

Sofia Honorio, Hangwen Li, and Dean G. Tang

ABSTRACT

The cancer stem cell (CSC) theory posits that only a small population of tumor cells within the tumor has the ability to reinitiate tumor development and is responsible for tumor homeostasis and progression. Tumor initiation is a defining property of putative CSCs, which have been reported in both blood malignancies and solid tumors. Here we provide evidence that both cultured prostate cancer cells and xenograft prostate tumors contain stem-like cells that can initiate serially transplantable tumors. We also present a hypothetical model of tumorigenic hierarchy of cancer cells in prostate tumors. Further studies on these important tumorigenic cells will help to understand prostate tumor biology and to develop novel diagnostic and prognostic markers and therapeutic agents.

Key Words: Prostate cancer, Cancer stem cells, Tumor progenitors, Clonal and clonogenic assays, Side population, Self-renewal, Transplantation sites, Sphere-formation assays

INTRODUCTION

Prostate cancer (PCa) is the most common noncutaneous malignant neoplasm in men in the Western world, accounts for the deaths of approximately 30,000 men per year in the United States, and constitutes the second leading cause of cancer-related deaths in American men (1). Since PCa is a disease of aging men, the number of afflicted men is increasing rapidly as the population of males over the age of 50 grows worldwide. PCa is generally diagnosed through an elevated prostate-specific antigen (PSA) level or abnormal digital rectal exam (2). PSA is a protein produced by normal epithelial cells of the prostate gland as well as PCa cells. It is present in small quantities in the serum of men without cancer, but is routinely elevated in the presence of PCa and in other benign prostate disorders such as infection, inflammation, and benign prostatic hyperplasia (BPH). Serum PSA as a screening tool sets the upper limit of normal at 4 ng/ml; levels above that point identify men who should be considered for prostate biopsy (3). As a result of PSA screening, most cancers are now discovered while they are still localized to the gland, which makes metastatic disease at the time of diagnosis a relatively rare event.

Localized disease is treated and often can be cured by surgery (prostatectomy) and radiation therapy (brachytherapy). The treatment of choice for advanced disease is androgen deprivation by either surgical or chemical castration (2). While this approach leads to tumor regression in 70–80% of patients with advanced PCa, most patients eventually relapse with hormone-refractory metastatic PCa that remains incurable by current treatment regimens.

PCa generally develops slowly, sometimes over a period of 20–30 years (3). Prostate carcinomas are multifocal (on average ≥ 5 cancer foci per patient) and highly heterogeneous. It is very common to find areas of cancer adjacent to prostatic intraepithelial neoplasia (PIN), considered to be the precursor lesions of PCa, and normal glands in human prostatectomy specimens. This degree of heterogeneity has resulted in the Gleason scoring/grading system (4), in which the two most common histologic patterns are assigned a grade of 1–5 according to decreasing degree of differentiation (i.e., grade 1 corresponding to a well-differentiated histological pattern while grade 5 to a poorly differentiated pattern); these two grades are summed and reported as the total Gleason score, which serves as a prognostic indicator of clinical behavior (5). Generally, PCa with a total Gleason score of 5–7 are considered to be intermediate grade/moderately differentiated and those with a score of 8–10 high grade/poorly differentiated.

CELLULAR ORGANIZATION OF THE PROSTATIC GLAND

The adult human prostate has three morphological zones: peripheral, transitional, and central. BPH occurs mainly in the transitional zone, while prostate carcinoma arises primarily in the peripheral zone. In contrast with the ductal-acinar histology of the human prostate, the rodent prostate gland consists of four distinct lobes: anterior, dorsal and lateral (collectively referred to as the dorsolateral lobe), and ventral. Although there is no clear analogy between the lobular structure of the rodent prostate and the zonal architecture of the human prostate, several studies claim that the dorsolateral lobe is most similar to the human peripheral zone (6).

The prostate is a hormonally regulated glandular organ whose growth accelerates at sexual maturity due to androgen actions on both stroma and epithelial cells (7). The prostatic glands contain two types of epithelial cells, i.e., the luminal secretory cells and the basal cells, and rare neuroendocrine (NE) cells (Fig. 1). External to the basal cells are also some transient (or reactive) “stromal” cell types whose identities remain unclear. These cells together form the pseudostratified prostatic glands. Basal cells form a layer of flattened cells along the basement membrane of each prostatic duct. Luminal cells form a layer of columnar shaped cells above the basal layer; they are the major cell type of the prostate and perform secretory function. NE cells often transverse both basal and luminal layers and secrete neuropeptides that support epithelial growth and viability. The prostatic epithelium is surrounded by a stromal component that includes fibroblasts, myofibroblasts, and smooth muscle cells that guide the growth and differentiation of the epithelium. Blood vessels, peripheral nerves and ganglia, and tissue infiltrating white blood cells are additional constituent cell elements of the normal adult human prostate.

The two epithelial cells express distinct markers (Fig. 1; Table 1) (8–37). While luminal cells express the low molecular weight cytokeratins (CK) 8 and 18, androgen receptor (AR), PSA, prostatic acid phosphatase (PAP), CD57, and 15-lipoxygenase 2 (15-LOX2), basal cells express the high molecular weight CK5 and 14, CD44, Bcl-2, p63, telomerase, and GST- π . NE cells are androgen-independent cells and express chromogranin A, synaptophysin, and neuron-specific enolase (NSE). They also produce and secrete various neuropeptides such as serotonin, bombesin, calcitonin, neurotensin, and parathyroid hormone-related protein (6).

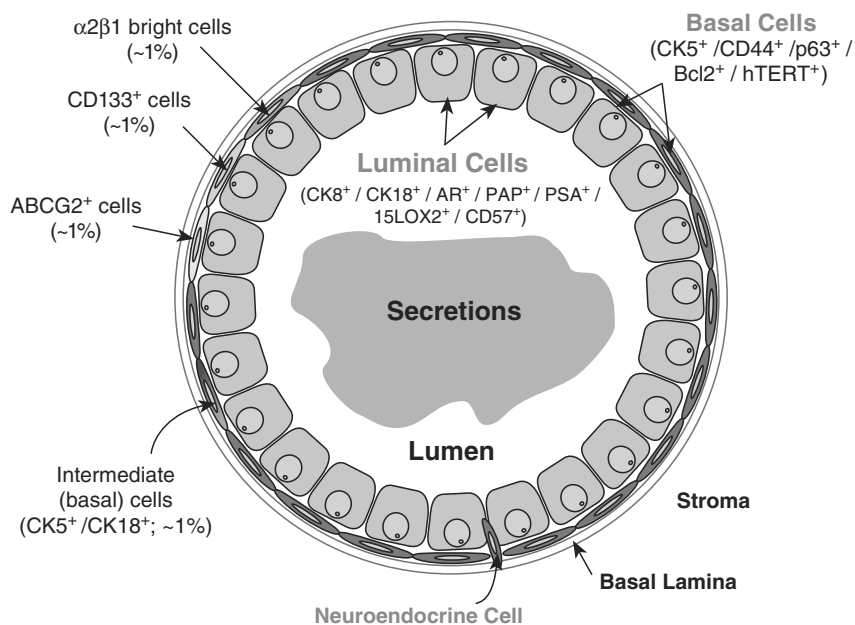


Fig. 1. Cartoon showing the general structure of a human prostatic gland.

