Scott D. Cramer *Editor*

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# Preface

Prostate cancer is common and kills people every day. Recently, understanding of the biology of adult tissue stem cells and their cognate cancers has identified striking similarities in normal stem cells and tumor-initiating cells, or the so-called cancer stem cells. The defining properties of a stem cell are self-renewal and multilineage differentiation. Many cancers possess tumor-initiating cells with these properties. Several groups have been investigating these principles in the prostate from multiple perspectives. *Stem Cells and Prostate Cancer* is meant to synthesize current directions in research on prostate stem cells and prostate cancer tumor-initiating cells.

Similarities between normal prostate stem cells and prostate tumor-initiating cells, for instance the ability for self-renewal and multilineage differentiation, have focused attention on normal stem cell biology. There are now very good data, summarized in this book, which demonstrate self-renewal and multilineage differentiation of populations of adult prostate cells from both mouse and humans. Although few studies have taken these experiments to clonal resolution, the mounting evidence is for one or more stem cell populations in the adult prostate. There is controversy regarding multiple aspects of prostate stem cell biology: Is it the cell of origin of prostate cancer or is it a more differentiated prostate cell that "gains" more stem-like properties, is there one prostate stem cell or multiple stem cells in different compartments, and what is the role of stem cells in castrate-resistant disease? These concepts and more are addressed by leaders in the field of *Stem Cells and Prostate Cancer*. The potential significance of the prostate stem cell in prostate cancer development and in the etiology of castrate-resistant disease makes this area of high clinical and translational significance for basic, translational, and clinical scientists interested in disease models.

The topics covered in *Stem Cells and Prostate Cancer* range from hormonal control of the prostate stem cell, methods of identification and characterization of prostate stem cells and prostate tumor-initiating cells, the role of the stem cell niche in differentiation, the tumor microenvironment, targeting the stem cell for prevention, and the use of stem cell models for validating prostate cancer genetics. The authors and topics were chosen to represent the spectrum of research in prostate stem cells from some of the best in the field. Each chapter represents a unique view on prostate

stem cells. In general, I have had a very light hand in editing these chapters so that the intent, tone, and perspective remain those of the contributing authors.

One underlying technique that is described in virtually all chapters is tissue recombination developed and refined by Jerry Cunha over several decades of pioneering research. In tissue recombination, fetal urogenital sinus mesenchyme, dissected from rodent embryos, is recombined with prostate epithelium and regrafted into a mouse host. This technique is described in multiple places in *Stem Cells and Prostate Cancer*. Originally these studies were used to demonstrate the instructive power of the mesenchyme in prostate epithelial development. Through multiple iterations of the model, this technique has guided our understanding of hormonal control of prostate development, endocrine targets in cancer, the contribution of tumor-associated fibroblasts to prostate cancer development and, most recently as described in this book, the use in evaluating prostate stem cells. The reader will find it clear that no definitive description of work on prostate stem cells is without discussion of the valuable contributions of tissue recombination to the field. My hat goes off to Jerry for his pioneering work that has facilitated our progress in the prostate stem cell field in uncountable ways.

Aurora, CO, USA Scott D. Cramer

# Contents

# Chapter 1
# Prostate Stem Cells, Hormones, and Development

**Gail S. Prins and Wen-Yang Hu**

**Abstract** While it is established that prostate cancer is a hormone-dependent disease, the cell(s) of origin of prostate cancer, i.e., the tumor-initiating cells, is still in debate. Strong evidence has emerged which indicates that prostate cancer can originate from both basal and luminal epithelial cell populations. In addition, prostate epithelial stem cells are candidates for the tumor-initiating cell based on work in hematopoietic and breast cancers and because of the growing acceptance of the cancer stem cell paradigm. To appreciate the interrelationships between the multiple cells of origin of prostate cancer, it may be necessary to first fully understand the prostate stem cell differentiation lineage during normal development and adult tissue maintenance as well as the factors that regulate stem cell self-renewal and lineage commitment. Recent advances in stem cell research have permitted isolation of prostate stem cells and shed light on the hierarchical relationship between the epithelial stem cells and their differentiated lineage. Furthermore, prostate cancer stem cells have been isolated and characterized from several prostate tumors which may provide an explanation for the known clinical and molecular heterogeneity of human prostate cancers. Although prostate stem cells and prostate cancer stem cells appear to be androgen receptor negative, new findings have established key roles for several other hormones in regulating prostate stem cells and their niche. Together, this new knowledge should allow for greater insight into the details of prostate development and to increased understanding of prostate cancer initiation and progression. In this chapter we will highlight recent advances in hormone modulation of prostate stem cells and their early progeny in development, normal tissue homeostasis, and cancer.

