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Exploring the Origins of the Normal Prostate and Prostate Cancer Stem Cell

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Abstract Prostate epithelial stem cells (PSCs) are primed by the urogenital mesenchyme to initiate bud formation and branching morphogenesis, ultimately culminating in a glandular structure composed of luminal, basal and neuroendocrine cells. Identity of this cell has remained elusive however cell populations enriched for cells exhibiting stem cell characteristics express the stem cell markers $CD133^{+}$, $\alpha 2\beta 1^{hi}$, CD44 and Sca-1 along with embryonic stem cell factors including Oct-1, Nanog, Sox2 and nestin. Androgens are critical to prostate organogenesis and play a major role in normal prostate function and the development of prostate cancer. Cell lineage is another variable in the development of prostate cancer. This review discusses the embryonic prostate stem cell niche, normal prostate development, isolation and characterization of normal prostate and prostate cancer stem cells, and current concepts on the origin of prostate cancer.

Keywords Stem cells · Cancer stem cells · Prostate · Review

Introduction

Stem cells sit at the top of the lineage hierarchy. They are self-renewing and multipotential, proliferating and capable of differentiating into the cell types that make up an organ. The prostate epithelial stem cell differentiates into three functionally distinct lineages; basal, luminal secretory and neuroendocrine. Basal cells express high molecular weight cytokeratins (CK) such as CK5 and CK14. They underpin the luminal cells which are characterized by the expression of low molecular weight CK, including CK 8 and CK18. Luminal cells produce prostatic secretions and prostate specific antigen (PSA), processes which are often regulated by androgens. Neuroendocrine cells produce neuropeptides including serotonin, bombesin, calcitonin and somatostatin. Luminal cells express the androgen receptor (AR).

Positive identification of the normal prostate stem cell and the prostate cancer stem cell (CSC) remain elusive. Analogous to normal PSCs, prostate CSCs likely possess the ability to self-renew and differentiate into the heterogeneous lineages of cancer cells which characterize a tumor [1]. They are thought to arise during the process of aging where they acquire epigenetic modifications and genetic mutations that result in dysregulation of normal cell processes while maintaining a self-renewal phenotype [2, 3]. Cell lineage is key to the development of prostate cancer since >95–99% of human PCa is derived from the luminal cell lineage. Less than 1% to 5% of PCa is derived from the neuroendocrine cell lineage and PCa derived from the basal cell lineage has not been reported. These observations imply that luminal secretory cells are more sensitive to the local microenvironment and more susceptible to epigenetic modifications and genetic mutations through exposure to insults such as oxidative stress and DNA damage.

The following review summarizes what is currently known about the embryonic prostate stem cell niche and prostate development, discusses approaches utilized to isolate and characterization of normal PSCs and CSCs, and presents current concepts on the origin of prostate

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CSCs and their implication in the development of prostate cancer.

The Embryonic Prostate Stem Cell Niche

Little is known of the embryonic prostate stem cell (PSC) niche. In the 9-week old embryonic human prostate, condensation of the mesenchyme surrounding the urogenital sinus at the level of the mesonephric duct openings is the first sign of prostate development [4]. Epithelial bud formation occurs in week 10 [5]. Although mesenchymal cells appear to have close contact with the basal lamina, the basement membrane remains continuous and separates the epithelium from the mesenchyme [4]. Later in development, gaps in the basement membrane occur, allowing contact between the outgrowing epithelium and the underlying mesenchyme [6, 7]. In parallel, the testicular Leydig cells mature, exhibit characteristics of steroid-secreting cells, implying that androgens regulate prostate development [4, 8]. Thus, the human prostate embryonic niche requires signals from the mesenchyme and testicular androgens to initiate prostate development and facilitate epithelial cell differentiation.

In the rodent, prostate organogenesis is also initiated by mesenchymal cells of the embryonic urogenital sinus [9]. Identity of the mesenchymal cells which initiate organogenesis and the epithelial cells which respond to these signals has remained elusive. Nuclear androgen receptor (AR) labeling was initially detected in mesenchymal cells between embryonic (E) day E14.5 and E16.5, whereas epithelial AR expression appeared at 10 days postnatally with the onset of differentiation [10]. By 10 weeks of age, epithelial AR labeling became more prominent while mesenchymal labeling declined [10]. In the testicular feminization mouse model (Tfm/Y), a defective androgen receptor blocked androgen signaling in the developing male organs, causing the Wolffian ducts to degenerate and a female-like ureter, a shortened vagina, and external female genitalia to develop [11]. Thus, prostatic structures did not develop in Tfm/Y males. Tissue recombination studies demonstrated that wild type mesenchyme recombined with Tfm/Y epithelium resulted in prostatic induction and ductal development [11] however, secretory function was impaired [12]. In contrast, Tfm/Y mesenchyme recombined with wild type epithelium histologically developed into tissue with vaginal-like characteristics and loss of androgen-regulated protein expression [13]. Collectively, these studies suggest that organogenesis and outgrowth of the prostatic buds is mediated by signals from AR^+ mesenchymal cells. Postnatally, epithelial AR regulates the final stages of differentiation and secretory function of the prostatic epithelium.

Human Prostate Development

Epithelial bud formation occurs during week 10 of development and the beginning of cannulization at the proximal ends is observed by the end of week 11 [6]. Initially, the epithelium resembles that of the stratified urethral epithelium; however they appear to contain more organelles [6]. Cells with neuroendocrine characteristics also appear in the basal regions of the buds [6, 14]. The number of epithelial outgrowths increases between weeks 11 and 14, developing into tubulo-acinar glands [6]. At this stage, they consist of three to five layers of cells, most of which are apolar and round in shape. The prostatic epithelium expresses primarily non-secretory acid phosphatase in lysosomes at week 10 [5, 15]. However, some cells contain acid phosphatase-positive apical granules, suggesting the beginning acid phosphatase secretion [5, 15]. Aumüller et al. did not observe secretory acid phosphatase or prostate specific antigen (PSA) expression in the prenatal prostate [14]. The secretions also stain positive for PAS and Alcian blue, indicating that epithelial cells synthesize neutral and acidic mucopolysaccharides [14]. Limited polarization of apical cells and the appearance of dense secretory granules occur at week 13, and during weeks 15 to 16, epithelial outgrowth increase [6]. The basal lamina becomes discontinuous in some places and presumably under the influence of the mesenchyme, epithelial cell differentiation occurs [6]. Triangular-shaped cells similar to postnatally-mature basal cells appear in the basal region of the gland [6, 14]. Connective tissue surrounding the prostatic epithelium increases and the inner zone forms the lamina propria of the acinar epithelium [6].

