of isolated cells from adult epithelial tissues, colony and sphere-forming assays are believed to measure progenitor activity rather than true stem cell function. For example, far greater numbers of mammary stem cell-enriched Lin⁻CD24⁺CD29^{hi} cells can generate clonogenic colonies *in vitro* than can repopulate mammary gland structures *in vivo* (Asselin-Labat et al. 2010). Therefore, *in vivo* assays are the most stringent tests of stem cell activity (Fig. 2.2), three of which will be described in the following section.

2.2.2.1 *In Vivo* Tissue-Regeneration Assays to Measure Prospectively Isolated Stem Cell Populations

Cunha and colleagues first described a tissue fragment recombination assay where mid-gestation urogenital sinus, the region destined to develop into the prostate, can

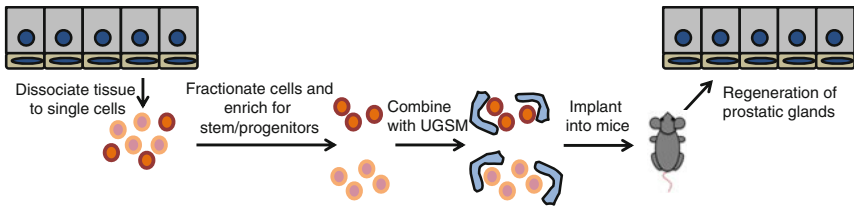
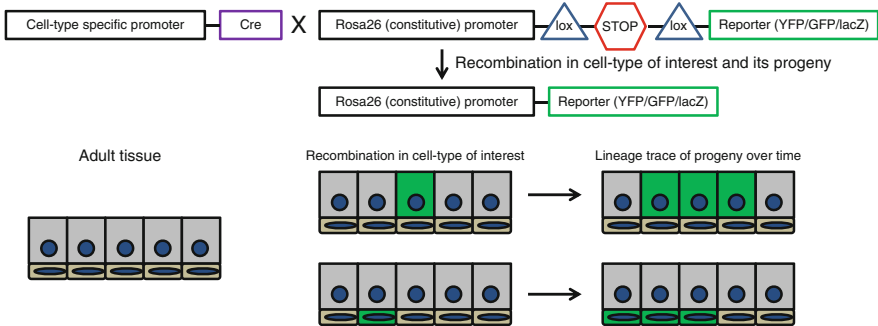
a *In vivo* tissue-regeneration**b** *In vivo* lineage tracing

Fig. 2.2 *In vivo* stem cell assays. **(a)** In the *in vivo* tissue-regeneration assay, prostate glands are dissociated to single cells and enriched/depleted subsets of cells are fractionated, combined with Urogenital Sinus Mesenchyme (UGSM), and implanted back into immune-deficient mice, either under the renal capsule or skin. Over 6–12 weeks, dissociated cells will regenerate prostatic glands. **(b)** An alternative approach is *in vivo* lineage tracing. The Cre recombinase, expressed in a cell-type-specific manner, will remove a STOP codon leading to constitutive expression of a reporter protein in the cell type of interest and all of its progeny

be physically separated into urogenital sinus mesenchyme (UGSM) and urogenital sinus epithelium (UGSE) and put back together under the renal capsule of immune-deficient mice (Cunha and Lung 1978). After a period of weeks to months, the recombined tissue will develop into prostatic glands. Our laboratory has made two important adaptations to utilize this assay with dissociated adult cells (Xin et al. 2003). First, we found that UGSM could support the regeneration of dissociated adult mouse prostate cells. The adult prostate could regenerate glands that resembled the native prostate gland (Xin et al. 2003). Since the epithelium was coming from the adult prostate, a second important adaptation was made to allow greater experimental control and perform transplantations without carefully timed matings. Xin et al. (2003) showed that UGSM could be dissociated to single cells, expanded in culture, frozen and thawed, and maintain its inductive activity on adult epithelium (Xin et al. 2003).

Since total dissociated adult mouse prostate cells could regenerate glands, the pool must contain stem-like tissue-regenerating cells, which could then be isolated using various approaches. Mouse prostate glandular regeneration is an extremely robust assay, used by many labs (Xin et al. 2003; Burger et al. 2005; Shi et al. 2007; Leong et al. 2008; Wang et al. 2009). A parallel dissociated cell tissue-regeneration assay using freshly isolated naïve benign primary human epithelial cells is more

difficult for two reasons. First, obtaining fresh human tissue requires a considerable degree of effort and coordination between surgeons, pathologists, and researchers in a timely manner (Goldstein et al. 2011). Secondly, human prostate tissue is most commonly isolated from aged men who suffer from a disorder either in the prostate or in a neighboring tissue, such as the bladder. In contrast, studies with mouse tissue are generally performed on young, healthy tissue. Despite these difficulties, we demonstrated the use of an analogous tissue-regeneration assay where naïve human epithelial cells could regenerate human prostate glands *in vivo* (Goldstein et al. 2010, 2011).