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## 1.1 Prostate Gland Development

The prostate gland develops embryologically from the endodermal urogenital sinus (UGS) under the influence of androgens produced by fetal Leydig cells upon chorionic gonadotropin stimulation. In humans, prostate development occurs during the second and third trimester and is complete at the time of birth (Lowsley 1912; Prins 1993). The prostatic portion of the urethra develops from the *pelvic* (middle) part of the UGS, and prostate development initiates when UGS epithelium in this region penetrates into the surrounding mesenchyme to form the primordial prostate buds. The glandular epithelium of the prostate differentiates from these endodermal UGS cells and the associated mesenchyme differentiates into the prostate stroma, which primarily contains fibroblasts and smooth muscle cells (Prins 1993; Donjacour and Cunha 1993, 1995; Prins and Putz 2008; Moore and Persaud 2008). While the human prostate does not consist of separate lobes, four morphologically distinct prostatic zones have been identified by McNeal. The prostatic urethra divides the prostate into an anterior fibromuscular zone and a posterior glandular portion that contains the peripheral, central, and transition zones (McNeal 1983).

A significant amount of information about prostate gland development at the morphologic, cellular, and molecular levels has been derived from studies using rodent models. In contrast to humans, the rodent prostate gland is rudimentary at birth and undergoes the majority of its development during the first 15 days of life. Although the developmental process is continuous, the development of the rodent prostate can be categorized into five distinct stages involving determination, initiation or budding, branching morphogenesis, differentiation, and pubertal maturation (Prins and Putz 2008). Determination of the prostate occurs before there is clear morphological evidence of a developing structure and involves expression of molecular signals that commit a specific field of UGS epithelial cells to a prostatic cell fate. Development of the prostate phenotype commences as UGS epithelial stem and progenitor cells form outgrowths or buds that penetrate into the surrounding UGS mesenchyme (UGM) in the ventral, dorsal, and lateral directions caudal to the bladder (Cunha et al. 1983; Cunha 1973, 1976, 1984). At birth, the ventral, dorsal, and lateral rodent prostate lobes primarily consist of unbranched, solid, elongating buds or ducts, and subsequent outgrowth and patterning occur postnatally (Fig. 1.1a). During this time, proliferation of epithelial cells occurs primarily at the leading edge of the ducts (i.e., distal tips) (Prins et al. 1992). Branching morphogenesis begins when the elongating UGS epithelial buds contact the prostate mesenchymal pads that are peripheral to the periurethral smooth muscle. At that point, secondary, tertiary, and further branch points are established with continued outgrowth in the proximal-to-distal direction and with increased complexity (Hayashi et al. 1991; Timms et al. 1994). Epithelial and mesenchymal cell differentiation is temporally coordinated with branching morphogenesis. Lumenization of the solid epithelial cords begins in the proximal ducts and spreads to the distal tips, occurring concomitantly with epithelial differentiation into separate basal and luminal cell layers to form a simple columnar epithelium (Fig. 1.1b).

