Prostate cancer stem/progenitor cells

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Abstract

The cancer stem cell (CSC) theory posits that only a small population of tumor cells within the tumor has the ability to re-initiate 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

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Introduction

Prostate cancer (PCa) is the most common non-cutaneous 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 to 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 to 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). Externally 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)***.

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 re-administration 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 indicates 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 ***(24)***. In the human prostate, several pieces of evidence suggest that the basal cell layer may contain stem-like cells. Firstly, most (>80%) proliferating cells are found in this compartment ***(41)***. Secondly, 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 ***(8)***). Thirdly, 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)***. Fourthly, 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 ***(8)***.

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 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 **(*8*)**. 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 $\alpha2\beta1$ or CD133 (reviewed in ***(8)***). 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 G_0/G_1 phase of the cell cycle ***(47)***, a characteristic of SCs. The ***ABCG2-expressing cells*** in the benign prostate localize mainly in the basal layer ***(9,10)***, constitute <1% of total basal cell population, and share essentially the same transcriptome as the SP cells ***(10)***. 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 ***(9)***. Interestingly, both SP and $ABCG2^+$ cells express genes indicative of a SC phenotype ***(10)***. 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 co-cultured 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 ***(11)***. The ***$\alpha2\beta1^{hi}$ cells*** comprise 1 to 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 ***(17)***. Further characterization reveals that this proliferation and developmental potentials are preferentially harbored by the ***CD133-expressing cells*** within the $CD44^{+}\alpha2\beta1^{hi}$ population, which represent ~0.75% of the human prostate basal cells ***(12)***. It has been proposed that the $CD44^{+}\alpha2\beta1^{hi}CD133^{+}$ cells, constituting ~1% of the total epithelial cells, represent prostate SCs whereas $CD44^{+}\alpha2\beta1^{hi}CD133^{-}$ cells represent the progenitor cells or transit amplifying cells ***(12,50)***. In support, the $\alpha2\beta1^{hi}CD133^{+}$ cells are AR-negative while the $\alpha2\beta1^{hi}CD133^{-}$ 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 ***(8,75)***. 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 ***(8,76)*** 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 anti-tumor 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 ***(14,24,80-82)***. Nevertheless,

some reports have identified intermediate cells coexpressing basal and luminal cell markers in PCa ***(83)***. In addition, PSCA (prostate stem cell antigen), a putative marker of normal late-intermediate prostate cells, is also found to be upregulated in PCa ***(36,84)***. 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 ***(8,85)***. *Second*, for both long-term cultured and xenograft-derived PCa cells, generally a large number of cells needs be injected into the animals to re-initiate a tumor ***(8,49,85-87)***, 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 found holoclones ***(8,85)***, 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 tumor-initiating ability.

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 $\alpha2\beta1$ ***(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 $ABCG2^+\alpha2\beta1^+$ and relatively quiescent, slow-cycling CSCs that are $CD44^+ABCG2^-\alpha2\beta1^-$ (Fig. 2). In support of this conjecture, all $ABCG2^+$ cells and most (i.e., 70-80%) of the $\alpha2\beta1^+$ cells are included in the $CD44^+$ cell population and overall the $CD44^+\alpha2\beta1^+$ and $CD44^+\alpha2\beta1^-$ LAPC-9 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 $CD44^+\alpha2\beta1^+$ or $CD44^+\alpha2\beta1^-$ cells ***(86,87)***, suggesting that FACS sorting using either CD44 alone or CD44/$\alpha2\beta1$ combination is purifying practically the same PCa cell population. Primary human tumors also reveal that ~75% of the $\alpha2\beta1^+$ cells are localized in the $CD44^+$ PCa cell population.