It is important to note that while the tissue-recombination assay can be easily adapted based on cell type and species of origin, the approach requires taking adult cells out of their niche in the prostate gland. The process of gland dissociation could disrupt critical cell–cell interactions, remove signals from supportive cell populations, and preferentially select for one cell type over another in transplanted preparations. As mentioned in the introduction, an alternative approach to retain the native structure is genetic lineage tracing.

2.2.2.2 In Vivo Lineage Tracing of Prostate Stem/Progenitor Cells and Their Progeny

The most common lineage tracing experiments utilize a cell-type or lineage-specific promoter to drive expression of the Cre recombinase either in a constitutive or regulated manner, reviewed by Fuchs and Horsley (2011). The Cre enzyme is a bacteriophage topoisomerase that specifically recognizes a short 34-base-pair stretch of DNA called a lox sequence, made up of two inverted repeats and a spacer region (Lakso et al. 1992). Mice engineered to express Cre from a lineage-specific promoter are most commonly bred with a reporter strain where lox sites flanking a STOP codon are placed between the ubiquitously expressed Rosa26 promoter and a reporter protein, such as green fluorescent protein (GFP) or lacZ (which can be detected upon the addition of the β -galactosidase substrate) (Soriano 1999; Barker et al. 2007; Fuchs and Horsley 2011). Using this strategy, the lox sites are excised allowing reporter expression only in the presence of the Cre recombinase. Since the recombination event occurs at the DNA level in the stem cell, all cells derived from that stem cell will retain the recombined allele and exhibit reporter expression. This strategy allows for robust tracing of the progeny from the original cells engineered to express the Cre protein. Therefore, the specificity and sensitivity of Cre expression in the cell type of interest are of vital importance for interpreting results.

2.2.2.3 In Vivo Castration and Androgen-Mediated Regeneration to Demonstrate Stem Cell Activity

The most impressive display of prostate stem cell activity is in its capacity to survive androgen ablation or castration and promote regeneration of the gland upon

administration of androgen (Tsujimura et al. 2002). The involution following castration and regeneration upon androgen add-back can be repeated almost indefinitely. Lukacs et al. (2008) utilized the tissue-recombination approach to demonstrate that adult prostate cells contain long-term self-renewing stem-like cells that can survive androgen deprivation and mediate regeneration following androgen add-back (Lukacs et al. 2008). Adult dissociated cells were combined with UGSM and implanted under the renal capsule of intact mice. Recipient mice were then subjected to rounds of castration-induced involution and androgen-mediated regeneration (Lukacs et al. 2008). In a manner analogous to the native prostate, regenerated tissue under the renal capsule was also capable of castration resistance and self-renewal. The lineage tracing approach has been combined with castration/regeneration to mark cells in the castrated or involuted state, using an inducible Cre recombinase to label cells with a fluorescent reporter protein, and then demonstrate the labeled progeny of those cells after androgen-mediated regeneration (Wang et al. 2009). These experiments prove that castration-resistant cells contribute to local tissue regeneration after addition of androgen.

2.3 Identification of Stem Cell Populations

2.3.1 *Isolated Stem Cells in Dissociated Mouse Tissues*

Having developed assays to measure stem-like activity, numerous groups have now defined methods to purify cell populations enriched for stem/progenitor cells. Purification methods have been primarily based on cell-surface markers combined with Fluorescence-Activated Cell Sorting (FACS) although methods to enrich for functional or enzymatic activity have also been used for stem/progenitor purification. While the majority of studies from numerous laboratories implicate basal-like cells from the mouse prostate as stem/progenitor cells (Lawson et al. 2007, 2010; Goldstein et al. 2008; Burger et al. 2009; Choi et al. 2012), recent studies have also proven that the luminal epithelial layer contains stem cells (Wang et al. 2009; Choi et al. 2012).

2.3.1.1 Evidence for Stem Cells with a Basal Localization

The stem cell antigen-1 (Sca-1), a marker of primitive stem/progenitor cells in many adult tissues (Spangrude et al. 1988; Welm et al. 2002; Kim et al. 2005), was used by two different groups to enrich for cells from the mouse prostate capable of tissue regeneration in vivo (Burger et al. 2005; Xin et al. 2005). While Sca-1+ cells, isolated from dissociated adult mouse prostate by FACS, can efficiently regenerate prostatic glands under the renal capsule, Sca-1- cells are devoid of this activity (Xin et al. 2005; Burger et al. 2009). A subset of Sca-1+ cells concentrated in the region