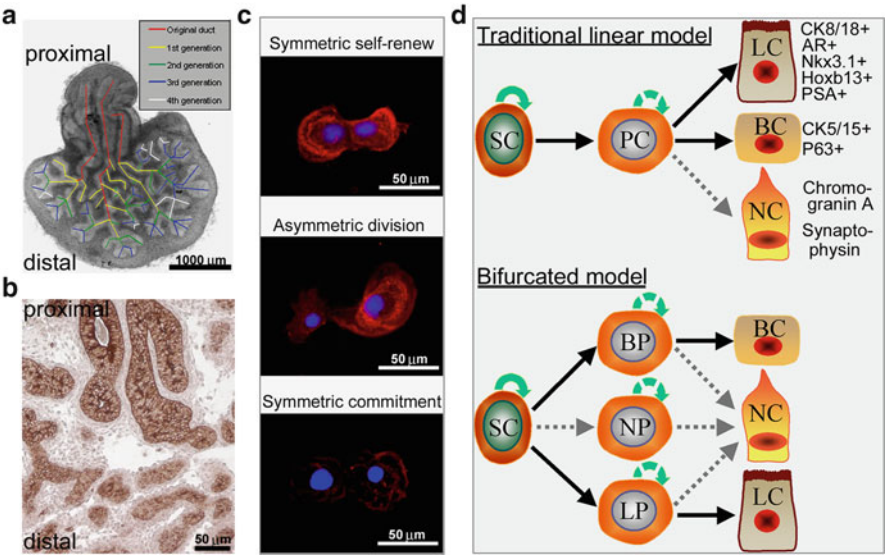


**Fig. 1.1** Prostate development and stem cells hierarchy. (**a**) Branching morphogenesis of rat ventral prostate lobe. The gland was removed at birth and cultured for 90 h as described (Huang et al. 2009). Images were taken every 30 min to track the branching events and a color-coded skeleton is used to indicate the generation of branches according to the following convention: The three primary buds that emerged from the UGS are considered the original ducts (*red*), branches that formed off the primary ducts are considered the first generation (*yellow*), and branches that formed off the first generation and elongate are considered the second generation (*green*), third generation (*blue*), and fourth generation (*white*). (**b**) Day 4 rat ventral prostate immunostained for CK8/18 reveals the proximal-to-distal spread of epithelial differentiation and lumen formation. Ducts in the proximal region of the gland have initiated lumenization concomitant with differentiation of luminal cells positive for CK8/18. In contrast, distal regions contain solid epithelial ducts with minimal luminal cell differentiation. (**c**) Immunolabeling of human prostate stem cells (isolated from day 4 prostaspheres) with stem cell marker CD49f identifies three types of stem cell divisions: (1) symmetric renewal, one stem cell gives rise to two identical daughter stem cells ($CD49f^{high}$); (2) asymmetric division, one stem cell gives rise to one daughter stem cell ($CD49f^{high}$) and one daughter progenitor cell ($CD49f^{low}$); and (3) symmetric commitment, one stem cell gives rise to two differentiated daughter cells ($CD49f^{low}$). (**d**) Prostate stem cell hierarchical models: in the traditional linear hierarchy model (*top*), self-renewing prostate stem cells (SC) give rise to intermediate, transit-amplifying progenitor cells (PC). These cells have high proliferative capacity and enter differentiation pathways to give rise to terminally differentiated luminal cells (LC), basal cells (BC), and neuroendocrine cells (NC). In the bifurcated model (*bottom*), a common SC with self-renewal capacity undergoes asynchronous cell division to give rise to lineage-restricted basal progenitor cells (BP) and luminal progenitor cells (LP). These PCs possess transient self-renewal capacity and terminally differentiate into basal and luminal epithelial cells. The lineage of neuroendocrine cells (NC) is unclear and may arise from the hierarchical intermediate BP and LP that produce BC and LC, or it may have a separate neuroendocrine progenitor (NP) origin

Mature prostate ducts contain three phenotypically and functionally distinct epithelial cell types (basal, luminal, and neuroendocrine cells) embedded in a fibromuscular stroma (Cunha et al. 1983, 1987; Cunha 1973; Long et al. 2005; Prins 1993; Prins and Putz 2008). Prostate basal epithelial cells are located adjacent to the

basal lamina and express p63, cytokeratin (CK) 5, and CK 14. They are largely androgen receptor (AR) negative and are independent of direct androgen action for survival (Prins and Birch 1995). Luminal epithelial cells, which comprise the majority of the prostate epithelium, are cuboidal and short columnar exocrine cells with an apical surface towards the ductal lumen. They are characterized by the expression of CK8/18, NKX3.1, and AR (Bhatia-Gaur et al. 1999; Hayward et al. 1996; Isaacs et al. 1981; Robinson et al. 1998; Wang et al. 2001a). Luminal cells are dependent on androgens for viability and function, producing prostatic secretory proteins such as prostate-specific antigen (PSA), prostatic acid phosphatase (PAP) in humans, and prostate-binding protein (PBP) in rodents. Neuroendocrine cells are dendritic-like intraepithelial regulatory cells with a hybrid phenotype having both neural and epithelial characteristics. They are a minor population scattered throughout the basal layer and are identified by the expression of neuroendocrine markers chromogranin A and synaptophysin (Rumpold et al. 2002).

## 1.2 Prostate Epithelial Stem Cells and Lineage Hierarchy

Adult prostate stem cells have been identified in human and rodent prostate glands where they play an essential role in tissue replenishment throughout life (Bhatt et al. 2003; Garraway et al. 2010; Goldstein et al. 2008; Isaacs 2008; Kasper 2007; Lawson et al. 2010; Leong et al. 2008; Liu et al. 2011; Xin et al. 2007). This rare cell type self-renews and has potential to differentiate into the three distinct epithelial cell types, essential characteristics of bonafide stem cells. Prostate stem cells are relatively growth quiescent, occasionally dividing to self-renew and generate daughter progenitor cells. Studies across multiple systems as well as the prostate epithelium have characterized three types of stem cell divisions (Fig. 1.1c): (1) symmetric division, aka symmetric self-renewal, which generates two identical stem cells; (2) asymmetric division which generates a single self-renewing stem cell and a daughter cell that has entered the earliest stage of differentiation (progenitor cell); and (3) symmetric commitment division whereby a stem cell produces two daughter progenitor cells (Morrison and Kimble 2006; Scaffidi and Misteli 2011; Tomasetti and Levy 2010; Wu et al. 2007). Unlike the stem cell, the daughter progenitor cell has transit-amplifying capacity through rapid cell divisions. As the progenitor cell proliferative potential is exhausted, it undergoes terminal differentiation.

While prostate stem cells are present in several regions of rodent prostatic ducts, accumulated prostate stem cells with considerable growth potential have been found in the proximal region of ducts close to the UGS, and the survival of these cells does not require the presence of androgens (Tsujimura et al. 2002; Goto et al. 2006). Primitive proximal prostate stem cells that are able to regenerate functional prostatic tissue *in vivo* are also programmed to reestablish a proximal-distal ductal axis. In contrast, prostate stem cells in the distal region of the prostate duct have more limited growth potential and require androgens for survival (Goto et al. 2006). See Chapter 6 for a more complete discussion of the prostate stem cell niche.