Immature prostatic glands are retained until the onset of puberty [14]. Stratified epithelium in the fetal and prepubertal human prostate appears to stain positive for all cytokeratins until after puberty when only secretory (or luminal) and basal cells are present [14, 16]. Intermediate forms between basal and luminal cells were not observed in the developing human prostate [14]. By 14 years of age, secretory PAP and PSA immunoreactivity are detected in prostatic epithelium and PAS-positive cells gradually become less and are absent in the normal adult prostate [4, 17].

Rodent Prostate Development

Unlike the human prostate which is organized into a central, peripheral and transitional zone surrounded by a thick non-muscular stromal capsule [18], the rodent prostate consists of paired anterior (or coagulating), dorsal, lateral and ventral lobes which surround the urethra. However, rodent prostate development follows a similar

pattern of differentiation with a differentiation gradient extending from proximal end to distal tips of the gland. On embryonic day 16 (E16), the urogenital sinus is committed to form prostate and the prostate buds are visible by E18.5 [19]. In rats, the solid cords of cuboidal epithelial cells protrude as paired lobes from the urethral epithelium into the mesenchymal pad. Cannulization of the epithelium occurs between 7 and 17 days postnatally and secretory granules are first observed during weeks 2 and 3 [4]. During the second week, mesenchymal cells adjacent to the basal lamina differentiate into fibroblasts whereas the peripherally located cells differentiate into smooth muscle cells [4]. Cessation of outgrowth and maturation of the prostate gland is complete at 5 weeks of age [20].

Morphological studies of the adult rodent prostate by Sugimura et al. [20] and Lee et al. [21] have shown that the prostatic lobes are tree-like branching structures which can be subdivided into proximal, intermediate and distal segments. Distal epithelium consisted of tall columnar cells which could undergo mitosis, intermediate epithelium were tall columnar, secretory cells and distal epithelial was composed of low columnar or cuboidal cells which stained positive for cathepsin D, a marker for apoptosis. It has been postulated that to maintain epithelial cell number, cells proliferating in the distal segments would migrate down the branching structures toward the proximal region to replace those cells undergoing apoptosis [20, 21].