most proximal to the urethra also express the Wnt target gene *Axin2*, indicating a potential role for Wnt signaling in maintaining these progenitor cells in their niche (Ontiveros et al. 2008). *Sca-1* is expressed both in stromal and epithelial cells, indicating that additional markers are necessary to isolate epithelial stem cells (Lawson et al. 2010). Lawson et al. (2007) found that integrin alpha 6 (CD49f), which is a stem cell marker in several adult tissues (Stingl et al. 2006; Rock et al. 2009; Notta et al. 2011), could further enrich for cells in the mouse prostate capable of tissue regeneration in vivo (Lawson et al. 2007, 2010). Up to 1 in every 44 cells in this population exhibited colony formation in vitro on either irradiated 3T3 mouse fibroblast feeder cells (Lawson et al. 2007) or on Matrigel (Lawson et al. 2010). These stem cells were identified by depleting for lineage (Lin) antibodies against hematopoietic (CD45+), endothelial (CD31+), and red blood cells (Ter119) to generate a Lin⁻Sca-1⁺CD49f^{hi} (LSC) profile. LSC cells identified a basally located stem cell population in the mouse prostate (Lawson et al. 2010).

The type I transmembrane protein Trop2, which is overexpressed in numerous cancers and associated with poor prognosis (Ohmachi et al. 2006; Fong et al. 2008a, b; Muhlmann et al. 2009; Kobayashi et al. 2010), was found to be highly expressed on a subset of mouse prostate LSC cells enriched for in vitro and in vivo stem-like activity (Goldstein et al. 2008). By gating on high levels of Trop2, up to 1/11 LSC Trop2^{hi} cells from the mouse prostate could generate spheres in vitro (Goldstein et al. 2008). In vivo, DsRed labeled LSC Trop2^{hi} cells could regenerate glands containing basal, luminal, and neuroendocrine cells. Importantly, neuroendocrine cells in regenerate glands were labeled with the DsRed protein, indicating that they were derived from donor stem cells capable of tri-lineage differentiation upon transplantation (Goldstein et al. 2008).

A single study implicates expression of the hematopoietic stem cell and germ cell marker *ckit*/CD117 (Manova et al. 1990; Ikuta and Weissman 1992) on putative mouse prostate epithelial stem cells and human prostate basal cells (Leong et al. 2008). However, numerous groups have found *ckit* expression in the adult human prostate to localize exclusively to the non-epithelial compartment on immune-infiltrating mast cells and specialized stromal cells termed interstitial cells of Cajal (ICC) (Van der Aa et al. 2003; Shafik et al. 2005; Nguyen et al. 2011). Other groups have reported an absence of *ckit* expression in mouse prostate luminal cells (Wang et al. 2009) or on adult mouse prostate epithelium (Blum et al. 2009).

While several surface markers can be combined to isolate basal stem cells from the mouse prostate, other approaches have also been utilized for mouse prostate stem cell purification. Cells with high levels of aldehyde dehydrogenase (ALDH) enzymatic activity can be labeled with a substrate that gets trapped inside of target cells and fluoresces at a detectable wavelength for FACS isolation. Burger et al. (2009) showed that Aldefluor^{bright} cells are enriched for stem cell activity and that these cells predominantly localize to the basal cell layer (Burger et al. 2009). Finally, using a cyan fluorescent protein (CFP) reporter expressed from the basal cell-specific keratin 5 promoter, K5-CFP+ cells show stem cell properties when isolated and grown in vitro or in vivo (Peng et al. 2011).

2.3.2 Identification of Stem Cells Using a Lineage Tracing Approach

2.3.2.1 Luminal Stem Cells in the Castrated/Regressed Prostate

Using a genetic lineage tracing approach, Wang et al. (2009) identified a luminal stem cell population in the castrated/regressed mouse prostate (Wang et al. 2009). Although the androgen target gene and homeobox transcription factor Nkx3.1 is dramatically downregulated following castration, rare luminal cells in the regressed/involuting mouse prostate gland remain in Nkx3.1+. By engineering an inducible Nkx3.1 promoter to drive expression of the Cre allele, Shen and colleagues were able to label *CAstration-Resistant* Nkx3.1+ luminal cells (CARNs) and their progeny with YFP (Wang et al. 2009). After androgen add-back and regeneration of the gland, the authors found evidence of basal, luminal, and neuroendocrine cells labeled with YFP, indicating that CARNs represent a stem cell population capable of multi-lineage differentiation. CARNs were isolated in the castrated state and subjected to in vivo tissue regeneration to show that this stem cell population can also regenerate prostatic glands upon transplantation under the kidney capsule (Wang et al. 2009).

2.3.2.2 Parallel Identification of Unipotent Basal and Luminal Stem Cells in the Adult Mouse Prostate

Given the findings that in the normal prostate, basal cells are the predominant cell type capable of tissue regeneration upon transplantation, but rare luminal cells in the castrated prostate can regenerate prostate tissue, Choi et al. (2012) performed lineage tracing on both basal and luminal cells in the normal adult murine prostate (Choi et al. 2012). Using a K14 promoter driving expression of Cre to label basal cells and their progeny and a K8 promoter driving Cre to mark luminal cells and their progeny, Xin and colleagues found that both basal and luminal cells are predominantly self-sustained lineages, presumably due to the coexistence of distinct unipotent lineage-restricted stem cells (Choi et al. 2012). Even after serial castration and regeneration, basal cells only gave rise to new basal cells, while luminal cells only gave rise to new luminal cells. A discussion of seemingly conflicting results (as basal cells and CARN cells are multipotent upon transplantation, but lineage-marked basal and luminal cells are unipotent) is included in Sect. 2.4.