Although the lineage hierarchy for the prostate epithelium has not been settled, epithelial differentiation of stem cells into differentiated basal, luminal, and neuroendocrine cells has been documented in the rodent prostate and in isolated human prostate cancer stem cells (Hudson 2004; Isaacs 2008; Kasper 2007; Long et al. 2005; Robinson et al. 1998; Wang et al. 2001a, b). These studies on stem cell differentiation have been observed with changing patterns of cytokeratins, cell-specific markers, and alterations in AR expression, an early marker of luminal epithelial cell differentiation. Two models have been proposed for the prostate epithelial stem cell lineage into differentiated basal, luminal, and neuroendocrine cell types (Fig. 1.1d). In the traditional linear hierarchy model, self-renewing prostate stem cells residing in the basal cell layer undergo asymmetric cell division giving rise to daughter progenitor cells with high proliferative potential, aka the transit-amplifying cells. In response to signals from the stem cell niche, these cells enter early differentiation pathways to eventually form separate basal, luminal, and neuroendocrine cells (Hudson 2004; Hudson et al. 2000; Isaacs and Coffey 1989). Phenotypic intermediate-type cells that co-express basal and luminal markers have been observed both *in vitro* and *in vivo* (Garraway et al. 2003; Long et al. 2005; Prins et al. 1995; Robinson et al. 1998). This suggests that basal and luminal cells are hierarchically related through common progenitor cells that give rise to differentiated basal cells and luminal cells. In the bifurcated model, basal cells and luminal cells represent separate epithelial cell lineages that originate from a common stem cell. These lineages may be sustained by intermediate transit-amplifying cells and/or lineage-restricted basal and luminal cell progenitors (Hudson 2004; Long et al. 2005; Wang et al. 2001a). The lineage of neuroendocrine cells is unclear. Neuroendocrine cells may arise from the hierarchical prostate epithelial stem and progenitor cells that produce basal and luminal cells, or they may have a separate progenitor cell origin as shown in the bifurcated model.

## 1.3 Prostate Stem Cell Isolation and Characterization

It is widely accepted that adult stem cells are involved in normal tissue maintenance throughout life while cancer stem cells support cancer growth (Presnell et al. 2002; Smith et al. 2007). Although the cell(s) of origin for prostate cancer may include luminal, basal, neuroendocrine, progenitor, and stem cells (Goldstein et al. 2010a, b; Kasper 2008, 2009; Wang and Shen 2011), it is increasingly evident that the resultant prostate cancers contain cancer stem cells that continuously seed and maintain tumor growth (Gu et al. 2007; Maitland et al. 2011). While conventional therapies for prostate cancer eradicate the majority of cells within a tumor, most patients with advanced cancer eventually progress to androgen-independent, metastatic disease that remains essentially incurable by current treatment strategies. Recent evidence has shown that cancer stem cells are a subset of tumor cells that appear to be therapy-resistant and are responsible for maintaining cancer growth which may be the underlying cause of disease relapse (Cocciadiferro et al. 2009;

Maitland and Collins 2008; Miki and Rhim 2008; Oldridge et al. 2012; Wang et al. 2012). Thus understanding the regulation of both normal stem cells and cancer stem cells may provide new insight into the origin and treatment of prostate cancer. Towards this end, identification and characterization of these rare cell populations has been a major research effort during the past decade with marked progress being realized utilizing flow cytometry and prostasphere culture (Garraway et al. 2010; Liu and True 2002; Xin et al. 2005).

Flow cytometry sorts cell populations by their specific cell surface CD markers, and expression profiles of CD markers have been extensively described for both normal and neoplastic prostate cell types (Liu et al. 2004; Liu and True 2002). Importantly, multiple CD molecules are enriched in prostate stem cells including Sca-1 (Xin et al. 2005), α2β1 integrin (Collins et al. 2001), CD133 (Richardson et al. 2003; Vander Griend et al. 2008), CD44 (Liu et al. 2004), CD117 (Leong et al. 2008), CD49f, and Trop2 (Garraway et al. 2010; Goldstein et al. 2008, 2010a). Combinations of antibodies specific for these markers have been used to isolate stem-like cells by FACS from dissociated prostate tissues or epithelial cell cultures, and their stem cell capabilities have been tested using various *in vivo* systems (Goldstein et al. 2008; Guo et al. 2012; Leong et al. 2008). An example of this approach using 2-channel flow cytometry from primary prostate epithelial cell cultures is shown in Fig. 1.2a. It is important to note, however, that there is no current consensus on the antigenic profile required for isolating a pure stem cell population from prostate epithelium by flow cytometry. The disadvantages of FACS include relative low cell yield when using multiple stem cell markers and cell damage following dissociation, labeling, and sorting.