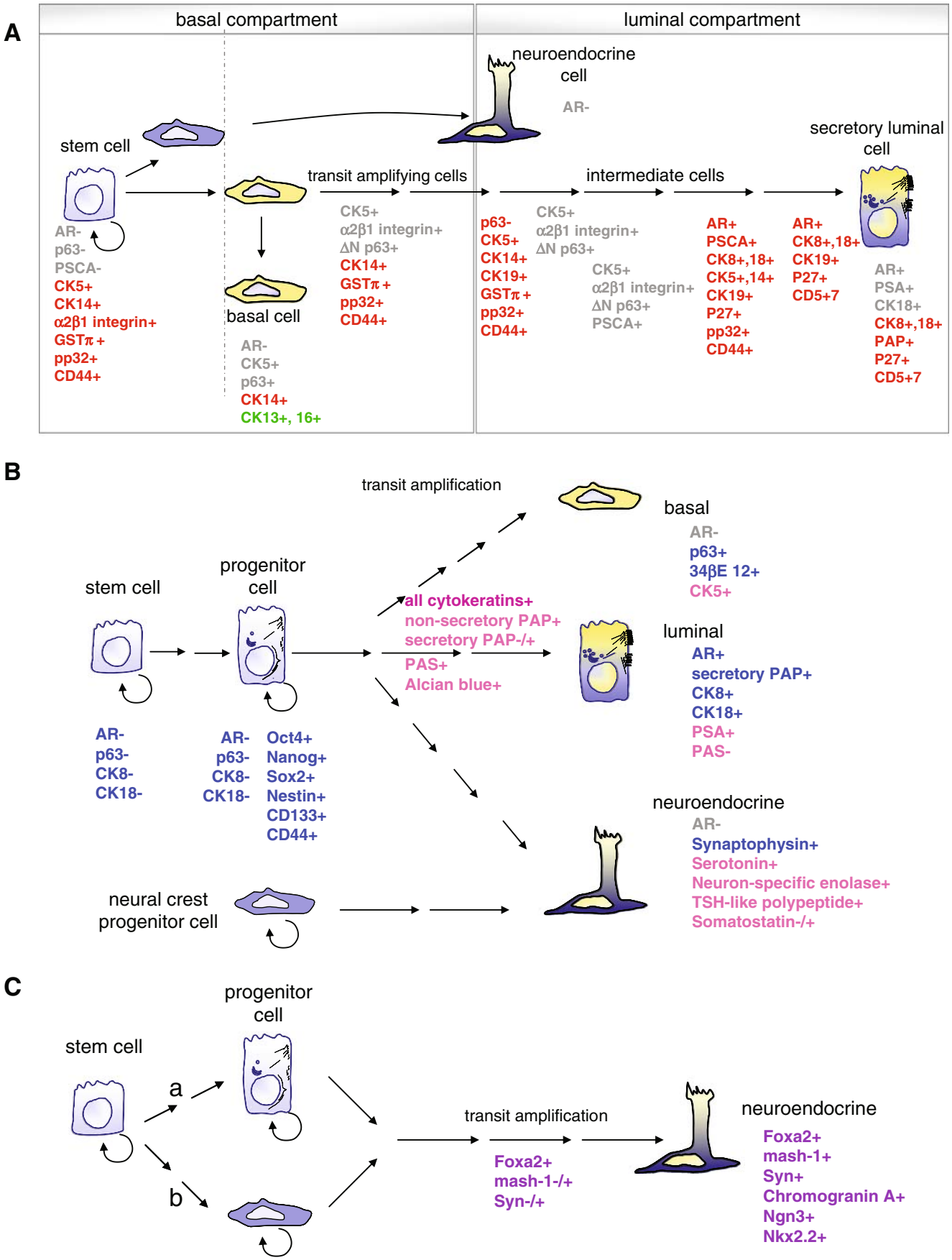
Origin of the Prostate Stem Cell

Although the developing human and rodent prostate have been extensively analyzed, the PSCs which initiate this process have not yet been identified. One model proposes that PSCs reside in the basal cell compartment (Fig. 1a) [22]. These cells proliferate and undergo transit amplification, expressing phenotypes intermediate between basal and luminal cells [22]. It is generally thought that PCPs do not express AR or p63 [23]. Regression/regeneration studies have shown that even after more than 30 rounds, androgen treatment could still restore secretory glandular structure [24]. Basal cells preferentially survived androgen ablation whereas 90% of luminal epithelial cells were lost through programmed cell death [25]. The surviving epithelial cells appeared androgen independent; however they remained responsive to exogenous androgen treatment [26]. Basal cells exhibited higher proliferation rates than luminal cells in normal and hyperplastic acini [27]. Loss of Pten resulted in the expansion of a Sca-1^{+}/Bcl-2^{+} prostate stem/progenitor-like cell population [28]. Approximately 70% of the cells expressing proliferation-associated antigens such as Ki-67 were phenotypically basal cells [27]. Neonatal rat prostatic buds co-expressed basal cell cytokeratins 5 and 14 as well as luminal cell cytokeratins 8 and 18 [26]. In archival human tissues representing primary prostate carcinoma and high grade prostatic epithelial neoplasia (PIN), De Marzo et al. identified an intermediate layer of $p27^{Kip1}$-negative cells sandwiched between $p27^{Kip1}$-positive basal and luminal cells and hypothesized that these cells were competent for entry into the cell cycle [22].

The p53 homologue p63 is expressed in basal cells of many organs. p63 null mice did not develop a prostate, suggesting that p63-expressing basal cells were required for prostate organogenesis [29]. Since p63 null mice died at birth, complementation experiments were performed where p63 null blastocysts were injected with $p63^{+/+}$ β-galactosidase positive Rosa26 embryonic stem cells [30]. Both basal and secretory cells in the resulting chimeric prostates expressed β-galactosidase exclusively, supporting the hypothesis that the basal cell compartment contained prostate stem cells [30]. Other intermediate cell markers (summarized in Fig. 1a) have been identified in the adult prostate as well as in cell culture [22, 31, 32].

An alternative model proposes that basal, luminal and neuroendocrine cells may represent separate epithelial cell lineages (Fig. 1b). In the prenatal human prostate, primitive epithelial cells are present from bud formation onward and neuroendocrine-like cells appear at the basal regions of the developing buds around the same time [6, 14, 33]. Fetal and prepubertal human stratified epithelium appeared positive for all cytokeratin antibodies [16] and intermediate phenotypes were not observed in the developing human prostate [14]. In tissue rescue experiments, Cunha and colleagues demonstrated that embryonic p63 null urogenital sinus developed into prostate when engrafted under the renal capsule of male mice [34]. Although the grafts contained luminal and neuroendocrine cells, basal cells were absent, supporting the hypothesis that these cell types arose from separate epithelial cell lineages [34]. In label-retaining experiments, Tsujimura et al. identified BrdU-retaining cells in both basal and luminal cells in the proximal region of the ductal structure [24]. Smaller numbers of labeled cells were observed in the intermediate and distal regions of the ventral prostatic lobes, implying that adult PSCs were not to be restricted to one region of the prostatic branching structure or to one epithelial cell compartment [24]. Yet other studies have shown that testosterone replacement promoted ^{3}H-thymidine incorporation and cell division in both secretory and basal cells of prostates in castrated mice [35, 36].

In conclusion, it is still not certain whether the PSC originates in the basal cell compartment or whether each epithelial lineage arises from a distinct stem/progenitor cell.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CK8-
CK18-
Oct4+
Nanog+
Sox2+
Nestin+
CD133+
CD44+
all cytokeratins+
non-secretory PAP+
secretory PAP-/+
PAS+
Alcian blue+
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CK8+
CK18+
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PAS-
neuroendocrine
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Synaptophysin+
Serotonin+
Neuron-specific enolase+
TSH-like polypeptide+
Somatostatin-/+
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transit amplification
Foxa2+
mash-1-/+
Syn-/+
neuroendocrine
Foxa2+
mash-1+
Syn+
Chromogranin A+
Ngn3+
Nkx2.2+

◀ **Fig. 1** Models for ontogeny of the epithelial lineages in the prostate. **a** The epithelial lineages are derived from PSCs which reside in the basal cell compartment. **b** Basal, luminal and neuroendocrine cells represent separate epithelial cell lineages. **c** Ontogeny of the neuroendocrine lineage. **a** common prostatic stem/progenitor cell; **b** neural crest stem/progenitor cell. References: *Grey font* [22, 26], *Red font* [65], *Green font* [66], *Blue font* [54], *Dark pink font* [16], *Pale pink font* [14], *Purple font* [40]

Neuroendocrine Cell Development

During development, cells with neuroendocrine (NE) characteristics arise in the basal regions of the developing epithelial buds [6, 14]. These cells do not appear to proliferate, suggesting that they are post-mitotic and terminally differentiated [37]. In mice carrying LNCaP xenografts, NE cells were found to promote prostate epithelial cell survival and androgen-regulated gene transcription post castration [38]. Normal NE cells may play a similar role in regulating proliferation and AR signaling in adjacent secretory cells [37]. Since prostatic NE cells secrete neuropeptides such as serotonin, TSH-like polypeptide, and somatostatin [14], one hypothesis is that they are derived from neural crest. Alternatively, they may arise from local stem cells since NE cells of a closed phenotype express basal cell cytokeratins and NE cells of an open phenotype co-express PSA and chromogranin A [37]. NE Chromogranin A (CgA) positive cells in the stroma reach the outer aspects of the basement membrane on the forming ducts and shortly thereafter, CgA-positive cells appear within the basal compartment of the forming ducts, suggesting that stromal NE cells induce epithelial NE cell differentiation (Aumüller, personal communication).