2.3.3 Markers of Isolated Basal Stem Cells in Dissociated Human Prostate Tissues

Using in vitro assays, it was shown that basal cells from the human prostate can give rise to luminal-like cells in vitro, suggesting a linear relationship between stem cells that reside within the basal layer and their luminal progeny (Robinson et al. 1998).

Collins et al. (2001) demonstrated that CD44+ basal cells from human prostate specimens expressing high levels of alpha-2 integrin preferentially adhere to collagen and form colonies on extracellular matrix-coated plates (Collins et al. 2001). Human prostate basal colony-forming cells can be further enriched in the CD133+ subset when grown on ECM proteins and with irradiated mouse embryonic fibroblasts as feeder cells (Richardson et al. 2004). In vivo, alpha2+ and CD133+ basal cells from the human prostate can generate epithelial structures at a low efficiency (Collins et al. 2001; Richardson et al. 2004). Human prostate basal cells isolated based on mouse prostate stem cell markers Trop2 and CD49f can form spheres at an average rate of almost 1/3, demonstrating significant progenitor activity within the phenotypic fraction Trop2+ CD49f^{hi} (Goldstein et al. 2008). Trop2 and CD49f isolate basal cells that can generate glands upon transplantation into immune-deficient mice that are indistinguishable from primary human prostate tubules (Goldstein et al. 2010). These collective data show that human prostate basal cells show stem cell activity in both in vitro and in vivo assays.

2.4 Unresolved Questions for Future Research

As described in Sect. 2.3.2.2, lineage tracing in the adult mouse prostate demonstrated that basal cells give rise to basal cells and luminal cells give rise to luminal cells in a unipotent manner (Choi et al. 2012). Using a dissociated cell tissue-regeneration assay, numerous groups have identified cells capable of multi-lineage differentiation capacity (Burger et al. 2005, 2009; Xin et al. 2005, 2007; Lawson et al. 2007, 2010; Goldstein et al. 2008, 2010; Leong et al. 2008; Wang et al. 2009). Do these assays (tissue recombination using embryonic mesenchyme vs. lineage tracing) measure different activities?

Experiments in the mouse skin indicate that hair follicle bulge stem cells are capable of generating all epidermal lineages upon transplantation, which mimics a wound healing type of response, but they only give rise to hair follicles under normal conditions in the intact skin by lineage tracing (Blanpain and Fuchs 2009). Mouse mammary gland stem cells, identified based on expression of basal cell-surface markers such as high levels of integrins CD49f and CD29, can reconstitute an entire mammary gland upon transplantation into a cleared fat pad (Shackleton et al. 2006; Stingl et al. 2006). However this multi-lineage differentiation capacity is not observed in the intact postpubertal adult mouse mammary gland using lineage tracing tools, as unipotent basal stem cells only give rise to basal cells and lineage-restricted luminal cells are limited to generating adult luminal cells (Van Keymeulen et al. 2011). Interestingly, all adult mouse mammary cells derive from a common embryonic precursor cell, marked by keratin 14 (Van Keymeulen et al. 2011). These collective data suggest that in the prostate and other epithelial tissues, a transplantation approach may push unipotent stem cells toward a more primitive multipotent state, most similar to early development. The use of embryonic mesenchyme in the prostate-regeneration assay may aid adult cells in adopting this embryonic-like fate.

Besides the predominant epithelial cell types, basal and luminal, rare neuroendocrine cells are also found in the developing and adult prostate. However the stem cell that gives rise to neuroendocrine cells remains uncertain. Given the presence of neuroendocrine cells outside of the epithelial glands in the developing human urogenital sinus region, Aumuller et al. (1999) proposed that neuroendocrine cells are derived from the neural crest or ectodermal lineage (Aumuller et al. 1999). However studies by our group and others showed that labeled basal stem cells (Goldstein et al. 2008) or labeled CARN cells (Wang et al. 2009) can give rise to labeled neuroendocrine cells, indicating that they can derive from an endodermal origin in the prostate epithelium.

Through the identification of a panel of cell-surface markers including CD49f, Dick and colleagues have recently demonstrated the purification of single human hematopoietic stem cells capable of long-term engraftment and multi-lineage differentiation (Notta et al. 2011). Isolating stem cells to such a high degree of purity allows for the investigation of their unique properties. Future studies will be necessary to determine whether mouse or human prostate stem cells can be purified to such a degree. Given the emerging role of tissue stem cells in the initiation of mouse (Wang et al. 2006, 2009; Lawson et al. 2010; Choi et al. 2012) and human (Goldstein et al. 2010; Taylor et al. 2012) prostate cancer, identification of unique targets and pathways regulating stem cells will be useful for the future detection and elimination of stem cells in malignant transformation.