NORMAL HUMAN PROSTATE STEM/PROGENITOR CELLS

The adult rodent prostate can undergo multiple rounds of castration-induced regression and testosterone-induced regeneration (38): androgen withdrawal results in glandular involution due to apoptosis of the terminally differentiated, androgen-dependent cells, while testosterone readministration restores the gland structure and its secretory function (39), supposedly owing to the reconstitution of the luminal cell compartment by basal cells (40). These data indicate that a population of stem cells (SCs), endowed with self-renewal and differentiation capacities, probably resides in the basal layer. This theory is further supported by findings that mice null for the basal cell marker p63 are born without prostate (26). In the human prostate, several pieces of evidence suggest that the basal cell layer may contain stem-like cells. First, most (>80%) proliferating cells are found in this compartment (41). Second, molecules important in maintaining SC self-renewal and proliferation (e.g., telomerase, p63), survival (Bcl-2), and detoxification (GST- π) preferentially localize in the basal layer (reviewed in (37)). Third, clonal analysis of dissociated adult human prostate epithelial cells reveals that only a small fraction (0.5–5%) of cells, all displaying basal cell characteristics, possess tremendous proliferative capacity (42). Fourth, when recombined with rat urogenital sinus mesenchyme (rUGM) and implanted under the renal capsule, the basal-like prostate epithelial cells can, like their murine counterpart, generate glandular structures (43). Finally, like other adult stem/progenitor cells, a small population of basal-like human prostate epithelial cells retains some developmental plasticity since, when cultured on mouse fibroblast feeder layers, they are able to “transdifferentiate” into neuronal/glial cells (37).

Strictly speaking, a prostate SC should be a cell that has the ability to regenerate the whole prostatic gland, much like what has been demonstrated for murine mammary SCs (44, 45). In this sense, the

Table 1
Representative marker expression in basal vs. luminal cells in human (and mouse) prostate^a

	<i>Basal cells</i>	<i>Luminal cells</i>
Surface ^b	<u><i>ABCG2</i></u> (also BCRP; Bcrp-1) (8, 9) <u><i>CD44</i></u> (10) <u><i>CD133</i></u> (12, 13) <u><i>CD104</i></u> (<i>integrin β4</i>) (11, 15) <u><i>CD138</i></u> (<i>syndecan</i>) (15) <u><i>α2β1 integrin</i></u> (18, 19) <i>Notch-1</i> ^c (20, 21) <i>Her-2/neu</i> (22) <i>Sca-1</i> (mouse); mainly in the proximal tubules also localized in luminal cells (23, 24)	<u><i>CD57</i></u> (10) <u><i>CD26</i></u> (<i>Dipeptidyl peptidase I</i>) (11) <u><i>CD13</i></u> (14) <u><i>CD10</i></u> (16) <u><i>CD38</i></u> (17) <i>Jagged-1</i> ^c
Cytoskeleton	<u><i>CK5/CK14</i></u>	<u><i>CK8/CK18</i></u>
Nuclear	<u><i>Sox9</i></u> (25); <i>p63</i> (26); telomerase (27, 28)	<i>AR</i> ; <u><i>Nkx3.1</i></u> (29)
Cytosolic	<u><i>Bcl-2</i></u> (30) <u><i>GST-π</i></u> (33)	<u><i>15-LOX2</i></u> (31, 32) <u><i>Probasin</i></u> (mouse) (34) <i>PAP</i> ; <i>PSA</i> <u><i>PSCA</i></u> ^d (identifying TACs) (35)

Note: CD49a (*integrin α1*) is very specific for human prostate stromal cells, so are COL6A3, CD56, and CD90 (Thy-1) (11). For mouse prostate, stromal, basal, luminal, and hematopoietic cells can be isolated by: CD34⁺, CD24⁺CD49f⁻, CD24⁺CD49f⁺, and CD45⁺ Ter119⁺ phenotype, respectively (36).

^a There are many differences between mouse and human prostates other than structural. For example, the basal cells in mouse prostate are only very scattered and do not form a continuous basal cell layer as in human. Mouse prostate epithelial cells express little PSA and no 15-LOX2, whereas probasin is unique to mouse prostate.

^b The underlined surface molecules are homogeneously expressed in most basal or luminal cells while the rest surface markers are expressed in a subset of cells.

^c Notch-1 is the receptor and Jagged-1 the ligand. These two markers have been identified from studies done in mouse prostate. It is not totally clear whether Jagged-1 is expressed only in the luminal cells.

^d PSCA has been shown to be expressed in late intermediate epithelial cells that are still double positive for CK5/CK18.

true prostate SCs have not been identified. For this reason, prostatic cells with certain SC properties such as extended proliferative and anchorage-independent growth capacities and partial differentiation abilities (e.g., to form ductal or acinar structures in Matrigel or when mixed with rUGM and transplanted under the renal capsule) are often called prostate SCs, progenitor cells, or stem/progenitor cells (37). Several candidate populations of NHP stem/progenitor cells have been reported. These include CK5 and CK18 double positive (CK5⁺/CK18⁺) intermediate cells, the side population (SP) cells and cells expressing the surface molecules CD44, ABCG2, integrin α2β1, or CD133 (reviewed in (37)). The CK5⁺/CK18⁺ intermediate cell population constitutes ~1% of the basal cells (46). Since CK5 and CK18 are intracellular cytoskeletal proteins, the CK5⁺/CK18⁺ intermediate basal cells have not been prospectively purified and their putative stem/progenitor cell properties have not been directly tested. Putative prostatic SCs appear to be enriched in the SP(47, 48), whose phenotype is mediated by multi-drug resistance family proteins such as MDR-1 and ABCG2 (reviewed in (49)). The SP in benign prostate constitutes 0.5–3% of epithelial cells and the SP cells cultured in Matrigel containing androgen have the ability to form acinus-like spheroids (48). The majority of the SP cells