The transcription factor FoxA2 is expressed in neuroendocrine small cell carcinomas and in undifferentiated adenocarcinoma [39]. A few clusters of cells expressing the transcription factor mouse achaete-scute homolog-1 (mash-1) are detected in prostatic intraepithelial neoplasia (PIN) and as invasive NE cancer develops, neurogenin3 (Ngn3) and Nkx2.2. are over-expressed [40]. Foxa2 has been identified as a pancreatic progenitor marker [41]. Pancreatic endocrine progenitor cells express high levels of Nkx2–2, and Ngn3 is expressed in Islet of Langerhans progenitor cells [41]. This transcription factor signature suggests that NE cells of the prostate may differentiate in a similar sequence as the islet cells of the endocrine pancreas.

Isolation and Characterization of Normal PSCs and Prostate CSCs

PSCs typically represent approximately 1% of the total cell population and several approaches have been used to isolate and characterize these cells. A commonly used method is fluorescence activated cell sorting (FACS) based on the expression of stem cell surface markers. CD133 (or prominin-1) is specifically localized to cellular protrusions and is expressed in hematopoietic stem and progenitor cells as well as in other non-hemopoietic and endothelial and stem cells [42]. In the prostate, approximately 1% of basal cells expressed $CD133^{+}/\alpha2\beta1^{hi}$ [43]. Prostate cells expressing $CD133^{+}$ exhibited characteristics of stem cells including prostasphere formation and the development of prostatic-like acini in immunocompromised male mice [43]. Subpopulations of prominin-positive cells also co-expressed cytokeratin 14 or TERT and gave rise to more numerous and larger branching ducts consisting of luminal and basal epithelial cells compared to their prominin-negative counterpart [44].

Molecular profiling of $CD133^{+}/\alpha2\beta1^{hi}$ and $CD133^{-}$ cells isolated from benign prostatic hyperplasia specimens revealed that $CD133^{+}/\alpha2\beta1^{hi}$ cells exhibited an expression profile associated with embryonic stem cells and embryonic development along with other groups representing ion homeostasis, response to chemical and biotic stimuli, cell communication and cell proliferation [45]. In contrast, $CD133^{-}$ cells expressed a profile resembling transit amplifying cells, with genes involved in cell cycle progression, RNA transcription and protein biosynthesis [45]. Key regulators of self-renewal and differentiation such as the TGFβ, BMP, Wnt, sonic hedgehog and Notch pathways were also differentially regulated in $CD133^{+}/\alpha2\beta1^{hi}$ and $CD133^{-}$ cells [45]. Another study isolating CSC from human PCa tissues determined that approximately 0.1% of cells in a tumor expressed $CD44^{+}/\alpha_2\beta_1{}^{hi}/CD133^{+}$ [46]. AR could be re-expressed in these cells under the appropriate microenvironment, implying that the stem cell niche was crucial in regulating CSC phenotype and function [46].

Stem cell antigen-1 (Sca-1) is expressed by stem/progenitor cells in a number to tissues including hemopoietic cells, cardiac tissue, mammary gland, skin, muscle and testis [47]. In the developing prostate, Sca-1 has been identified in the proximal region of the prostatic gland [47]. Greater than 60% of these cells co-expressed α-6 integrin/CD49f and Bcl-2 [47]. Furthermore, they regenerated normal functional prostatic ducts in an *in vivo* transplantation assay [47]. $Sca\text{-}1^{+}$ could regenerate tubular structures and cells expressing Sca-1 were androgen-independent since castration resulted in a concomitant enrichment for $Sca\text{-}1^{+}$ cells [48]. Over-expression of constitutively active Akt resulted in glandular structures that contained both normal prostatic architecture and mouse PIN [48]. The human Sca-1 homologue has not yet been identified [49].

$CD44^{+}$ is expressed in normal PSC as well as in prostate CSCs [46]. $CD44^{+}$ cells were more proliferative and inherently more tumorigenic and metastatic than $CD44^{-}$ prostate cancer cells [50]. A limited number of $CD44^{+}$ cells

underwent asymmetric division, one of the hallmarks of slow-cycling stem cells [50]. LNCaP prostate cancer cells expressing $CD44^{+}/CD24^{-}$ exhibited stem cell characteristics, including forming prostaspheres in cell culture, colonies in soft agar and tumors in NOD/SCID [51].

Flow cytometry-separated side populations have been used to identifying cells with stem cell characteristics. Side population cells isolated from LAPC-9 prostate tumors and the rat C6 glioma and MCF-7 cell lines were more tumorigenic that non-side population cells [52]. Side populations of U373 cells exhibited stem cell characteristics in that they sustained clonal growth and gave rise to non-side population cells in vivo [52]. Other techniques have utilized stem cell behavior. The ATP-binding cassette membrane transporter ABCG2 is associated with multi-drug resistance and has been used to determine the side population phenotype [52]. Hoechst 33342 dye efflux has enabled isolation of an enriched stem cell side population from benign and malignant prostatic tissue [53]. These cells developed spheroids and branching structures in 3-dimentional Matrigel culture [53].

Clonal selection of human prostate cancer epithelial cells is a further method for studying CSCs. Human telomerase reverse transcriptase (hTERT) was used to immortalize primary human prostate cancer epithelial (HPET) cells [54]. The resulting HPET cell lines regenerated tumors in mice that resembled the original patient tumor with respect to Gleason score and histopathology [54]. Furthermore, tumors generated from single-cell derived clones contained cells representing the three epithelial cell lineages of the prostate, i.e., luminal secretory, basal and neuroendocrine cells [54]. HPET cells expressed the $AR^{-}/p63^{-}$ signature of the prostate stem cell as well as embryonic stem and early progenitor markers such as Oct-4, Nanog, Sox2, nestin, CD44, CD133 and c-kit (Fig. 2b) [54]. Thus the HPET model supports the CSC definition which states that tumors contain a reservoir of self-renewing cells that undergo self-renewal and maintain the heterogeneous cell population of the tumor [1]. Whether HPET cells are resistant to therapy and can therefore survive to repopulate the tumor during progression to therapy resistant disease remains to be determined.