References

- Abate-Shen C, Shen MM (2000) Molecular genetics of prostate cancer. *Genes Dev* 14:2410–2434
- Asselin-Labat ML, Vaillant F, Sheridan JM, Pal B, Wu D, Simpson ER, Yasuda H, Smyth GK, Martin TJ, Lindeman GJ et al (2010) Control of mammary stem cell function by steroid hormone signalling. *Nature* 465:798–802
- Aumuller G, Leonhardt M, Janssen M, Konrad L, Bjartell A, Abrahamsson PA (1999) Neurogenic origin of human prostate endocrine cells. *Urology* 53:1041–1048
- Barclay WW, Axanova LS, Chen W, Romero L, Maund SL, Soker S, Lees CJ, Cramer SD (2008) Characterization of adult prostatic progenitor/stem cells exhibiting self-renewal and multilineage differentiation. *Stem Cells* 26:600–610
- Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ et al (2007) Identification of stem cells in small intestine and colon by marker gene *Lgr5*. *Nature* 449:1003–1007
- Barrandon Y, Green H (1987) Three clonal types of keratinocyte with different capacities for multiplication. *Proc Natl Acad Sci USA* 84:2302–2306
- Beauchamp JR, Heslop L, Yu DS, Tajbakhsh S, Kelly RG, Wernig A, Buckingham ME, Partridge TA, Zammit PS (2000) Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. *J Cell Biol* 151:1221–1234
- Berardi AC, Wang A, Levine JD, Lopez P, Scadden DT (1995) Functional isolation and characterization of human hematopoietic stem cells. *Science (New York, NY)* 267:104–108
- Blanpain C, Fuchs E (2009) Epidermal homeostasis: a balancing act of stem cells in the skin. *Nat Rev Mol Cell Biol* 10:207–217
- Blum R, Gupta R, Burger PE, Ontiveros CS, Salm SN, Xiong X, Kamb A, Wesche H, Marshall L, Cutler G et al (2009) Molecular signatures of prostate stem cells reveal novel signaling pathways and provide insights into prostate cancer. *PLoS One* 4:e5722

- Bonkhoff H, Wernert N, Dhom G, Remberger K (1991) Basement membranes in fetal, adult normal, hyperplastic and neoplastic human prostate. *Virchows Archiv* 418:375–381
- Burger PE, Xiong X, Coetzee S, Salm SN, Moscatelli D, Goto K, Wilson EL (2005) Sca-1 expression identifies stem cells in the proximal region of prostatic ducts with high capacity to reconstitute prostatic tissue. *Proc Natl Acad Sci USA* 102:7180–7185
- Burger PE, Gupta R, Xiong X, Ontiveros CS, Salm SN, Moscatelli D, Wilson EL (2009) High aldehyde dehydrogenase activity: a novel functional marker of murine prostate stem/progenitor cells. *Stem Cells* 27:2220–2228
- Choi N, Zhang B, Zhang L, Ittmann M, Xin L (2012) Adult murine prostate basal and luminal cells are self-sustained lineages that can both serve as targets for prostate cancer initiation. *Cancer Cell* 21:253–265
- Collins AT, Habib FK, Maitland NJ, Neal DE (2001) Identification and isolation of human prostate epithelial stem cells based on alpha(2)beta(1)-integrin expression. *J Cell Sci* 114: 3865–3872
- Cotsarelis G, Cheng SZ, Dong G, Sun TT, Lavker RM (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
- Cunha GR, Lung B (1978) The possible influence of temporal factors in androgenic responsiveness of urogenital tissue recombinants from wild-type and androgen-insensitive (Tfm) mice. *J Exp Zool* 205:181–193
- Dontu V, Abdallah WM, Foley JM, Jackson KW, Clarke MF, Kawamura MJ, Wicha MS (2003) In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. *Genes Dev* 17:1253–1270
- Emonard H, Calle A, Grimaud JA, Peyrol S, Castronovo V, Noel A, Lapiere CM, Kleinman HK, Foidart JM (1987a) Interactions between fibroblasts and a reconstituted basement membrane matrix. *J Invest Dermatol* 89:156–163
- Emonard H, Grimaud JA, Nusgens B, Lapiere CM, Foidart JM (1987b) Reconstituted basement-membrane matrix modulates fibroblast activities in vitro. *J Cell Physiol* 133:95–102
- English HF, Santen RJ, Isaacs JT (1987) Response of glandular versus basal rat ventral prostatic epithelial cells to androgen withdrawal and replacement. *Prostate* 11:229–242
- Feldman BJ, Feldman D (2001) The development of androgen-independent prostate cancer. *Nat Rev Cancer* 1:34–45
- Fong CJ, Sherwood ER, Sutkowski DM, Abu-Jawdeh GM, Yokoo H, Bauer KD, Kozlowski JM, Lee C (1991) Reconstituted basement membrane promotes morphological and functional differentiation of primary human prostatic epithelial cells. *Prostate* 19:221–235
- Fong D, Moser P, Krammel C, Gostner JM, Margreiter R, Mitterer M, Gastl G, Spizzo G (2008a) High expression of TROP2 correlates with poor prognosis in pancreatic cancer. *Br J Cancer* 99:1290–1295
- Fong D, Spizzo G, Gostner JM, Gastl G, Moser P, Krammel C, Gerhard S, Rasse M, Laimer K (2008b) TROP2: a novel prognostic marker in squamous cell carcinoma of the oral cavity. *Mod Pathol* 21:186–191
- Fuchs E, Horsley V (2011) Ferreting out stem cells from their niches. *Nat Cell Biol* 13:513–518
- Garraway IP, Sun W, Tran CP, Perner S, Zhang B, Goldstein AS, Hahm SA, Haider M, Head CS, Reiter RE et al (2009) Human prostate sphere-forming cells represent a subset of basal epithelial cells capable of glandular regeneration in vivo. *The Prostate*
- Garraway IP, Sun W, Tran CP, Perner S, Zhang B, Goldstein AS, Hahm SA, Haider M, Head CS, Reiter RE et al (2010) Human prostate sphere-forming cells represent a subset of basal epithelial cells capable of glandular regeneration in vivo. *The Prostate* 70: 491–501
- Goldstein AS, Lawson DA, Cheng D, Sun W, Garraway IP, Witte ON (2008) Trop2 identifies a subpopulation of murine and human prostate basal cells with stem cell characteristics. *Proc Natl Acad Sci USA* 105:20882–20887
- Goldstein AS, Huang J, Guo C, Garraway IP, Witte ON (2010) Identification of a cell of origin for human prostate cancer. *Science (New York, NY)* 329:568–571