Side-population analysis utilizing flow cytometry in combination with functional properties of stem cells is a convenient tool to characterize stem-like cells within mixed epithelial cell populations. Stem cell side-populations were first identified in hematopoietic stem cells enriched from heterogeneous cell populations based upon their unique ability to actively extrude Hoechst 33342 (Brown et al. 2007; Goodell et al. 1996). ABCG2 is a member of the family of ATP-binding cassette (ABC) transporters, and it pumps several endogenous and exogenous compounds out of cells including Hoechst 33342. Widely expressed in a variety of stem cells, ABCG2 was found to be a molecular determinant of the side-population phenotype and is recognized as a universal marker of stem-like cells (Ding et al. 2010; Zhou et al. 2001). The side-population assay, based on exclusion of Hoechst dyes, has proven to be a valuable method for identifying and sorting stem and early-stage progenitor cells in a variety of tissues and species. Application of this approach to assess putative prostatic stem cell numbers from heterogeneous prostate epithelial cell populations is shown in Fig. 1.2b (Bhatt et al. 2003; Brown et al. 2007; Mathew et al. 2009).

Another approach for enrichment and characterization of prostate stem cell populations is the prostasphere assay which utilizes a three-dimensional (3D) culture system to form spheroid structures (Garraway et al. 2010; Hu et al. 2011, 2012; Lukacs et al. 2010; Xin et al. 2007). First used for the isolation and characterization

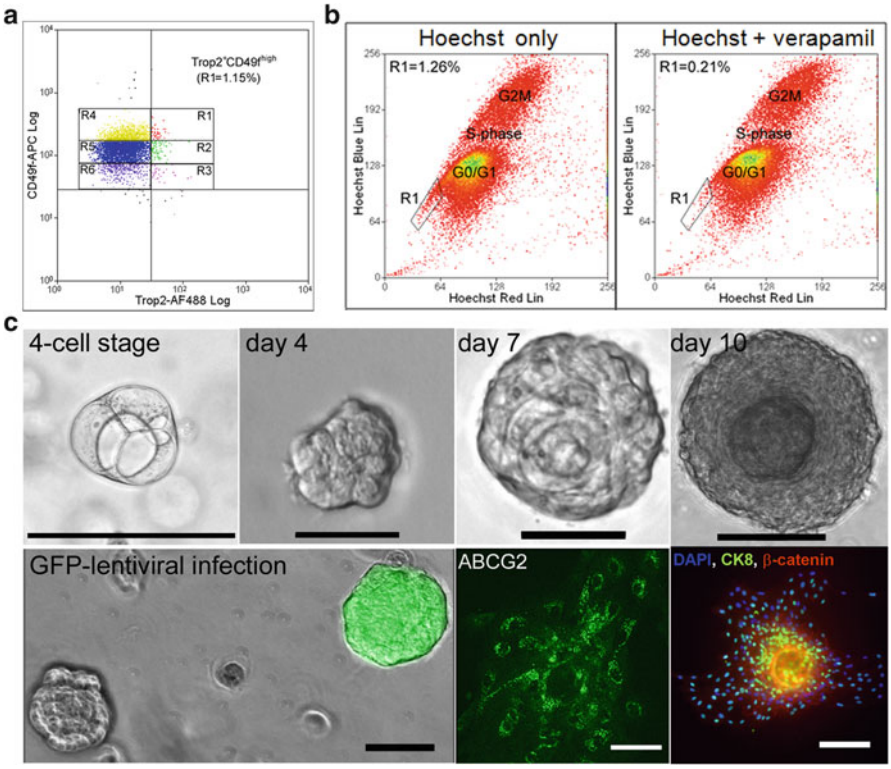


**Fig. 1.2** Methodological approaches for prostate stem cell isolation and characterization. (**a**) Flow cytometry analysis of normal human prostate epithelial cells (PrEC) from primary cell culture following labeling with Trop2-AF488 and CD49f-APC antibodies. A subpopulation of $Trop2^{+}CD49f^{high}$ (R1 = 1.15%, *red*) represents prostate stem cells. (**b**) Hoechst 33342 dye efflux fluorescence-activated flow cytometry analysis reveals a side population in human PrEC (gated as *R1*) that actively excludes the dye. PrEC were stained with 5 μg/mL of Hoechst 33343 either in the absence or presence of 50 μM of verapamil hydrochloride, an ABCG2 inhibitor. Windows for the side population are determined by comparison of cells without and with verapamil in each FACS analysis. (**c**) Prostate stem/progenitor cells isolation using prostasphere assay. Human PrEC from disease-free organ donors were established in primary 2D cultures and transferred to 3D Matrigel-slurry culture as described (Hu et al. 2011). Under these conditions, ~0.2–1% of primary PrEC cells (stem cell population) survive and undergo self-renew to form spheroid structures termed prostaspheres. By day 4 of culture, prostaspheres 30–40 μm in diameter are visible, increasing in size through transit amplification to 60–100 μm by day 7. To confirm clonality of the spheroids, mixed primary PrEC cells with or without lentiviral-GFP were transferred to 3D Matrigel cultures. At day 7, formed prostaspheres were either entirely GFP+ or GFP− (*bottom left*), indicating the clonal origin of prostaspheres. Day 7 prostasphere cells express multiple prostate stem cell markers (Hu et al. 2011) including the transporter protein ABCG2 (*bottom middle*). By day 10 of culture, prostaspheres grow >150 μm in diameter and form a visible double layer of cells (*top right*). Immunocytochemistry of a day 10 prostasphere shows central cells as differentiating $CK8^{+}$ (*green*) luminal cells (*lower right*) and peripheral cells $p63^{+}$ basal cells (Hu et al. 2011). Bar = 50 μm