are in the Go/G1 phase of the cell cycle (47), a characteristic of SCs. The *ABCG2*-expressing cells in the benign prostate localize mainly in the basal layer (8, 9), constitute <1% of total basal cell population, and share essentially the same transcriptome as the SP cells (9). It has been proposed that the *ABCG2*⁺ cells mark prostate SCs and that *ABCG2* functions to efflux androgen to keep these cells under the undifferentiated state (8). Interestingly, both SP and *ABCG2*⁺ cells express genes indicative of a SC phenotype (9). As of now, neither SP cells nor *ABCG2*⁺ cells have been shown to have the ability to regenerate prostatic glands in vivo. *CD44* is expressed in nearly all basal cells in the human prostatic glands. Purified *CD44*⁺ prostate basal cells, when cocultured with stromal cells in the presence of Matrigel and dihydrotestosterone (DHT), can produce PSA, presumably due to the differentiation of *CD44*⁺ cells into luminal cells (10). The $\alpha 2\beta 1^{\text{hi}}$ cells comprise 1–15% of the *CD44*⁺ basal cell population and seem to possess higher in vitro colony-forming efficiency as well as the ability to generate prostatic-like acini when subcutaneously engrafted with stromal cells into the flanks of male, athymic nude mice (19). Further characterization reveals that this proliferation and developmental potentials are preferentially harbored by the *CD133*-expressing cells within the *CD44*⁺ $\alpha 2\beta 1^{\text{hi}}$ population, which represent ~0.75% of the human prostate basal cells (12). It has been proposed that the *CD44*⁺ $\alpha 2\beta 1^{\text{hi}}$ *CD133*⁺ cells, constituting ~1% of the total epithelial cells, represent prostate SCs whereas *CD44*⁺ $\alpha 2\beta 1^{\text{hi}}$ *CD133*⁻ cells represent the progenitor cells or transit amplifying cells (12, 50). In support, the $\alpha 2\beta 1^{\text{hi}}$ *CD133*⁺ cells are AR-negative while the $\alpha 2\beta 1^{\text{hi}}$ *CD133*⁻ cells are AR-positive (50). None of these purported prostate SCs have been demonstrated to regenerate the whole prostatic gland at the single cell level and the interrelationships among these reported prostate stem/progenitor cells are presently unclear.

STEM-LIKE CELLS IN TUMORS AND PCa STEM/PROGENITOR CELLS

Tumor development to a certain degree resembles and has been compared as “caricatures” of normal tissue histogenesis and organogenesis (51). Indeed, most human tumors are heterogeneous in their cellular composition (52–54). Although many posit that tumor cell heterogeneity is of a genetic basis associated with inherent high genomic instability in tumor cells, the heterogeneous cellular composition in tumors has also been hypothesized, early on, to be the consequence of abnormal tumor stem cell differentiation (55). This latter postulate, called “cancer stem cell (CSC) hypothesis” was recently revived (56) mainly due to progresses made on studies of normal tissue stem cells. The CSC hypothesis has two central tenets – tumors are derived from transformation of normal stem cells or their progeny (i.e., progenitor or even differentiated cells) and every tumor contains a small population of stem-like cells that possess a unique ability to drive tumor formation and maintain tumor homeostasis (56). In support of the first tenet, both CML (chronic myelogenous leukemia; (57)) and AML (acute myelogenous leukemia; (58)) appear to have arisen from the committed progenitor cells that have acquired self-renewing capabilities. In support of the second tenet, stem-like cells or CSCs that can initiate serially transplantable tumors in mice recapitulating the heterogeneous nature of patient tumors have been reported not only in leukemia but also in solid tumors including breast cancer, glioma, melanoma, colon and liver cancers, head and neck squamous cell carcinoma, and pancreatic cancer (Table 2) (59–72).

Leukemic stem cells (LSCs), although constituting a minority (~0.1%) of the total cell population, are the only cells that can transfer the disease to NOD/SCID mice (73). In the past 5 years, putative CSCs, or tumor-initiating cells, have been reported for many human solid tumors (Table 2). Several important principles have emerged from these studies. First, most CSCs have been identified using cell surface markers for the corresponding normal tissue stem/progenitor cells, suggesting that normal and cancer SCs share some phenotypic markers. Second, interestingly, although no markers may be

truly SC-specific, CD44 and CD133 have been used to identify many types of CSCs. For example, CD44 has been used to enrich for breast, colon, pancreatic, liver, and head and neck CSCs, whereas CD133 for CSCs in lung and colon cancers and glioma (Table 2). Some other markers may be tumor specific, e.g., breast CSCs have a (CD44⁺)CD24⁻ phenotype (59) whereas pancreatic CSCs possess the (CD44⁺)CD24⁺ phenotype (67). Third, in a particular tumor, CD44 and CD133 may identify distinct and/or overlapping populations of tumor stem/progenitor cells. For instance, both CD133 (63–65) and CD44 (66) have been utilized as the positive selection marker for colon CSCs. The same two markers have also been employed to independently select for pancreatic CSCs (67, 68). In both cases, the interrelationship (inclusive, exclusive, or hierarchical) between the CD133 and CD44 selected CSCs remains unclear. These observations (63–68) emphasize the important point that the CSC population is likely heterogeneous, as elucidated in LSCs (74), and also raise the possibility that combining CD44 and CD133 may enrich for more primitive CSCs. Fourth, CSCs are only operationally or functionally defined. Perhaps one of the most important criteria is that putative CSCs possess an enhanced ability to initiate serially transplantable tumors that phenotypically recapitulate patient tumor histology (37, 75). In all of the above-mentioned CSC studies (Table 2), “naked” tumor cells were injected into the immunodeficient mice, implying that putative CSCs possess an intrinsic ability to establish a “niche” in a foreign microenvironment. Fifth, nevertheless, reconstitution of CSC activity and tumor development of human tumor cells in mice represents an extremely challenging task (37, 76) involving numerous variables associated with both tumors (availability, heterogeneity, stage/grade, size, quality, digestion/purification/implantation methods, etc.) and recipient mice (strains, degree of immune deficiency, pre-conditioning, injection/implantation sites, etc.). Consequently, different tumors have a wide variety of “empirical” details that cannot be interpreted readily and reconciled scientifically. For instance, although some tumorigenic subsets were implanted “orthotopically,” many others were injected at ectopic sites, in particular, subcutaneously (s.c) or under the kidney capsule (Table 2). Sixth, as predicted, CSCs seem to be more resistant to antitumor therapeutics including chemotherapy and radiation (68, 77–79). Of clinical significance, the abundance of CSCs significantly increases in breast cancer patients that have received prior therapies (77).