Clonally derived holoclones are thought to contain self-renewing stem cells whereas meroclones and paraclones consist of transit amplifying cells [55]. Holoclones derived from the PC-3 prostate cancer cell line could be serially passaged and were capable of regenerating all three types of clones [56]. Furthermore, they expressed high levels of CD44, $\alpha 2\beta 1$ integrin and β-catenin [56]. In contrast, meroclones and paraclones exhibited a more differentiate phenotype in that expression of these stem cell markers was decreased, they could not be continuously propagated and they did not initiate tumor development [56].

Stem Cells and Self-renewal in Prostate Cancer

Organ stem cells are long-lived and during the process of aging, they can acquire epigenetic modifications and genetic mutations through exposure to insults such as oxidative stress and DNA damage, resulting in the dysregulation of normal cell processes [2, 3]. The epigenetic progenitor model is a multi-step process in which epigenetic disruption of stem/progenitor cells is mediated by tumor progenitor genes. Subsequently, monoclonal genetic mutations in gatekeeper genes are acquired along with genetic and epigenetic plasticity [3]. This process could explain the late onset of prostate cancer and other adult cancers, tumor heterogeneity, latent cancers, the consequence of environmental effects, progression to recurrent disease, and the genetics of cancer risk [3].

Tumor characteristics could be determined by the stage at which these genetic alterations occurred (Figs. 2, 3a,b). For example, mutations in embryonic stem cells would give rise to childhood tumors such as Wilms tumors and other embryonal tumors including teratocarcinomas and retino-

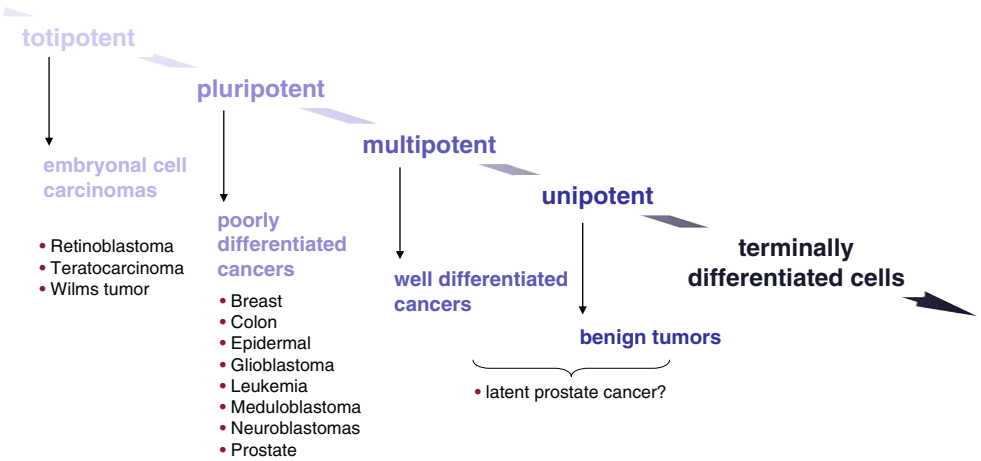

Fig. 2 The epigenetic progenitor origin of cancer. Epithelial or neuroendocrine cells may acquire epigenetic modifications and genetic mutations through DNA damage, oxidative stress, etc. during the process of differentiation. The phenotypic characteristics of the cancer would therefore be determined by the stem or progenitor cell-of-origin