of neural stem cells, it is widely accepted that only stem-like cells have the capacity to survive and proliferate to form spheroids in 3D culture. Using a Matrigel-slurry culture system in our laboratory, 0.2–1% of 2D cultured primary prostate epithelial cells (PrEC) are capable of survival and proliferation to form free-floating prostaspheres that are clonal in origin (Fig. 1.2c). Immunofluorescent labeling with multiple prostate stem cell markers confirms their stemness characteristics (Hu et al. 2011). That these spheroids consist of stem cells is best demonstrated by their ability to form fully differentiated and functional human prostate basal and luminal cells *in vivo* when reconstituted with inductive UGM (Hu et al. 2011). This prostasphere culture system closely mimics the *in vivo* situation as the cells are grown in a suspended semisolid gel, which allows the development of intercellular interactions. Several key variables contribute to the formation of these prostaspheres from PrEC including the age of the prostate donor, cell plating density, culturing techniques, and passage number, all of which influence the homogeneity or heterogeneity of the spheroids. The major advantages of the prostasphere assay are the functional isolation of prostate stem cells, expansion of the stem cell numbers *in vitro,* and the ability to manipulate them *in vitro* which provides research opportunities to identify regulation of stem and progenitor cell proliferation and differentiation.

At early stages of formation, the prostaspheres consist of stem-like cells undergoing synchronous self-renewal and asynchronous cell division to generate daughter early-stage progenitor cells that have not yet differentiated along cell lineages (Fig. 1.1c). By days 3–4 of culture, prostaspheres that are ~30 μm in diameter and consist of 20–40 cells are visible to the naked eye. Through rapid cell proliferation, they continue to grow with diameters reaching ~80–100 μm by day 7 (Fig. 1.2c). At this stage, cell markers indicate that the majority of cells express Nanog, Trop2, CD49f$^{high}$, ABCG2, CD133, CD44, and SSEA4 with no immunostaining for p63, CK8, NKX3.1, and HOXB13 suggesting that the day 7 spheroid cells consist of prostate stem and progenitor cell populations. Gene expression analysis of day 7 spheroids by real-time qRT-PCR supports their stem/progenitor cell status with lack of luminal cell gene expression (Fig. 1.3). Interestingly, although the cells are p63 negative by immunocytochemistry, p63 mRNA levels similar to parental PrEC cells are observed suggesting that early differentiation towards a basal cell lineage has initiated. With continued culture through day 10 to day 30, spheroid cells undergo cytodifferentiation, forming double-layered prostaspheres with sizes of 150–200 μm (Fig. 1.2c). Immunostaining of day 10 prostaspheres reveals that peripheral cells are p63-positive basal-type cells while centrally located cells are positive for CK8/18 and NKX3.1 indicating their differentiation towards a luminal phenotype (Fig. 1.2c). With continued culture under basal conditions through day 30, prostaspheres form branching-type structures and undergo functional differentiation as indicated by PSA gene induction (Hu et al. 2011). Furthermore, their growth and differentiation can be driven by various conditions including coculture with stromal cells and treatment with differentiating factors such as hepatocyte growth factor (HGF) (Schalken 2007) or hormones as described below.

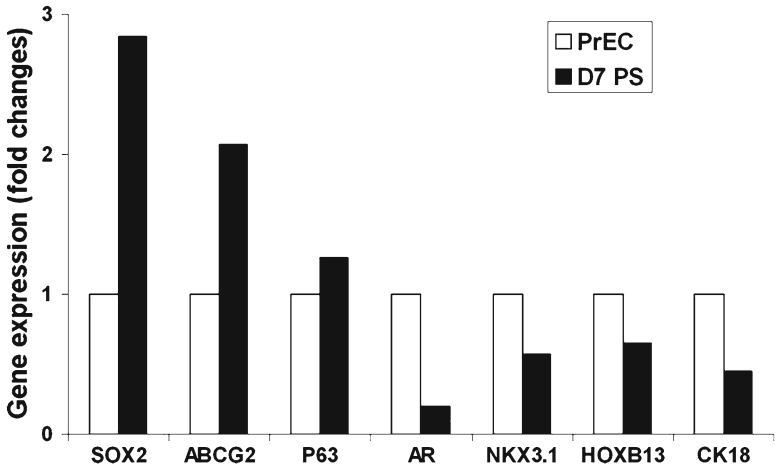


**Fig. 1.3** Day 7 Prostasphere gene expression analyzed by real-time qPCR. Relative to the normal parental PrEC cells in 2D primary culture, day 7 prostasphere cells express increased levels of prostate stem cell markers Sox2, ABCG2, and basal cell marker p63 and low to negligible (<30–35 Ct cycles) levels of luminal cell differentiation markers including AR, NKX3.1, HOXB13, and CK18