Third, the $\alpha2\beta1^+$ and $\alpha2\beta1^-$ cells are not significantly different in terms of their tumorigenicity, which can be explained by the fact that ~30% of the $CD44^+$ cells are localized in the $\alpha2\beta1^-$ cell population ***(87)***. In fact, the $\alpha2\beta1^-$ population appears to be slightly enriched in tumorigenic cells. For example, 100,000 of $\alpha2\beta1^-$ Du145 cells orthotopically implanted in the DP can initiate tumor in 4 of the 4 injections whereas the same number of unfractionated Du145 cells cannot initiate any tumor development. Also, all tumors derived from the $\alpha2\beta1^-$ cells contain small numbers of $\alpha2\beta1^+$ cells. Remarkably, in tumors derived from high numbers (i.e., 100,000) of the $\alpha2\beta1^-$ LAPC4 or LAPC9 cells, more $\alpha2\beta1^+$ cells are observed than in unsorted tumors ***(87)***. All these observations support the hypothesis that $\alpha2\beta1^-$ population contains more primitive cells that can 'regenerate' $\alpha2\beta1^+$ cells. Furthermore, when injected s.c, 100 $\alpha2\beta1^-$ LAPC-9 cells, like the unsorted cells, can initiate 50% tumor development whereas 10 times more $\alpha2\beta1^+$ cells are required to achieve similar tumor take ***(87)***. These data suggest that the ~30% of the $CD44^+$ PCa cells that are $\alpha2\beta1^-$ might harbor primitive self-renewing CSCs (Fig. 2). ***Fourth***, the $CD44^+\alpha2\beta1^-$ cells and $CD44^-\alpha2\beta1^+$ cells behave very similarly, in terms of their tumor-initiating abilities, to the $\alpha2\beta1^-$ and $\alpha2\beta1^+$ cells, respectively. Also, we have previously shown that 1,000 $CD44^-$ LAPC-9 cells injected s.c can initiate tumor development in 5 of the 6 injections ***(86)***, suggesting that there exist tumorigenic cells in the $CD44^-$ cell population. Also, 1,000 highly purified $CD44^-\alpha2\beta1^+$ cells initiate tumor development in 9 of the 10 implantations ***(87)***, suggesting that tumorigenic cells in $CD44^-$ population might all be $\alpha2\beta1^+$ (i.e., having the $CD44^-\alpha2\beta1^+$ 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 % of $CD44^+$ cells is always higher

than that of α2β1, supporting that CD44 marks both CSCs and tumor progenitors whereas α2β1 expression identifies a subset of tumor progenitors (Fig. 2). ***Finally***, since 100 LAPC9 SP cells can initiate tumor development whereas ≥1000 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 3 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.

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Figure legends

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

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

Figure 3. The LAPC9 SP cell-initiated tumors can be serially passaged. 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 (1º) 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).

Table 1. Representative Marker Expression in Basal vs. Luminal Cells in Human (and Mouse) Prostate[a]

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

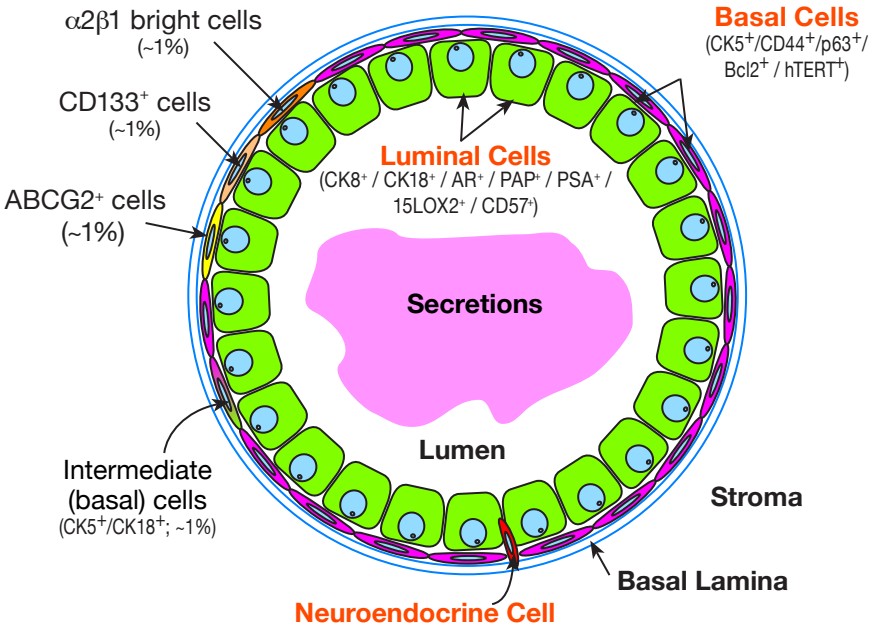
[d]PSCA has been shown to be expressed in late intermediate epithelial cells that are still double positive for CK5/CK18.

Note: CD49a (integrin α1) is very specific for human prostate stromal cells, so are COL6A3, CD56, and CD90 (Thy-1) ***(15)***. For mouse prostate, stromal, basal, luminal, and hematopoietic cells can be isolated by: CD34+, CD24+CD49f-, CD24+CD49f+, and CD45+Ter119+ phenotype, respectively ***(37)***.