- Goldstein AS, Drake JM, Burnes DL, Finley DS, Zhang H, Reiter RE, Huang J, Witte ON (2011) Purification and direct transformation of epithelial progenitor cells from primary human prostate. *Nat Protoc* 6:656–667
- Hudson DL (2004) Epithelial stem cells in human prostate growth and disease. *Prostate Cancer Prostatic Dis* 7:188–194
- Hudson DL, O'Hare M, Watt FM, Masters JR (2000) Proliferative heterogeneity in the human prostate: evidence for epithelial stem cells. *Lab Invest* 80:1243–1250
- Ikuta K, Weissman IL (1992) Evidence that hematopoietic stem cells express mouse c-kit but do not depend on steel factor for their generation. *Proc Natl Acad Sci USA* 89:1502–1506
- Isaacs JT (1985) Control of cell proliferation and cell death in the normal and neoplastic prostate: a stem cell model. In: Rodgers CH et al (eds) *Benign prostatic hyperplasia*. Department of Health and Human Services, Washington, DC, pp 85–94
- Jaks V, Barker N, Kasper M, van Es JH, Snippert HJ, Clevers H, Toftgard R (2008) Lgr5 marks cycling, yet long-lived, hair follicle stem cells. *Nat Genet* 40:1291–1299
- Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, Jacks T (2005) Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 121:823–835
- Kobayashi H, Minami Y, Anami Y, Kondou Y, Iijima T, Kano J, Morishita Y, Tsuta K, Hayashi S, Noguchi M (2010) Expression of the GA733 gene family and its relationship to prognosis in pulmonary adenocarcinoma. *Virchows Arch* 457:69–76
- Lakso M, Sauer B, Mosinger B Jr, Lee EJ, Manning RW, Yu SH, Mulder KL, Westphal H (1992) Targeted oncogene activation by site-specific recombination in transgenic mice. *Proc Natl Acad Sci USA* 89:6232–6236
- Lavker RM, Sun TT (2000) Epidermal stem cells: properties, markers, and location. *Proc Natl Acad Sci USA* 97:13473–13475
- Lawson DA, Witte ON (2007) Stem cells in prostate cancer initiation and progression. *J Clin Invest* 117:2044–2050
- Lawson DA, Xin L, Lukacs RU, Cheng D, Witte ON (2007) Isolation and functional characterization of murine prostate stem cells. *Proc Natl Acad Sci USA* 104:181–186
- Lawson DA, Zong Y, Memarzadeh S, Xin L, Huang J, Witte ON (2010) Basal epithelial stem cells are efficient targets for prostate cancer initiation. *Proc Natl Acad Sci USA* 107(6):2610–2615
- Leong KG, Wang BE, Johnson L, Gao WQ (2008) Generation of a prostate from a single adult stem cell. *Nature* 456:804–808
- Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, Asselin-Labat ML, Gyorki DE, Ward T, Partanen A et al (2009) Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. *Nat Med* 15:907–913
- Litvinov IV, Vander Griend DJ, Xu Y, Antony L, Dalrymple SL, Isaacs JT (2006) Low-calcium serum-free defined medium selects for growth of normal prostatic epithelial stem cells. *Cancer Res* 66:8598–8607
- Lukacs RU, Lawson DA, Xin L, Zong Y, Garraway I, Goldstein AS, Memarzadeh S, Witte ON (2008) Epithelial stem cells of the prostate and their role in cancer progression. *Cold Spring Harb Symp Quant Biol* 73:491–502
- Lukacs RU, Goldstein AS, Lawson DA, Cheng D, Witte ON (2010a) Isolation, cultivation and characterization of adult murine prostate stem cells. *Nat Protoc* 5:702–713
- Lukacs RU, Memarzadeh S, Wu H, Witte ON (2010b) Bmi-1 is a crucial regulator of prostate stem cell self-renewal and malignant transformation. *Cell Stem Cell* 7:682–693
- Manova K, Nocka K, Besmer P, Bachvarova RF (1990) Gonadal expression of c-kit encoded at the W locus of the mouse. *Development* 110:1057–1069
- Muhlmann G, Spizzo G, Gostner J, Zitt M, Maier H, Moser P, Gastl G, Muller HM, Margreiter R, Ofner D et al (2009) TROP2 expression as prognostic marker for gastric carcinoma. *J Clin Pathol* 62:152–158
- Mulholland DJ, Xin L, Morim A, Lawson D, Witte O, Wu H (2009) Lin-Sca-1+CD49fhigh stem/progenitors are tumor-initiating cells in the Pten-null prostate cancer model. *Cancer Res* 69:8555–8562