The cellular origin of PCa is unknown. Because the majority of tumor cells in early PCa have a luminal cell phenotype expressing CK8, CK18, AR, and PSA, it has been proposed that PCa may arise from the transformation and dedifferentiation of luminal cells (15, 26, 80–82). Nevertheless, some reports have identified intermediate cells coexpressing basal and luminal cell markers in PCa (83). In addition, prostate stem cell antigen (PSCA), a putative marker of normal late-intermediate prostate cells, is also found to be upregulated in PCa (35, 84). These data suggest that the disease might originate in an intermediate or transit-amplifying epithelial cell population. Furthermore, all PCa still contain a minor fraction of basal-like cells that express CD44, p63, ABCG2, or CD133, which identify normal prostate stem/progenitor cells. Therefore, it also remains possible that PCa might arise from normal prostate SCs.

Regardless of its origin, PCa seems to contain stem-like tumor cells, as evidenced by several observations. First, in long-term cultured PCa cells, only a small percentage of cells can establish serially passageable clones, colonies, or spheres (37, 85). Second, for both long-term cultured and xenograft-derived PCa cells, generally a large number of cells needs be injected into the animals to reinitiate a tumor (37, 49, 85–87), suggesting that PCa cells are not all equal in their tumor-initiating abilities. Third, PCa cells in culture, like keratinocytes, can form clones with distinct morphologies, i.e., holoclone, meroclone, and paraclone. Strikingly, only holoclones can be serially passaged and can initiate serially transplantable tumors (85). Since <10% PCa cells can form holoclones (37, 85), these observations again suggest that PCa cells are heterogeneous with respect to their tumor-initiating abilities and that only a small population of PCa cells have tumor-initiating ability.

Table 2
CSC studies in human solid tumors (2003–2008)

Tumor type	Samples	Marker	Mice	Transplantation	Results	Ref.
Breast cancer	9 (1 primary; 8 met.)	CD44 ⁺ CD24 ^{-lo} ESA ⁺ (FACS)	NOD/SCID mice pretreated with VP16	Mammary fat pad	>50-fold enrichment in tumorigenicity	(59)
Breast cancer	4 xenotransplants (from 2 primary; 2 met)	ALDH ⁺ (FACS)	NOD/SCID mice	Humanized mammary fat pad	500 ALDH ⁺ cells gen- erate T; 20 ALDH ⁺ CD44 ⁺ CD24 ⁺ Lin ⁻ cells generate T	(60)
Brain tumors	7 primary tumors	CD133 ⁺ (MACS)	6–8 wk NOD/ SCID	Intracranial injection	CD133 ⁺ more tumori- genic	(61)
Prostate cancer	7 (4 primary, 1 benign, 2 LN mets)	CD44 ⁺ α2β1 ^{hi} CD133 ⁺ (MACS) purified from long-term cultured cells		No tumor experiments	Marker+ cells more clonogenic	(62)
Colon cancer	17 (6 primary, 10 liver and 1 retroperito- neal met.)	CD133 ⁺ (double MACS)	8 wk NOD/SCID irradiated	Renal capsule	1 CSC/57,000 T cells; 1 CSC/262 CD133 ⁺ cells	(63)
Colon cancer	19 primary (5 Dukes A)	CD133 ⁺ (FACS or MACS)	SCID	Subcutaneous	3,000 CD133 ⁺ cells generate T	(64)
Colon cancer	21 primary CRC	CD133 ⁺	5–6 wk nude mice	Subcutaneous	2,500 CD133 ⁺ cells generate T; 25 CD133 ⁺ -derived spheres generate T	(65)
Colon cancer	2 primary, 6 xenografts	EpCAMCD166 ⁺ CD44 ⁺ (FACS)	6–8 wk NOD/ SCID	Subcutaneous	150 EpCAMCD166 ⁺ CD44 ⁺ cells gener- ate T	(66)
Pancreatic cancer	10 (2 primary; 2 met.)	CD44 ⁺ CD24 ⁺ ESA ⁺	NOD/SCID	Subcutaneous + pancreas	>100-fold enrichment	(67)

(continued)

Table 2
(continued)

<i>Tumor type</i>	<i>Samples</i>	<i>Marker</i>	<i>Mice</i>	<i>Transplantation</i>	<i>Results</i>	<i>Ref.</i>
<i>Pancreatic cancer</i>	11 (6 met.); sorting for 7 L3.6pl metastatic line	CD133 ⁺ (MACS) CD133 ⁺ CXCR4 ⁺ (FACS)	8–12 wk nude mice	Pancreas	500 CD133 ⁺ cells generate T; the CD133 ⁺ CXCR4 ⁺ population mediates metastasis.	(68)
<i>Head & neck</i>	25 primary (3 recurrences); 9 for sorting (4 primary + 5 xenografts)	CD44 ⁺ Lin ⁻ (FACS)	NOD/SCID & Rag2 ^{-/-}	Subcutaneous	5,000 CD44 ⁺ Lin ⁻ cells generate T; only 13/25 HNSCC samples gave tumors	(69)
<i>Melanoma</i>	7 (1 primary; 4 LN & 2 visceral met.)	ABC5 ⁺ (MACS)	NOD/SCID	Subcutaneous	1 MMIC/1 million bulk T cells; primary xeno: 1 MMIC/160,000 ABC5 ⁺ cells; secondary xeno: 1 MMIC/120,000 ABC5 ⁺ cells	(70)
<i>Lung cancer</i>	19 (18 primary; 1 met.)	CD133 ⁺ (FACS)	4 wk SCID or nude	Subcutaneous	10 ⁴ CD133 ⁺ cells generate T	(71)
<i>Liver cancer</i>	28 primary (only 13 used)	CD45 ⁻ CD90 ⁺ (MACS)	SCID	Intrahepatic	CD45 ⁻ CD90 ⁺ more tumorigenic	(72)

The obvious challenge is to prospectively purify the stem-like cells out and further characterize their potential CSC properties. To this end, we have utilized three PCa xenograft models, i.e., Du145 (derived from brain metastasis), LAPC-4 (from a lymph node metastasis), and LAPC-9 (derived from a bony metastasis). Xenograft models have distinct advantages of being relatively genetically stable and providing a “renewable” source of specific populations of cells. Remarkably, these three xenograft tumors contain small populations of basal-like cells expressing ABCG2 (49), CD44 (86), and $\alpha 2\beta 1$ (87). We could also detect an SP, interestingly, only in LAPC-9 tumors (49). Since normal prostate epithelial cells expressing these markers or showing the SP phenotype have been proposed as stem/progenitor cells (see above), we tested whether these marker-expressing cells in the xenograft tumors might have CSC properties. We prospectively purified these marker-expressing or the SP cells out from xenografts and compared their initiating abilities with the corresponding marker-negative or non-SP cells. We also studied the potential CSC properties of the matched populations. These studies have revealed that prostate tumor cells seem to be organized as a tumorigenic hierarchy (Fig. 2).