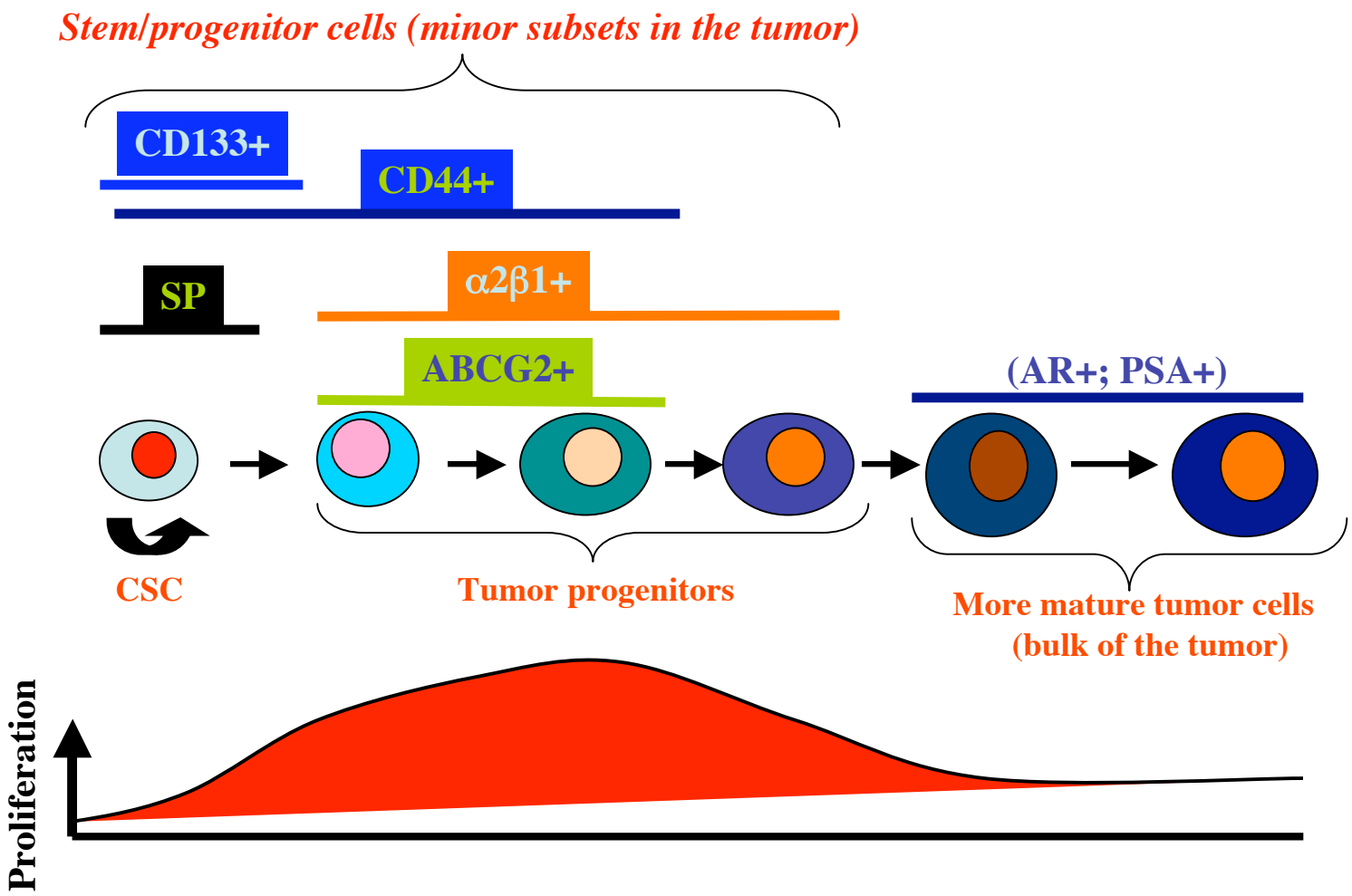
Table 2. CSC studies in human solid tumors (2003 - 2008)