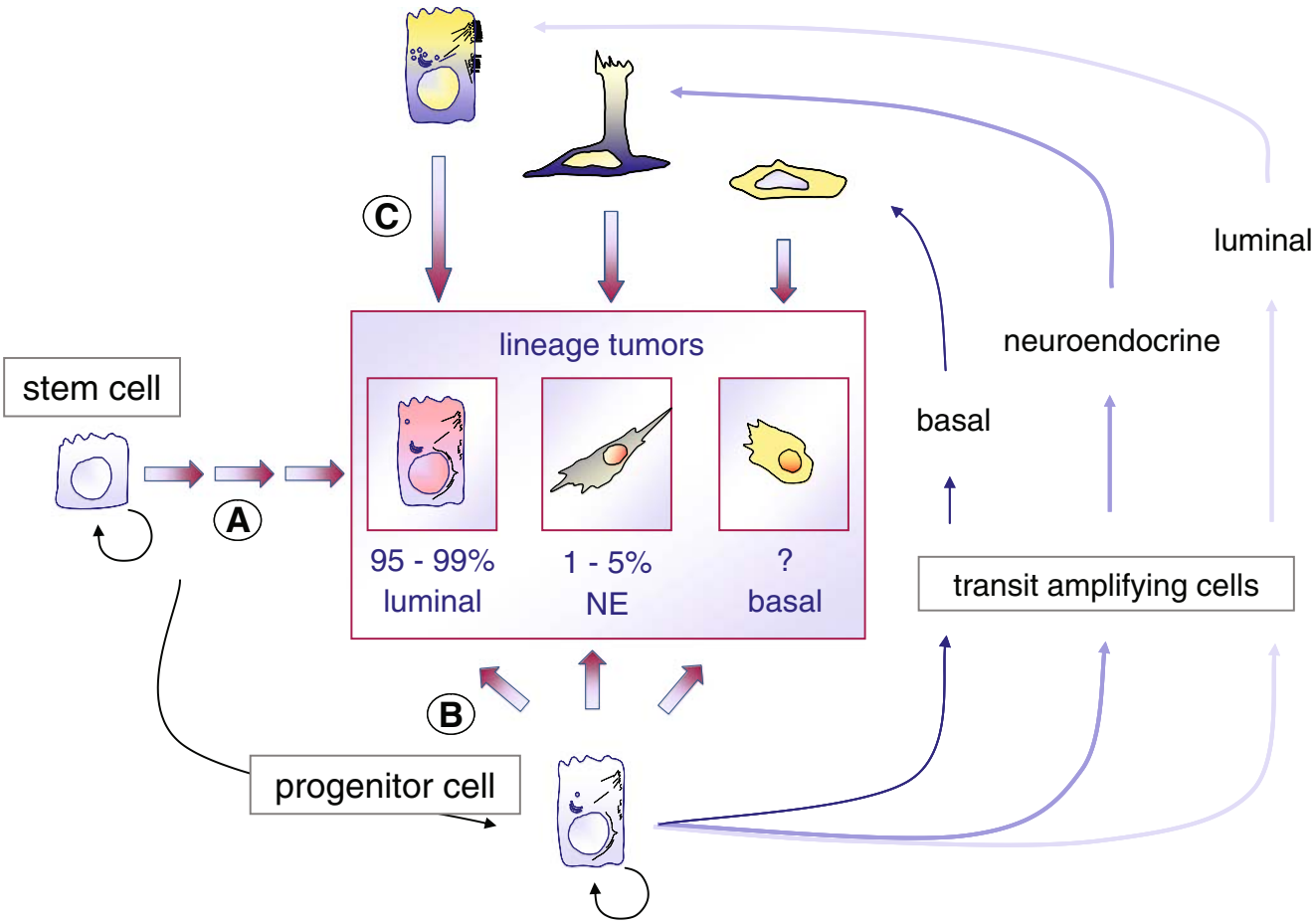

Fig. 3 Self-renewal in prostate cancer. The maintenance or reactivation of a self-renewal program appears central to tumorigenesis. **a** and **b** demonstrate the CSC hypothesis in which epigenetic changes and mutations in stem/progenitor cells give rise to prostate cancer. **c** Cancer cells acquire mutations which reactivate self-renewal. *NE* neuroendocrine

blastoma [57]. Mutations in early progenitor cells would promote the development of poorly differentiated cancers including solid tumors such as prostate, breast, and colon cancer [57]. In contrast, well-differentiated cancers would arise from late progenitor cells and benign tumors from cells just prior to terminal differentiation [57]. Prostate cancer has been found in 40% of cystoprostatectomy samples removed due to bladder cancer [58]. These cancers were characterized as histologically malignant but biologically benign tumors [58, 59]. They were small (<0.5 ml in volume) and low grade [58, 59]. Stamey et al. hypothesized that these cancers would not become clinically significant during the patient's life due to their extraordinarily slow doubling time [58]. An alternative hypothesis could be that these cancers arose from multi- or unipotent cells just prior to differentiation. If this were the case, they would form benign tumors which exhibited no/slow doubling times.

Although the CSC hypothesis has generated a lot of excitement, this process may not apply to all types of cancer. In a recent study comparing CD44(+) and CD24(+) breast cancer cell populations similarly isolated from pleural effusions, CD44(+) and CD24(+) cell appeared clonally related but not identical, since CD42(+) cells in some tumors showed additional genetic alterations [60]. These genetic differences suggested that clonal evolution could be an alternative interpretation [61]. The clonal genetic model of cancer does not consider epigenetic changes [61] but postulates that cancers arise through series of mutations in genes encoding proteins such as oncogenes and tumor suppressors with each mutation leading to the selective overgrowth of a monoclonal population of tumour cells [3].

Reactivation in a cancer cell of a portion of program associated with self-renewal may provide another mechanism for the development of CSCs (Fig. 3c). For example, chromosomal translocation can generate a novel fusion protein which reprograms a cell to express a self-renewal signature. In mixed lineage leukemia (MLL), the H3K4 methyl transferase domain is lost when as a consequence of chromosomal translocation; the N terminal of MLL is fused to the C terminal of potentially over 50 different partners

[62]. Acute myeloid leukemia (AML) is continuously regenerated by leukemia stem cells (LSC) [63]. AML can develop when expression of the fusion protein MLL-AF9 reactivates a portion of the self-renewal associated program normally expressed in hematopoietic stem cells [64]. Thus, the maintenance or reactivation of a self-renewal program appears central to cancer development and progression.

Concluding Remarks

Whether prostate cancer and other solid tumors develop CSC or from mature cancer cells which have regained a program for self-renewal remains to be determined. Cell lineage appears critical since >95–99% of human prostate cancers are derived from the luminal/secretory cell lineage. Identifying the cancer stem cell and elucidating the mechanisms that regulate stem cell phenotype would lead to the development of more effective therapeutic approaches for eradicating this disease.