Several pieces of evidence provide support for this model (Fig. 2). First, most tumorigenicity resides in the relatively small population of CD44⁺ cells, which range from ~1–20% in xenograft tumors (86, 87). In primary patient tumors, interestingly, the percentage of CD44⁺ cells seems to correlate with the Gleason grade, with Gleason grade 6–9 tumors having ~3, 9, 18, and 19% of CD44⁺ PCa cells (unpublished observations). Second, the CD44⁺ PCa cell population is still heterogeneous, encompassing tumor progenitor cells that are ABCG2⁺ $\alpha 2\beta 1$ ⁺ and relatively quiescent, slow-cycling CSCs that are CD44⁺ABCG2⁻ $\alpha 2\beta 1$ ⁻ (Fig. 2). In support of this conjecture, all ABCG2⁺ cells and most (i.e., 70–80%) of the $\alpha 2\beta 1$ ⁺ cells are included in the CD44⁺ cell population and overall the CD44⁺ $\alpha 2\beta 1$ ⁺ and LAPC-9 CD44⁺ $\alpha 2\beta 1$ ⁻ cells have very similar tumorigenicities. In fact, the tumorigenicity of CD44⁺ (i.e., sorted using a single marker) cells is also indistinguishable from that of CD44⁺ $\alpha 2\beta 1$ ⁺ or CD44⁺ $\alpha 2\beta 1$ ⁻ cells (86, 87), suggesting that FACS sorting using either CD44 alone or CD44/ $\alpha 2\beta 1$ combination is purifying practically the same PCa cell population. Primary human tumors also reveal that ~75% of the $\alpha 2\beta 1$ ⁺ cells are localized in the CD44⁺ PCa cell population.

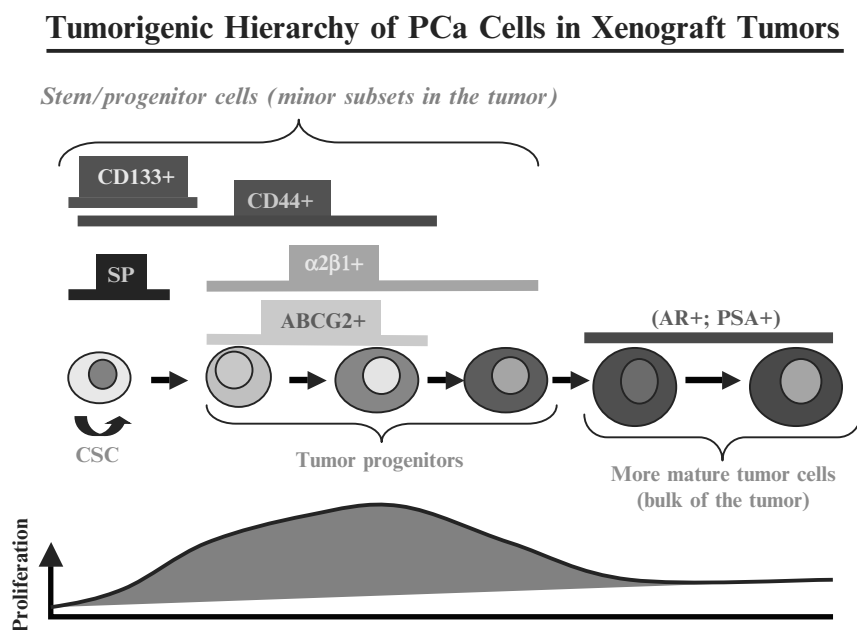


Fig. 2. Schematic depicting the tumorigenic hierarchy of tumor cells in xenograft tumors.

Third, the $\alpha 2\beta 1^+$ and $\alpha 2\beta 1^-$ cells are not significantly different in terms of their tumorigenicity, which can be explained by the fact that $\sim 30\%$ of the $CD44^+$ cells are localized in the $\alpha 2\beta 1^-$ cell population (87). In fact, the $\alpha 2\beta 1^-$ population appears to be slightly enriched in tumorigenic cells. For example, 100,000 of $\alpha 2\beta 1^-$ Du145 cells orthotopically implanted in the DP can initiate tumor in four of the four injections whereas the same number of unfractionated Du145 cells cannot initiate any tumor development. Also, all tumors derived from the $\alpha 2\beta 1^-$ cells contain small numbers of $\alpha 2\beta 1^+$ cells. Remarkably, in tumors derived from high numbers (i.e., 100,000) of the LAPC4 $\alpha 2\beta 1^-$ or LAPC9 cells, more $\alpha 2\beta 1^+$ cells are observed than in unsorted tumors (87). All these observations support the hypothesis that $\alpha 2\beta 1^-$ population contains more primitive cells that can “regenerate” $\alpha 2\beta 1^+$ cells. Furthermore, when injected s.c, 100 LAPC-9 $\alpha 2\beta 1^-$ cells, like the unsorted cells, can initiate 50% tumor development whereas ten times more $\alpha 2\beta 1^+$ cells are required to achieve similar tumor take (87). These data suggest that the $\sim 30\%$ of the $CD44^+$ PCa cells that are $\alpha 2\beta 1^-$ might harbor primitive self-renewing CSCs (Fig. 2). Fourth, the $CD44^+\alpha 2\beta 1^-$ cells and $CD44^-\alpha 2\beta 1^+$ cells behave very similarly, in terms of their tumor-initiating abilities, to the $\alpha 2\beta 1^-$ and $\alpha 2\beta 1^+$ cells, respectively. Also, we have previously shown that 1,000 LAPC-9 $CD44^-$ cells injected s.c can initiate tumor development in five of the six injections (86), suggesting that there exist tumorigenic cells in the $CD44^-$ cell population. Also, 1,000 highly purified $CD44^-\alpha 2\beta 1^+$ cells initiate tumor development in nine of the ten implantations (87), suggesting that tumorigenic cells in $CD44^-$ population might all be $\alpha 2\beta 1^+$ (i.e., having the $CD44^-\alpha 2\beta 1^+$ phenotype). These results emphasize the important concept that tumor progenitor cells, like the putative primitive CSCs, can be tumorigenic in regular tumor assays. Presumably, exhaustive serial tumor transplantation experiments can functionally distinguish putative CSCs from tumor progenitors. Fifth, in all xenograft models (Dnp53-T, Du145, LAPC-4, and LAPC-9) as well as primary patient samples we have studied, the percentage of $CD44^+$ cells is always higher than that of $\alpha 2\beta 1$, supporting that $CD44$ marks both CSCs and tumor progenitors whereas $\alpha 2\beta 1$ expression identifies a subset of tumor progenitors (Fig. 2). Finally, since 100 LAPC9 SP cells can initiate tumor development whereas $\geq 1,000$ $CD44^+$ cells are generally required to initiate tumor development, we hypothesize that the SP PCa cell population might contain more primitive CSCs than the $CD44^+$ cells (Fig. 2). In partial support, tumors initiated by the LAPC9 SP cells can be passaged for at least three generations (Fig. 3). Currently, the relationship between SP and $CD44^+$ cells remains unclear.