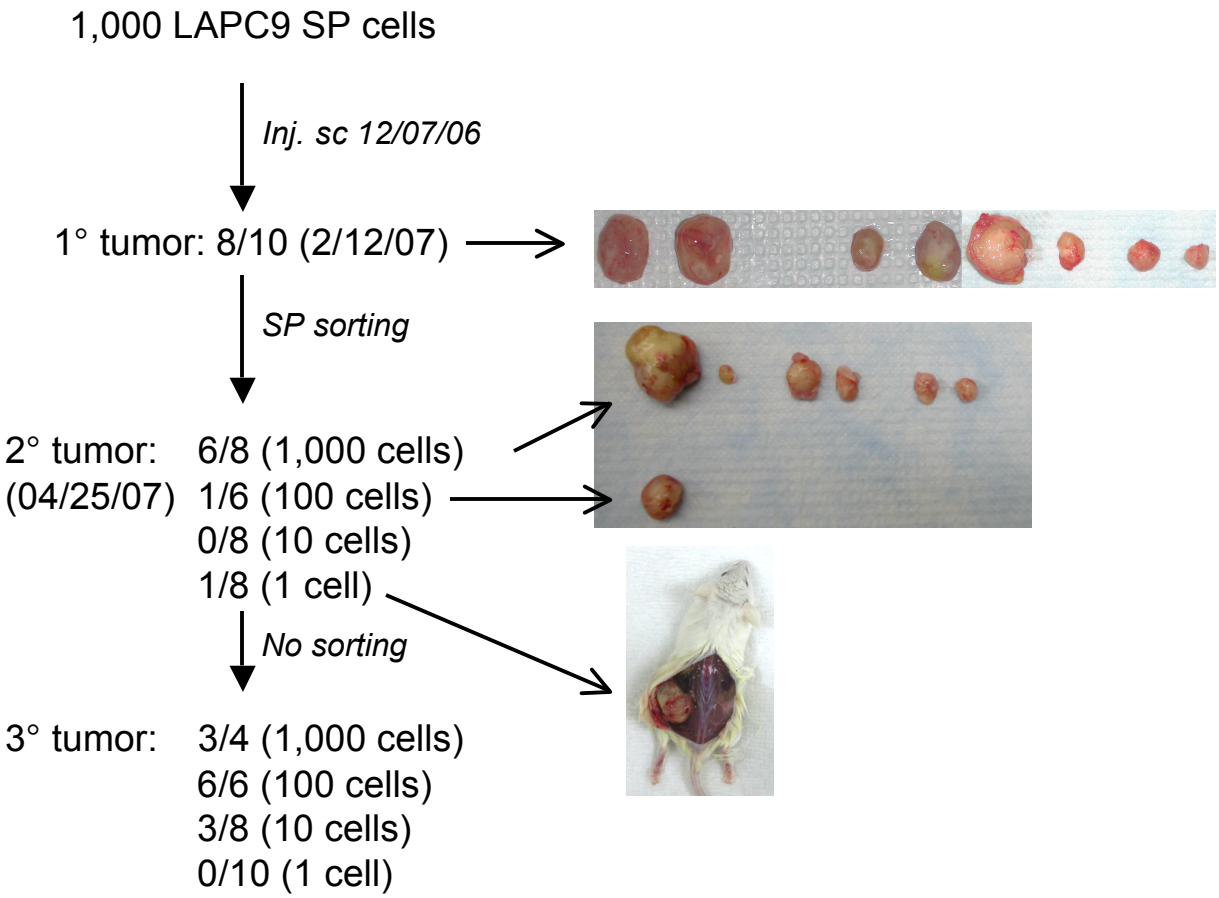
Tumor type	Samples	Marker	Mice	Transplantation	Results	Ref.
Breast cancer	9 (1 primary; 8 met.)	$CD44^+CD24^{-/lo}ESA^+$ FACS	NOD/SCID mice pretreated with VP16	mammary fat pad	>50 fold enrichment in tumorigenicity	59
Breast cancer	4 xenotransplants (from 2 primary; 2 met.)	$ALDH^+$ FACS	NOD/SCID mice	humanized mammary fat pad	500 $ALDH^+$ cells generate 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 tumorigenic	61
Prostate cancer	7 (4 primary, 1 benign, 2 LN mets)	$CD44^+\alpha2\beta1^{hi}CD133^+$ (MACS) purified from long-term cultured cells		***no tumor experiments***	marker+ cells more clonogenic	62
Colon cancer	17 (6 primary, 10 liver & 1 retroperitoneal 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 generate T	66
Pancreatic cancer	10 (2 primary; 2 met.)	$CD44^+CD24^+ESA^+$	NOD/SCID	subcutaneous+pancreas	>100 fold enrichment	67
Pancreatic cancer	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 $CD133^+CXCR4^+$ pop. mediates met.	68
Head & Neck	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
Melanoma	7 (1 primary; 4 LN & 2 visceral met.)	$ABCB5^+$ (MACS)	NOD/SCID	subcutaneous	1 MMIC/1 million bulk T cells 1^{ary} xeno: 1 MMIC/160,000 $ABCB5^+$ cells 2^{ary} xeno: 1 MMIC/120,000 $ABCB5^+$ cells	70
Lung cancer	19 (18 primary; 1 met.)	$CD133^+$ (FACS)	4 wk SCID or nude	subcutaneous	10^4 CD133+ cells generate T.	71
Liver cancer	28 primary (only 13 used)	CD45-CD90+ (MACS)	SCID	intrahepatic	CD45-CD90+ more tumorigenic	72

Honorio et al., Fig. 1



Tumorigenic Hierarchy of PCa Cells in Xenograft Tumors




1,000 LAPC9 SP cells
Inj. sc 12/07/06
1° tumor: 8/10 (2/12/07)
SP sorting
2° tumor: 6/8 (1,000 cells)
(04/25/07) 1/6 (100 cells)
0/8 (10 cells)
1/8 (1 cell)
No sorting
3° tumor: 3/4 (1,000 cells)
6/6 (100 cells)
3/8 (10 cells)
0/10 (1 cell)