## 1.4 Hormone Receptor Expression and Hormonal Regulation of Prostate Stem and Progenitor Cell Self-Renewal and Differentiation

Androgens are essential for prostate gland development and maintenance throughout life and are believed to play central roles in prostate cancer initiation and progression. Despite this, prostate epithelial stem and early progenitor cells are AR negative (Hu et al. 2011; Kasper 2009; Oldridge et al. 2012) and are thus not directly regulated by androgen action. As a result, any effects of androgens on prostate epithelial stem cell homeostasis and differentiation are most likely mediated through indirect actions on the stem cell niche which includes $AR^+$ stromal cells and, in the mature prostate, $AR^+$ luminal epithelial cells (Berry et al. 2008). Androgens have been shown to influence the secretion of multiple paracrine-acting factors by these cells during prostate development and in the adult tissue that may influence the stem cell niche including *Fgf*s, *Shh*, and *Wnt*s (Prins and Putz 2008). While several studies have shown that prostate cancer stem-like cells are similarly AR negative (Kasper 2009; Oldridge et al. 2012), there are scattered reports on direct androgen action and AR protein in prostate cancer-initiating cells and prostate cancer stem cell subpopulations (Sharifi et al. 2008; Vander Griend et al. 2010). In addition to androgens, a number of other hormones are known to regulate prostate growth and function and to influence growth and progression of prostate cancer including estrogens (Prins and Korach 2008), retinoids (McCormick et al. 1999; Schenk et al. 2009), prolactin (Dagvadorj et al. 2007), growth hormone, and IGF-1 (Chan et al. 1998; Wang et al. 2005). Further, there is

clear evidence that mammary gland stem cells and daughter progenitors are under direct regulation by several of these hormones (Asselin-Labat et al. 2010; Joshi et al. 2010). In this context, we investigated whether prostate stem and progenitor cells express other hormone receptors and respond to the non-androgenic hormones that are known to influence the prostate gland.

Past research on estrogen action in the prostate gland has focused entirely on estrogen receptor (ER)α, ERβ, and G protein-coupled receptor 30 (GPR30) within differentiated stromal, basal, and luminal cells. It is noteworthy that the different ERs within these cell types have apparent opposing actions; stromal cell ERα has proliferative and cancer-promoting actions (Ricke et al. 2008; Sissung et al. 2011) while ERβ in basal and luminal epithelial cells has antiproliferative and proapoptotic activity (McPherson et al. 2010). GPR30, expressed at the plasma membrane and endoplasmic reticulum and activated by estradiol, initiates growth arrest and induces necrosis in prostate cancer cells (Chan et al. 2010). Recently, our laboratory discovered that human prostate epithelial stem and progenitor cells from disease-free prostates express robust levels of ERα, ERβ, and GPR30 mRNA and protein (Hu et al. 2011). Further, prostaspheres cultured from primary PrEC in 1 nM estradiol-17β ($E_2$) exhibited a marked increase in spheroid size and number (Hu et al. 2011) with elevated expression of multiple stemness genes at day 7 of culture as compared to vehicle alone (Fig. 1.4a). Using a side-population analysis of primary PrEC, we noted a biphasic effect of estradiol with increased stem cell numbers at 1–10 nM $E_2$ but limited stimulation at higher doses (Fig. 1.4b). Taken together, these findings implicate prostate stem/progenitor cells as direct estrogen targets and indicate that estrogens support stem cell self-renewal and progenitor amplification and maintain their stemness state within the prostate gland. Moreover, these results raise the intriguing possibility that prostate stem and early progenitor cell populations may be susceptible targets of elevated estrogen levels in aging men (Vermeulen et al. 2002).

To evaluate whether prostate cancer stem-like cells may likewise express ERs and respond to estrogens, we examined ERα, ERβ, and GPR30 expression in stem and progenitor cells from prostate cancer specimens (Fig. 1.4c). Prostaspheres were cultured from primary prostate cancer cells (PCa-E) and matched benign prostate epithelial cells (EPZ) from the same patient at prostatectomy (kindly supplied by Dr. L Nonn, University of Illinois at Chicago). The PCa-E cells were cultured from pathologically confirmed cores containing >80% cancer cells and expressed significantly elevated AMACR and reduced NKX3.1 as compared to the EPZ cells. As shown in Fig. 1.4c, there was a sixfold increase in ERα mRNA and 8–12-fold increase in GPR30 expression in both the patient benign and cancerous prostasphere cells as compared to spheroids grown from normal donor PrEC. For ERβ, there was a fourfold increase in the PCa-E-derived stem/progenitor cells but not the benign EPZ cells relative to normal donor PrEC expression. Since the prostaspheres from PCa-E were mixed stem and progenitor cells that are not confirmed as prostate cancer stem-like cells, ERs were also evaluated in two human prostate cancer stem-like cell lines, HPET (Gu et al. 2007) and HuSLC (kindly supplied by Dr. S. Kasper, University of Cincinnati). Each cell line was generated from separate Gleason score 9 tumors, spontaneously immortalized and is capable of fully reestablishing the