An obvious question pertains to the phenotypic properties of the putative CSCs in the $CD44^+$ PCa cell population (Fig. 2). The $CD133^+$ cells may represent good candidates as they have been reported to mark normal prostate SCs (12) and potential prostate CSCs with higher clonogenic potential (although tumorigenic potential has not been studied; (62)). We have also found that primary patient tumor samples contain 0.25–1.4% $CD133^+$ cells and that the $CD133^+$ PCa cells purified from LAPC-4 xenograft and HPCa13 patient tumors possess higher clonal and clonogenic potentials (Patrawala et al., unpublished observations). Studies are underway to characterize the *in vivo* tumorigenicity of $CD44^+$ $CD133^+$ PCa cells and to determine whether they may represent human prostate CSCs. Of particular interest, $CD133$ has recently been used as a marker to prospectively identify brain and colon tumor-initiating cells (Table 2), suggesting that this surface molecule, whose biological functions are yet to be elucidated, may represent more or less a “universal” normal SC and CSC marker. Another potential candidate population of primitive prostate CSCs might be in SP (Fig. 2). Since emerging evidence indicates that putative CSCs in solid tumors are more resistant to chemotherapeutic drugs and radiation and that CSCs might represent metastasis-mediating cells (68), identification and further characterization of prostate CSCs in patient tumors may lead to novel prognostic and therapeutic targets.

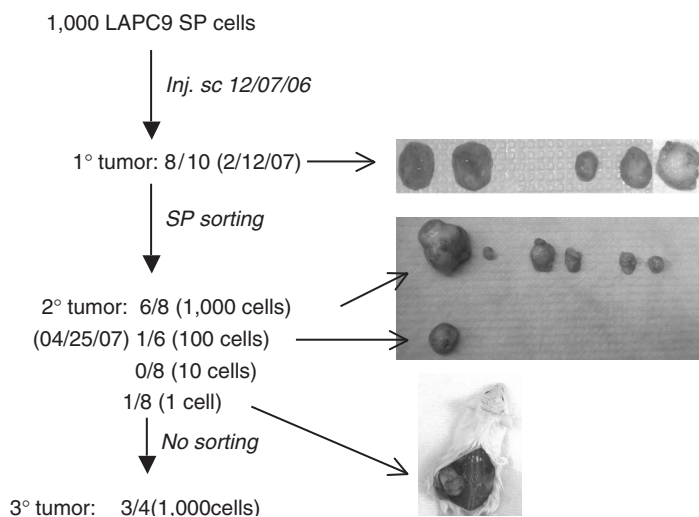


Fig. 3. The LAPC9 SP cell-initiated tumors can be serially passed. 1,000 SP cells were acutely purified from the maintenance tumors (49) and injected s.c into the male NOD/SCID mice (on Dec. 7, 2006). The first-generation (1°) tumors, with an incidence of 8/10 (i.e., 8 tumors out of 10 injections), were further sorted for SP cells, which were used at different numbers in secondary tumor development. The secondary tumor cells, without further SP sorting, were used in tertiary tumor development (incidences and cell numbers indicated). Shown at the right are corresponding tumor images. Note the tumor derived from a single SP cell (right bottom).

ACKNOWLEDGMENTS

We thank Mr. Kent Claypool for assistance in FACS, Histology and Animal Facility Cores for technical assistance, and other members (past and present) of the Tang lab for discussion and support. This work was supported in part by grants from NIH (R01-AG023374, R01ES015888, and R21-ES015893-01A1), American Cancer Society (RSG MGO-105961), Department of Defense (W81XWH-07-1-0616 and PC073751), Prostate Cancer Foundation, and Elsa Pardee Foundation (D.G.T), and by two Center grants (CCSG-5 P30 CA166672 and ES07784). H. Li was supported in part by a predoctoral fellowship from DOD (W81XWH-07-1-0132).

BIBLIOGRAPHY

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin* 2007;57:43–66.
2. National Comprehensive Cancer Network. 2007. NCCN Clinical Practice Guidelines in Oncology. Prostate cancer. v.2.2007. Available at: http://www.nccn.org/professionals/physician_gls/PDF/prostate.pdf.
3. Taichman RS, Loberg RD, Mehra R, Pienta KJ. The evolving biology and treatment of prostate cancer. *J Clin Invest* 2007;117:2351–61.
4. Gleason DF, Mellinger GT. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. *J Urol* 1974;111:58–64.
5. Bostwick DG, Foster CS. Predictive factors in prostate cancer: current concepts from the 1999 College of American Pathologists Conference on Solid Tumor Prognostic Factors and the 1999 World Health Organization Second International Consultation on Prostate Cancer. *Semin Urol Oncol* 1999;17:222–72.
6. Abate-Shen C, Shen MM. Molecular genetics of prostate cancer. *Genes Dev* 2000;14:2410–34.
7. Cunha GR, Harward SW, Wang YZ. Role of stroma in carcinogenesis of the prostate. *Differentiation* 2002;70:473–85.
8. Huss WJ, Gray DR, Greenberg NM, Mohler JL, Smith GJ. Breast cancer resistance protein-mediated efflux of androgen in putative benign and malignant prostate stem cells. *Cancer Res* 2005;65(15):6640–50.