References

1. Clarke, M. F., Dick, J. E., Dirks, P. B., et al. (2006). Cancer stem cells—perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Research*, *66*, 9339–9344.
2. Bell, D. R., & Van Zant, G. (2004). Stem cells, aging, and cancer: inevitabilities and outcomes. *Oncogene*, *23*, 7290–7296.
3. Feinberg, A. P., Ohlsson, R., & Henikoff, S. (2006). The epigenetic progenitor origin of human cancer. *Nature Reviews Genetics*, *7*, 21–33.
4. Kellokumpu-Lehtinen, P., Santti, R., & Pelliniemi, L. J. (1979). Early cytodifferentiation of human prostatic urethra and Leydig cells. *Anatomical Record*, *194*, 429–443.
5. Kellokumpu-Lehtinen, P. (1983). Localization of acid phosphatase activity in testosterone-treated prostatic urethra of human fetuses. *Prostate*, *4*, 265–270.
6. Kellokumpu-Lehtinen, P., Santti, R., & Pelliniemi, L. J. (1980). Correlation of early cytodifferentiation of the human fetal prostate and Leydig cells. *Anatomical Record*, *196*, 263–273.
7. Kellokumpu-Lehtinen, P., Santti, R. S., & Pelliniemi, L. J. (1981). Development of human fetal prostate in culture. *Urological Research*, *9*, 89–98.
8. Kellokumpu-Lehtinen, P., & Pelliniemi, L. J. (1988). Hormonal regulation of differentiation of human fetal prostate and Leydig cells in vitro. *Folia Histochemica et Cytobiologica*, *26*, 113–117.
9. Cunha, G. R. (1972). Epithelio-mesenchymal interactions in primordial gland structures which become responsive to androgenic stimulation. *Anatomical Record*, *172*, 179–195.
10. Takeda, H., Mizuno, T., & Lasnitzki, I. (1985). Autoradiographic studies of androgen-binding sites in the rat urogenital sinus and postnatal prostate. *Journal of Endocrinology*, *104*, 87–92.
11. Cunha, G. R., Donjacour, A. A., Cooke, P. S., et al. (1987). The endocrinology and developmental biology of the prostate. *Endocrine Reviews*, *8*, 338–362.
12. Donjacour, A. A., & Cunha, G. R. (1993). Assessment of prostatic protein secretion in tissue recombinants made of urogenital sinus mesenchyme and urothelium from normal or androgen-insensitive mice. *Endocrinology*, *132*, 2342–2350.
13. Cunha, G. R., & Lung, B. (1978). The possible influence of temporal factors in androgenic responsiveness of urogenital tissue recombinants from wild-type and androgen- insensitive (Tfm) mice. *Journal of Experimental Zoology*, *205*, 181–193.
14. Aumueller, G., Seitz, J., & Riva, A. (1994). *Functional Morphology of Prostate Gland* pp. 61–112. Kluwer Academic.
15. Kellokumpu-Lehtinen, P. (1980). The histochemical localization of acid phosphatase in human fetal urethral and prostatic epithelium. *Investigative Urology*, *17*, 435–440.
16. Wernert, N., Seitz, G., & Achtstatter, T. (1987). Immunohistochemical investigation of different cytokeratins and vimentin in the prostate from the fetal period up to adulthood and in prostate carcinoma. *Pathology, Research and Practice*, *182*, 617–626.
17. Aumuller, G., Seitz, J., & Bischof, W. (1983). Immunohistochemical study on the initiation of acid phosphatase secretion in the human prostate. Cytochemistry and biochemistry of acid phosphatases IV. *Journal of Andrology*, *4*, 183–191.
18. McNeal, J. E. (1981). The zonal anatomy of the prostate. *Prostate*, *2*, 35–49.
19. Timms, B. G., Mohs, T. J., & Didio, L. J. (1994). Ductal budding and branching patterns in the developing prostate. *Journal of Urology*, *151*, 1427–1432.
20. Sugimura, Y., Cunha, G. R., & Donjacour, A. A. (1986). Morphogenesis of ductal networks in the mouse prostate. *Biology of Reproduction*, *34*, 961–971.
21. Lee, C., Sensibar, J. A., Dudek, S. M., Hiipakka, R. A., & Liao, S. T. (1990). Prostatic ductal system in rats: regional variation in morphological and functional activities. *Biology of Reproduction*, *43*, 1079–1086.
22. De Marzo, A. M., Meeker, A. K., Epstein, J. I., & Coffey, D. S. (1998). Prostate stem cell compartments: expression of the cell cycle inhibitor p27Kip1 in normal, hyperplastic, and neoplastic cells. *American Journal of Pathology*, *153*, 911–919.
23. Isaacs, J. T. (2008). Prostate stem cells and benign prostatic hyperplasia. *Prostate*, *68*(9), 1025–1034.
24. Tsujimura, A., Koikawa, Y., Salm, S., et al. (2002). Proximal location of mouse prostate epithelial stem cells: a model of prostatic homeostasis. *Journal of Cell Biology*, *157*, 1257–1265.
25. Kyprianou, N., & Isaacs, J. T. (1988). Identification of a cellular receptor for transforming growth factor-beta in rat ventral prostate and its negative regulation by androgens. *Endocrinology*, *123*, 2124–2131.
26. De Marzo, A. M., Nelson, W. G., Meeker, A. K., & Coffey, D. S. (1998). Stem cell features of benign and malignant prostate epithelial cells. *Journal of Urology*, *160*, 2381–2392.
27. Bonkhoff, H., Stein, U., & Remberger, K. (1994). The proliferative function of basal cells in the normal and hyperplastic human prostate. *Prostate*, *24*, 114–118.
28. Wang, S., Garcia, A. J., Wu, M., Lawson, D. A., Witte, O. N., & Wu, H. (2006). Pten deletion leads to the expansion of a prostatic stem/progenitor cell subpopulation and tumor initiation. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 1480–1485.
29. Signoretti, S., Waltregny, D., Dilks, J., et al. (2000). p63 is a prostate basal cell marker and is required for prostate development. *American Journal of Pathology*, *157*, 1769–1775.
30. Signoretti, S., Pires, M. M., Lindauer, M., et al. (2005). p63 regulates commitment to the prostate cell lineage. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 11355–11360.
31. Uzgare, A. R., Xu, Y., & Isaacs, J. T. (2004). In vitro culturing and characteristics of transit amplifying epithelial cells from human prostate tissue. *Journal of Cellular Biochemistry*, *91*, 196–205.
32. Liu, A. Y., & Peehl, D. M. (2001). Characterization of cultured human prostatic epithelial cells by cluster designation antigen expression. *Cell & Tissue Research*, *305*, 389–397.