- Nguyen DT, Dey A, Lang RJ, Ventura S, Exintaris B (2011) Contractility and pacemaker cells in the prostate gland. *J Urol* 185:347–351
- Notta F, Doulatov S, Laurenti E, Poepl A, Jurisica I, Dick JE (2011) Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. *Science* (New York, NY) 333:218–221
- Ogawa M (1993) Differentiation and proliferation of hematopoietic stem cells. *Blood* 81: 2844–2853
- Ohmachi T, Tanaka F, Mimori K, Inoue H, Yanaga K, Mori M (2006) Clinical significance of TROP2 expression in colorectal cancer. *Clin Cancer Res* 12:3057–3063
- Okada H, Tsubura A, Okamura A, Senzaki H, Naka Y, Komatz Y, Morii S (1992) Keratin profiles in normal/hyperplastic prostates and prostate carcinoma. *Virchows Archiv* 421:157–161
- Ontiveros CS, Salm SN, Wilson EL (2008) Axin2 expression identifies progenitor cells in the murine prostate. *Prostate* 68:1263–1272
- Peng W, Bao Y, Sawicki JA (2011) Epithelial cell-targeted transgene expression enables isolation of cyan fluorescent protein (CFP)-expressing prostate stem/progenitor cells. *Transgenic Res* 20:1073–1086
- Ploemacher RE, Brons RH (1989) Separation of CFU-S from primitive cells responsible for reconstitution of the bone marrow hemopoietic stem cell compartment following irradiation: evidence for a pre-CFU-S cell. *Exp Hematol* 17:263–266
- Reynolds BA, Weiss S (1996) Clonal and population analyses demonstrate that an EGF-responsive mammalian embryonic CNS precursor is a stem cell. *Dev Biol* 175:1–13
- Richardson GD, Robson CN, Lang SH, Neal DE, Maitland NJ, Collins AT (2004) CD133, a novel marker for human prostatic epithelial stem cells. *J Cell Sci* 117:3539–3545
- Robinson EJ, Neal DE, Collins AT (1998) Basal cells are progenitors of luminal cells in primary cultures of differentiating human prostatic epithelium. *Prostate* 37:149–160
- Rock JR, Onaitis MW, Rawlins EL, Lu Y, Clark CP, Xue Y, Randell SH, Hogan BL (2009) Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc Natl Acad Sci USA* 106:12771–12775
- Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML, Wu L, Lindeman GJ, Visvader JE (2006) Generation of a functional mammary gland from a single stem cell. *Nature* 439:84–88
- Shafik A, Shafik I, el-Sibai O (2005) Identification of c-kit-positive cells in the human prostate: the interstitial cells of Cajal. *Arch Androl* 51:345–351
- Shahi P, Seethamagari MR, Valdez JM, Xin L, Spencer DM (2011) Wnt and Notch pathways have interrelated opposing roles on prostate progenitor cell proliferation and differentiation. *Stem Cells* (Dayton, Ohio) 29:678–688
- Shen MM, Abate-Shen C (2010) Molecular genetics of prostate cancer: new prospects for old challenges. *Genes Dev* 24:1967–2000
- Shen MM, Wang X, Economides KD, Walker D, Abate-Shen C (2008) Progenitor cells for the prostate epithelium: roles in development, regeneration, and cancer. *Cold Spring Harb Symp Quant Biol* 73:529–538
- Shi X, Gipp J, Bushman W (2007) Anchorage-independent culture maintains prostate stem cells. *Dev Biol* 312:396–406
- Slack JM (2000) Stem cells in epithelial tissues. *Science* (New York, NY) 287:1431–1433
- Soriano P (1999) Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* 21:70–71
- Spangrude GJ, Heimfeld S, Weissman IL (1988) Purification and characterization of mouse hematopoietic stem cells. *Science* (New York, NY) 241:58–62
- Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, Choi D, Li HI, Eaves CJ (2006) Purification and unique properties of mammary epithelial stem cells. *Nature* 439:993–997
- Taylor RA, Toivanen R, Frydenberg M, Pedersen J, Harewood L, Australian Prostate Cancer B, Collins AT, Maitland NJ, Risbridger GP (2012) Human epithelial basal cells are cells of origin of prostate cancer, independent of CD133 status. *Stem Cells* (Dayton, Ohio) 30:1087–1096
- Thorgeirsson SS (1996) Hepatic stem cells in liver regeneration. *FASEB J* 10:1249–1256