9. Pascal LE, Oudes AJ, Petersen TW, Goo YA, Walashek LS, True LD, et al. Molecular and cellular characterization of ABCG2 in the prostate. *BMC Urol* 2007;7:6.
10. Liu AY, True LD, LaTray L, Nelson PS, Ellis WJ, Vessella RL, et al. Cell–cell interaction in prostate gene regulation and cyto-differentiation. *Proc Natl Acad Sci USA* 1997;94:10705–10.
11. Oudes AJ, Campbell DS, Sorensen CM, Walashek LS, True LD, Liu AY. Transcriptomes of human prostate cells. *BMC Genomics* 2006;7:92.
12. Richardson GD, Robson CN, Lang SH, Neal DE, Maitland NJ, Collins AT. CD133, a novel marker for human prostatic epithelial stem cells. *J Cell Sci* 2004;117:3539–45.
13. Miki J, Furusato B, Li H, Gu Y, Takahashi H, Egawa S, et al. Identification of putative stem cell markers, CD133 and CXCR4, in hTERT-immortalized primary nonmalignant and malignant tumor-derived human prostate epithelial cell lines and in prostate cancer specimens. *Cancer Res* 2007;67:3153–61.
14. Huang K, Takahara S, Kinouchi T, Takeyama M, Ishida T, Ueyama H, et al. Alanyl aminopeptidase from human seminal plasma: purification, characterization, and immunohistochemical localization in the male genital tract. *J Biochem* 1997;122:779–87.
15. Liu AY, True LD. Characterization of prostate cell types by CD cell surface molecules. *Am J Pathol* 2002;160:37–43.
16. Song J, Aumüller G, Xiao F, Wilhelm B, Albrecht M. Cell specific expression of CD10/neutral endopeptidase 24.11 gene in human prostatic tissue and cells. *Prostate* 2004;58:394–405.
17. Kramer G, Steiner G, Födinger D, Fiebiger E, Rappersberger C, Binder S, et al. High expression of a CD38-like molecule in normal prostatic epithelium and its differential loss in benign and malignant disease. *J Urol* 1995;154:1636–41.
18. Knox JD, Cress AE, Clark V, Manriquez L, Affinito KS, Dalkin BL, et al. Differential expression of extracellular matrix molecules and the alpha 6-integrins in the normal and neoplastic prostate. *Am J Pathol* 1994;145:167–74.
19. Collins AT, Habib FK, Maitland NJ, Neal DE. Identification and isolation of human prostate epithelial stem cells based on alpha(2)beta(1)-integrin expression. *J Cell Sci* 2001;114:3865–72.
20. Shou J, Ross S, Koeppen H, de Sauvage FJ, Gao WQ. Dynamics of notch expression during murine prostate development and tumorigenesis. *Cancer Res* 2001;61:7291–7.
21. Wang XD, Shou J, Wong P, French DM, Gao WQ. Notch1-expressing cells are indispensable for prostatic branching morphogenesis during development and re-growth following castration and androgen replacement. *J Biol Chem* 2004;279:24733–44.
22. Reiter RE, Sawyers CL. (2001). In: Chung LWK, Isaacs WB, Simons JW (eds). *Prostate Cancer: Biology, Genetics, and the New Therapeutics*. Humana Press: Totowa, NJ, 163–74.
23. Xin L, Lawson DA, Witte ON. The Sca-1 cell surface marker enriches for a prostate-regenerating cell subpopulation that can initiate prostate tumorigenesis. *Proc Natl Acad Sci USA* 2005;102:6942–7.
24. Burger PE, Xiong X, Coetzee S, Salm SN, Moscatelli D, Goto K, et al. 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* 2005;102:7180–5.
25. Wang H, McKnight NC, Zhang T, Lu ML, Balk SP, Yuan X. SOX9 is expressed in normal prostate basal cells and regulates androgen receptor expression in prostate cancer cells. *Cancer Res* 2007;67:528–36.
26. Signoretti S, Waltregny D, Dilks J, Isaac B, Lin D, Garraway L, et al. p63 is a prostate basal cell marker and is required for prostate development. *Am J Pathol* 2000;157:1769–75.
27. Myers RB, Grizzle WE. Changes in biomarker expression in the development of prostatic adenocarcinoma. *Biotech Histochem* 1997;72:86–95.
28. Bui M, Reiter RE. Stem cell genes in androgen-independent prostate cancer. *Cancer Metastasis Rev* 1999;17:391–9.
29. Ornstein DK, Cinquanta M, Weiler S, Duray PH, Emmert-Buck MR, Vocke CD, et al. Expression studies and mutational analysis of the androgen regulated homeobox gene NKX3.1 in benign and malignant prostate epithelium. *J Urol* 2001;165:1329–34.
30. McDonnell TJ, Troncoso P, Brisbay SM, Logothetis C, Chung LW, Hsieh JT, et al. Expression of the protooncogene bcl-2 in the prostate and its association with emergence of androgen-independent prostate cancer. *Cancer Res* 1992;52:6940–4.
31. Shappell SB, Boeglin WE, Olson SJ, Kasper S, Brash AR. 15-lipoxygenase-2 (15-LOX-2) is expressed in benign prostatic epithelium and reduced in prostate adenocarcinoma. *Am J Pathol* 1999;155:235–45.
32. Tang S, Bhatia B, Maldonado C, Yang P, Newman RA, Liu J, et al. Evidence that arachidonate 15-lipoxygenase 2 is a negative cell cycle regulator in normal prostate epithelial cells. *J Biol Chem* 2002;277:16189–201.
33. Cookson MS, Reuter VE, Linkov I, Fair WR. Glutathione S-transferase (GST- π) class expression by immunohistochemistry in benign and malignant prostate tissue. *J Urol* 1997;157:673–6.
34. Matuo Y, Nishi N, Murguruma Y, Yoshitake Y, Kurata N, Wada F. Localization of prostatic basic protein (“probasin”) in the rat prostates by use of monoclonal antibody. *Biochem Biophys Res Commun* 1985;130:293–300.
35. Tran CP, Lin C, Yamashiro J, Reiter RE. Prostate stem cell antigen is a marker of late intermediate prostate epithelial cells. *Mol Cancer Res* 2002;1:113–21.
36. Lawson DA, Xin L, Lukacs RU, Cheng D, Witte ON. Isolation and functional characterization of murine prostate stem cells. *Proc Natl Acad Sci USA* 2007;104:181–6.
37. Tang DG, Patrawala L, Calhoun T, Bhatia B, Choy G, Schneider-Broussard R, et al. Prostate cancer stem/progenitor cells: identification, characterization, and implications. *Mol Carcinog* 2007;46:1–14.
38. Isaacs JT, Coffey DS. Etiology and disease process of benign prostatic hyperplasia. *Prostate Suppl* 1989;2:33–50.
39. English HF, Kyprianou N, Isaacs JT. Relationship between DNA fragmentation and apoptosis in the programmed cell death in the rat prostate following castration. *Prostate* 1989;15:233–50.