33. Aumuller, G., Leonhardt, M., Renneberg, H., von Rahden, B., Bjartell, A., & Abrahamsson, P. A. (2001). Semiquantitative morphology of human prostatic development and regional distribution of prostatic neuroendocrine cells. *Prostate*, *46*, 108–115.
34. Kurita, T., Medina, R. T., Mills, A. A., & Cunha, G. R. (2004). Role of p63 and basal cells in the prostate. *Development*, *131*, 4955–4964.
35. Evans, G. S., & Chandler, J. A. (1987). Cell proliferation studies in the rat prostate: II. The effects of castration and androgen-induced regeneration upon basal and secretory cell proliferation. *Prostate*, *11*, 339–351.
36. English, H. F., Santen, R. J., & Isaacs, J. T. (1987). Response of glandular versus basal rat ventral prostatic epithelial cells to androgen withdrawal and replacement. *Prostate*, *11*, 229–242.
37. Bonkhoff, H., & Remberger, K. (1996). Differentiation pathways and histogenetic aspects of normal and abnormal prostatic growth: a stem cell model. *Prostate*, *28*, 98–106.
38. Jin, R. J., Wang, Y., Masumori, N., et al. (2004). NE-10 neuroendocrine cancer promotes the LNCaP xenograft growth in castrated mice. *Cancer Research*, *64*, 5489–5495.
39. Mirosevich, J., Gao, N., Gupta, A., Shappell, S. B., Jove, R., & Matusik, R. J. (2006). Expression and role of Foxa proteins in prostate cancer. *Prostate*, *66*, 1013–1029.
40. Gupta, A., Wang, Y.-Q., Browne, C., et al. (2008). Neuroendocrine differentiation in the 12T-10 transgenic prostate mouse model mimics endocrine differentiation of pancreatic beta cells. *The Prostate*, *68*, 50–60.
41. Jorgensen, M. C., Ahnfelt-Ronne, J., Hald, J., Madsen, O. D., Serup, P., & Hecksher-Sorensen, J. (2007). An illustrated review of early pancreas development in the mouse. *Endocrine Reviews*, *28*, 685–705.
42. Fargeas, C. A., Joester, A., Missol-Kolka, E., Hellwig, A., Huttner, W. B., & Corbeil, D. (2004). Identification of novel Prominin-1/CD133 splice variants with alternative C-termini and their expression in epididymis and testis. *Journal of Cell Science*, *117*, 4301–4311.
43. Richardson, G. D., Robson, C. N., Lang, S. H., Neal, D. E., Maitland, N. J., & Collins, A. T. (2004). CD133, a novel marker for human prostatic epithelial stem cells. *Journal of Cell Science*, *117*, 3539–3545.
44. Mundy, G. R. (2002). Metastasis to bone: causes, consequences and therapeutic opportunities. *Nature reviews Cancer*, *2*, 584–593.
45. Shepherd, C. J., Rizzo, S., Ledaki, I., et al. (2008). Expression profiling of CD133(+) and CD133(-) epithelial cells from human prostate. *Prostate*, *68*(9), 1007–1024.
46. Collins, A. T., Berry, P. A., Hyde, C., Stower, M. J., & Maitland, N. J. (2005). Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Research*, *65*, 10946–10951.
47. Burger, P. E., Xiong, X., Coetzee, S., et al. (2005). Sca-1 expression identifies stem cells in the proximal region of prostatic ducts with high capacity to reconstitute prostatic tissue. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 7180–7185.
48. Xin, L., Lawson, D. A., & Witte, O. N. (2005). The Sca-1 cell surface marker enriches for a prostate-regenerating cell subpopulation that can initiate prostate tumorigenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 6942–6947.
49. Li, Y., & Rosen, J. M. (2005). Stem/progenitor cells in mouse mammary gland development and breast cancer. *Journal of Mammary Gland Biology and Neoplasia*, *10*, 17–24.
50. Patrawala, L., Calhoun, T., Schneider-Broussard, R., et al. (2006). Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene*, *25*, 1696–1708.
51. Hurt, E. M., Kawasaki, B. T., Klarmann, G. J., Thomas, S. B., & Farrar, W. L. (2008). CD44(+)CD24(-) prostate cells are early cancer progenitor/stem cells that provide a model for patients with poor prognosis. *British Journal of Cancer*, *98*, 756–765.
52. Patrawala, L., Calhoun, T., Schneider-Broussard, R., Zhou, J., Claypool, K., & Tang, D. G. (2005). Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and A. *Cancer Research*, *65*, 6207–6219.
53. Brown, M. D., Gilmore, P. E., Hart, C. A., et al. (2007). Characterization of benign and malignant prostate epithelial Hoechst 33342 side populations. *Prostate*, *67*, 1384–1396.
54. Gu, G., Yuan, J., Wills, M. L., & Kasper, S. (2007). Prostate cancer cells with stem cell characteristics reconstitute the original human tumor in vivo. *Cancer Research*, *67*, 4807–4815.
55. Pellegrini, G., Ranno, R., Stracuzzi, G., et al. (1999). The control of epidermal stem cells (holoclones) in the treatment of massive full-thickness burns with autologous keratinocytes cultured on fibrin. *Transplantation*, *68*, 868–879.
56. Li, H., Chen, X., Calhoun-Davis, T., Claypool, K., & Tang, D. G. (2008). PC3 human prostate carcinoma cell holoclones contain self-renewing tumor-initiating cells. *Cancer Research*, *68*, 1820–1825.
57. Sell, S. (2004). Stem cell origin of cancer and differentiation therapy. *Critical Reviews in Oncology/Hematology*, *51*, 1–28.
58. Stamey, T. A., Freiha, F. S., McNeal, J. E., Redwine, E. A., Whittemore, A. S., & Schmid, H. P. (1993). Localized prostate cancer. Relationship of tumor volume to clinical significance for treatment of prostate cancer. *Cancer*, *71*, 933–938.
59. Adolfsson, J. (2007). Commentary: on the incidence of histological prostate cancer and the probable diagnosis of cases with tumours too small to produce symptoms or to attract attention on physical examination–the findings of Dr Arnold Rice Rich. *International Journal of Epidemiology*, *36*, 285–287.
60. Shipitsin, M., Campbell, L. L., Argani, P., et al. (2007). Molecular definition of breast tumor heterogeneity. *Cancer Cell*, *11*, 259–273.
61. Feinberg, A. P. (2007). Phenotypic plasticity and the epigenetics of human disease. *Nature*, *447*, 433–440.
62. Krivtsov, A. V., & Armstrong, S. A. (2007). MLL translocations, histone modifications and leukaemia stem-cell development. *Nature Reviews Cancer*, *7*, 823–833.
63. Jin, L., Hope, K. J., Zhai, Q., Smadja-Joffe, F., & Dick, J. E. (2006). Targeting of CD44 eradicates human acute myeloid leukemic stem cells. *Natural Medicines*, *12*, 1167–1174.
64. Krivtsov, A. V., Twomey, D., Feng, Z., et al. (2006). Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature*, *442*, 818–822.
65. Schalken, J. A., & van Leenders, G. (2003). Cellular and molecular biology of the prostate: stem cell biology. *Urology*, *62*, 11–20.
66. Feitz, W. F., Debruyne, F. M., Vooijs, G. P., Herman, C. J., & Ramaekers, F. C. (1986). Intermediate filament proteins as tissue specific markers in normal and malignant urological tissues. *Journal of Urology*, *136*, 922–931.