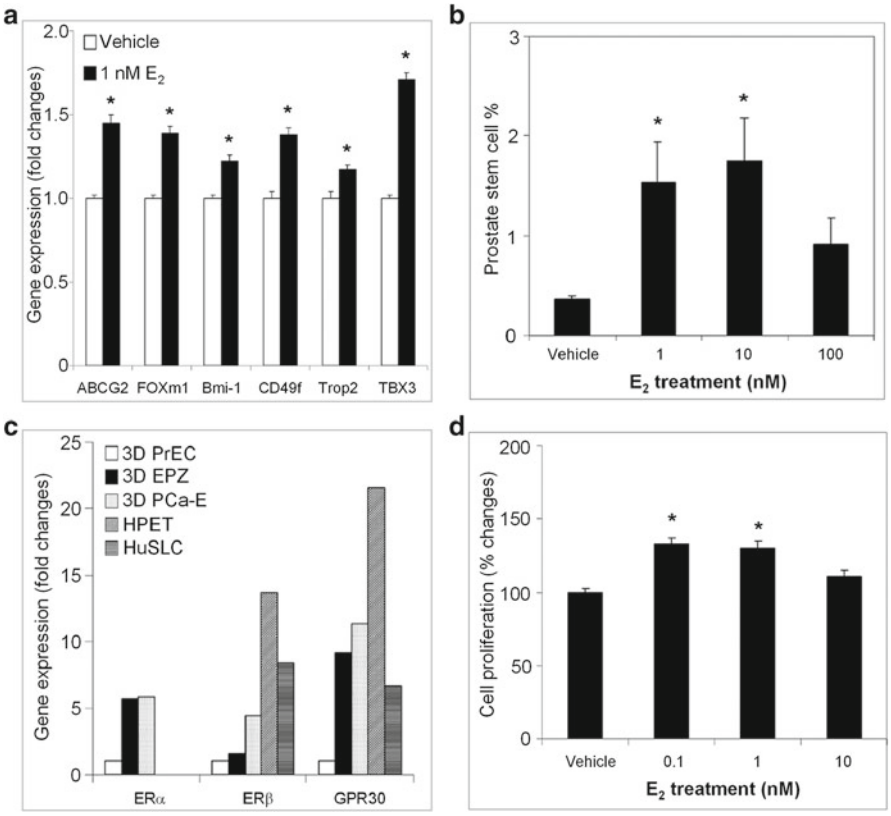


**Fig. 1.4** Modulation of prostate stem and progenitor cell populations by estrogen. (**a**) Prostaspheres were cultured from disease-free primary epithelial cells in the absence or presence of 1 nM of estradiol-17β ($E_2$) for 7 days, and gene expression was evaluated by qRT-PCR. $E_2$ significantly increased mRNA levels of stem cell markers ABCG2, FOXm1, Bmi-1, CD49f, Trop2, and TBX3. $^*P<0.05$ vs. vehicle; $n=4$. (**b**) 2D primary prostate epithelial cells from disease-free donors were cultured for 72 h in 1, 10, or 100 nM $E_2$, and the Hoechst 33342 exclusion based side-population analysis by flow cytometry was used to measure the percentage of stem-like cells. $^*P<0.05$ vs. vehicle by ANOVA; $n=6$. (**c**) ER expression by q RT-PCR in normal prostate stem-like cells and prostate cancer stem-like cells. A 3D prostasphere assay was used to isolate and amplify the stem/early-stage progenitor cell populations from disease-free primary prostate epithelial cultures (PrEC), matched prostate epithelial primary cultures from benign regions (EPZ) and prostate cancer cores with >80% cancer cells from the same patient (PCa-E). HPET and HuSLC are two human prostate cancer stem-like cell lines established from Gleason score 9 human prostate cancer (kindly supplied by Dr. Susan Kasper). Data is normalized to ER expression levels in PrEC-derived prostaspheres which was set as 1. (**d**) HuSLC cells were cultured in the absence or presence of 0.1–10 nM of $E_2$ for 72 h. Cell proliferation was evaluated by MTT assay. Treatment of 0.1 and 1 nM of $E_2$ significantly increased the HuSLC cell proliferation. $^*P<0.05$ vs. vehicle; $n=4$

original tumors *in vivo*. While both prostate cancer stem-like cell lines were negative for ERα, they expressed 10–15-fold higher ERβ and 7–20-fold higher GPR30 levels compared to prostasphere cells from normal prostate epithelium (Fig. 1.4c).