40. Bonkhoff H, Remberger K. Differentiation pathways and histogenetic aspects of normal and abnormal prostatic growth: a stem cell model. *Prostate* 1996;28:98–106.
41. Bonkhoff H, Stein U, Remberger K. The proliferative function of basal cells in the normal and hyperplastic human prostate. *Prostate* 1994;24:114–8.
42. Hudson DL, O'Hare M, Watt FM, Masters, JR. Proliferative heterogeneity in the human prostate: evidence for epithelial stem cells. *Lab Invest* 2000;80:1243–50.
43. Hayward SW, Haughney PC, Rosen MA, Greulich KM, Weier HU, Dahiya R, et al. Interactions between adult human prostatic epithelium and rat urogenital sinus mesenchyme in a tissue recombination model. *Differentiation* 1998; 63:131–40.
44. Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML, et al. Generation of a functional mammary gland from a single stem cell. *Nature* 2006;439:84–8.
45. Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, Choi D, et al. Purification and unique properties of mammary epithelial stem cells. *Nature* 2006;439:993–7.
46. van Leenders G, Dijkman H, Hulsbergen-van de Kaa C, Ruiters D, Schalken J. Demonstration of intermediate cells during human prostate epithelial differentiation in situ and in vitro using triple-staining confocal scanning microscopy. *Lab Invest* 2000;80:1251–8.
47. Bhatt RI, Brown MD, Hart CA, Gilmore P, Ramani VA, George NJ, et al. Novel method for the isolation and characterization of the putative prostatic stem cell. *Cytometry A* 2003;54:89–99.
48. Brown MD, Gilmore PE, Hart CA, Samuel JD, Ramani VA, George NJ, et al. Characterization of benign and malignant prostate epithelial Hoechst 33342 side populations. *Prostate* 2007;67:1384–96.
49. Patrawala L, Calhoun T, Schneider-Broussard R, Zhou J, Claypool K, Tang DG. Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2- cancer cells are similarly tumorigenic. *Cancer Res* 2005;65:6207–19.
50. Heer R, Robson CN, Shenton BK, Leung HY. The role of androgen in determining differentiation and regulation of androgen receptor expression in the human prostatic epithelium transient amplifying population. *J Cell Physiol* 2007;212:572–8.
51. Sell S, Pierce GB. Maturation arrest of stem cell differentiation is a common pathway for the cellular origin of teratocarcinomas and epithelial cancers. *Lab Invest* 1994;70:6–22.
52. Dexter DL, Kowalski HM, Blazar BA, Fligiel Z, Vogel R, Heppner GH. Heterogeneity of tumor cells from a single mouse mammary tumor. *Cancer Res* 1978;38:3174–81.
53. Heppner GH. Tumor heterogeneity. *Cancer Res* 1984;44:2259–65.
54. Weiss L. Cancer cell heterogeneity. *Cancer Metastasis Rev* 2000;19:345–50.
55. Pierce GB. Neoplasms, differentiations and mutations. *Am J Pathol* 1974;77:103–18.
56. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001;414:105–11.
57. Jamieson CH, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N Engl J Med* 2004;351:657–67.
58. Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, et al. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature* 2006;442:818–22.
59. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003;100:3983–8.
60. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* 2007;1:555–67.
61. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, et al. Identification of human brain tumour initiating cells. *Nature* 2004;432:396–401.
62. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005;65:10946–51.
63. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007;445:106–10.
64. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, et al. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007;445:111–5.
65. Todaro M, Alea MP, Di Stefano AB, Cammareri P, Vermeulen L, Iovino F, et al. Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. *Cell Stem Cell* 2007;1:389–402.
66. Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, et al. Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci USA* 2007;104:10158–63.
67. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, et al. Identification of pancreatic cancer stem cells. *Cancer Res* 2007;67:1030–7.
68. Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* 2007;1:313–23.
69. Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci USA* 2007;104:973–8.
70. Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, et al. Identification of cells initiating human melanomas. *Nature* 2008;451:345–9.
71. Eramo A, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio A, et al. Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ* 2008;15:504–14.

72. Yang ZF, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, et al. Significance of CD90(+) cancer stem cells in human liver cancer. *Cancer Cell* 2008;13:153–66.
73. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994;367:645–8.
74. Hope KJ, Jin L, Dick JE. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. *Nat Immunol* 2004;5:738–43.
75. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, et al. Cancer stem cells – Perspectives on current status and future directions: AACR workshop on cancer stem cells. *Cancer Res* 2006;66:9339–44.
76. Hill RP. Identifying cancer stem cells in solid tumors: case not proven. *Cancer Res* 2006;66:1891–5.
77. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, et al. let-7 regulates self renewal and tumorigenicity of breast cancer cells. *Cell* 2007;131:1109–23.
78. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 2006;444:756–760.
79. Wang JCY. Evaluating therapeutic efficacy against cancer stem cells: New challenges posed by a new paradigm. *Cell Stem Cell* 2007;1:497–501.
80. Okada H, Tsubura A, Okamura A, Senzaki H, Naka Y, Komatz Y, et al. Keratin profiles in normal/hyperplastic prostates and prostate carcinoma. *Virchows Arch A* 1992;421:157–61.
81. De Marzo AM, Meeker AK, Epstein JI, Coffey DS. Prostate stem cell compartments: expression of the cell cycle inhibitor p27Kip1 in normal, hyperplastic, and neoplastic cells. *Am J Pathol* 1998;153:911–9.
82. Nagle RB, Ahmann FR, McDaniel KM, Paquin ML, Clark VA, Celniker A. Cytokeratin characterization of human prostatic carcinoma and its derived cell lines. *Cancer Res* 1987;47:281–6.
83. Verhagen AP, Ramaekers FC, Aalders TW, Schaafsma HE, Debruyne FM, Schalken JA. Colocalization of basal and luminal cell-type cytokeratins in human prostate cancer. *Cancer Res* 1992;52:6182–7.
84. Reiter RE, Gu Z, Watabe T, Thomas G, Szigeti K, Davis E, et al. Prostate stem cell antigen: a cell surface marker overexpressed in prostate cancer. *Proc Natl Acad Sci USA* 1998;95:1735–40.
85. Li H, Chen X, Calhoun-Davis T, Claypool K, Tang DG. PC3 human prostate carcinoma cell holoclones contain self-renewing tumor-initiating cells. *Cancer Res* 2008;68:1820–5.
86. Patrawala L, Calhoun T, Schneider-Broussard R, Li H, Bhatia B, Tang S, et al. Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene* 2006;25:1696–708.
87. Patrawala L, Calhoun-Davis T, Schneider-Broussard R, Tang DG. Hierarchical organization of prostate cancer cells in xenograft tumors: the CD44+ α 2 β 1+ cell population is enriched in tumor-initiating cells. *Cancer Res* 2007;67:6796–805.