- Tsujimura A, Koikawa Y, Salm S, Takao T, Coetzee S, Moscatelli D, Shapiro E, Lepor H, Sun TT, Wilson EL (2002) Proximal location of mouse prostate epithelial stem cells: a model of prostatic homeostasis. *J Cell Biol* 157:1257–1265
- Uzgare AR, Xu Y, Isaacs JT (2004) In vitro culturing and characteristics of transit amplifying epithelial cells from human prostate tissue. *J Cell Biochem* 91:196–205
- Van der Aa F, Roskams T, Blyweert W, De Ridder D (2003) Interstitial cells in the human prostate: a new therapeutic target? *Prostate* 56:250–255
- Van Keymeulen A, Rocha AS, Ousset M, Beck B, Bouvencourt G, Rock J, Sharma N, Dekoninck S, Blanpain C (2011) Distinct stem cells contribute to mammary gland development and maintenance. *Nature* 479:189–193
- van Leenders G, Dijkman H, Hulsbergen-van de Kaa C, Ruiter D, Schalken J (2000) Demonstration of intermediate cells during human prostate epithelial differentiation in situ and in vitro using triple-staining confocal scanning microscopy. *Lab Invest* 80:1251–1258
- van Leenders GJ, Gage WR, Hicks JL, van Balken B, Aalders TW, Schalken JA, De Marzo AM (2003) Intermediate cells in human prostate epithelium are enriched in proliferative inflammatory atrophy. *Am J Pathol* 162:1529–1537
- Wang S, Garcia AJ, Wu M, Lawson DA, Witte ON, Wu H (2006) Pten deletion leads to the expansion of a prostatic stem/progenitor cell subpopulation and tumor initiation. *Proc Natl Acad Sci USA* 103:1480–1485
- Wang X, Kruithof-de Julio M, Economides KD, Walker D, Yu H, Halili MV, Hu YP, Price SM, Abate-Shen C, Shen MM (2009) A luminal epithelial stem cell that is a cell of origin for prostate cancer. *Nature* 461:495–500
- Welm BE, Tepera SB, Venezia T, Graubert TA, Rosen JM, Goodell MA (2002) Sca-1(pos) cells in the mouse mammary gland represent an enriched progenitor cell population. *Dev Biol* 245:42–56
- Xin L, Ide H, Kim Y, Dubey P, Witte ON (2003) In vivo regeneration of murine prostate from dissociated cell populations of postnatal epithelia and urogenital sinus mesenchyme. *Proc Natl Acad Sci USA* 100(Suppl 1):11896–11903
- Xin L, Lawson DA, Witte ON (2005) The Sca-1 cell surface marker enriches for a prostate-regenerating cell subpopulation that can initiate prostate tumorigenesis. *Proc Natl Acad Sci USA* 102:6942–6947
- Xin L, Lukacs RU, Lawson DA, Cheng D, Witte ON (2007) Self-renewal and multilineage differentiation in vitro from murine prostate stem cells. *Stem Cells*(Dayton, Ohio) 25:2760–2769
- Xue Y, Smedts F, Debruyne FM, de la Rosette JJ, Schalken JA (1998) Identification of intermediate cell types by keratin expression in the developing human prostate. *Prostate* 34:292–301
- Zhang L, Valdez JM, Zhang B, Wei L, Chang J, Xin L (2011) ROCK inhibitor Y-27632 suppresses dissociation-induced apoptosis of murine prostate stem/progenitor cells and increases their cloning efficiency. *PLoS One* 6:e18271



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