Oncogene (2011) 30, 1261–1271

www.nature.com/onc

REVIEW

Revisiting the concept of cancer stem cells in prostate cancer

ZA Wang and MM Shen

Departments of Medicine and Genetics and Development, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY, USA

The cancer stem cell (CSC) model proposes that cells within a tumor are organized in a hierarchical lineage relationship and display different tumorigenic potential, suggesting that effective therapeutics should target rare CSCs that sustain tumor malignancy. Here we review the current status of studies to identify CSCs in human prostate cancer as well as mouse models, with an emphasis on discussing different functional assays and their advantages and limitations. We also describe current controversies regarding the identification of prostate epithelial stem cells and cell types of origin for prostate cancer, and present potential resolutions of these issues. Although definitive evidence for the existence of CSCs in prostate cancer is still lacking, future directions pursuing the identification of tumor-initiating stem cells in the mouse may provide important advances in evaluating the CSC model for prostate cancer.
Oncogene (2011) **30,** 1261–1271; doi:10.1038/onc.2010.530; published online 29 November 2010

Keywords: cancer stem cell; prostate cancer; mouse models; cell of origin; tumor-initiating cell

The cancer stem cell (CSC) model has attracted considerable interest in recent years due to its implications in cancer prognosis and clinical management. In its strictest form, the CSC model posits a hierarchical organization of tumors, with cancer stem cells at the top of the lineage hierarchy being capable of indefinite self-renewal, unlike their progeny, which undergoes an epigenetic program of differentiation and loss of tumorigenicity (Reya *et al*., 2001; Marotta and Polyak, 2009; Rosen and Jordan, 2009; Shackleton *et al*., 2009). In this view, rare CSCs may represent the driving force of tumor malignancy, and therefore effective treatment could be achieved by specific targeting of the CSC population.

In contrast, the stochastic (clonal) evolution model proposes that most of the cancer cells within a tumor are highly tumorigenic and possess different genetic or epigenetic properties (Marotta and Polyak, 2009; Rosen and Jordan, 2009; Shackleton *et al*., 2009). Clones can arise in the tumor population that have distinct growth advantages and/or therapy resistance, and thereby drive the malignant evolution of the tumor. Thus, in the clonal evolution model, it is essential to eliminate nearly all cancer cells to achieve therapeutic efficacy. In theory, these two models do not need to be mutually exclusive. Cancers that follow the CSC model may as well undergo clonal evolution if more than one type of CSCs coexist or CSCs are under environmental selection (Barabe *et al*., 2007; Marotta and Polyak, 2009).

As it is not experimentally feasible to investigate the potential existence of CSCs in human tumors solely on the basis of these theoretical definitions, CSCs are instead defined in practical terms through the use of several functional assays. The most frequently used methodology involves xenotransplantation of flow-sorted populations of primary cancer cells into immunodeficient mice. In this assay, CSCs are defined as a subpopulation of cells within a primary tumor that can initiate tumor formation in mice following transplantation, unlike the remaining tumor cells. Using this assay, early studies identified CSC populations in hematological malignancies, such as the $CD34^+CD38^-$ population in acute myeloid leukemia (Lapidot *et al*., 1994; Bonnet and Dick, 1997; Wang and Dick, 2005; Barabe *et al*., 2007). Similar approaches were subsequently applied to solid tumors, leading to the identification of candidate CSC populations that were prospectively enriched using specific markers in breast ($CD44^+CD24^-Lin^-$), brain ($CD133^+$) and colon cancers ($CD133^+$) (Al-Hajj *et al*., 2003; Singh *et al*., 2004; O'Brien *et al*., 2007; Ricci-Vitiani *et al*., 2007). Overall, however, the available evidence supporting the identification of CSCs in solid tumors has been less convincing, at least in part because solid tumor cells exist in a complex microenvironment that is not readily modeled by xenotransplantation (Hill, 2006).

Much of the confusion in the literature arises through inconsistencies in nomenclature within the field. In particular, due to the wide use of xenotransplantation as a functional assay for CSCs, transformed cells that can initiate tumor formation in this assay are often referred to as CSCs in the literature. However, a *tumor-initiating cell* (TIC) represents a different concept from that of a CSC, as TICs unquestionably exist within tumors and their identification does not by itself imply a hierarchical organization of a tumor. Indeed, the majority of cells within a tumor could potentially possess TIC properties and nonetheless follow a clonal

Correspondence: Professor MM Shen, Departments of Medicine and Genetics and Development, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, 1130 St Nicholas Ave, New York, NY 10032, USA.
E-mail: mshen@columbia.edu
Received 1 September 2010; revised and accepted 13 October 2010; published online 29 November 2010

evolution model. Consequently, it is important to distinguish CSCs that have been strictly defined by their position and function within a lineage hierarchy *in vivo* from CSCs that have been identified as rare TICs in transplantation studies.

A similar confusion arises with respect to the *cell of origin* for cancer, which corresponds to a normal tissue cell that is the target for the initiating events of tumorigenesis. In principle, a normal adult stem cell could be a logical cell of origin for cancer, as it would retain the ability to self-renew and generate a hierarchy of differentiated lineages within a tumor. However, it is also possible that a cell of origin could correspond to a downstream progenitor cell or conceivably even a terminally differentiated cell that acquires stem cell properties during oncogenic transformation. For example, both hematopoietic stem cells as well as committed progenitor cells can initiate leukemia after transformation (Cozzio *et al*., 2003; Huntly *et al*., 2004; Passegue *et al*., 2004). More recently, activation of canonical Wnt signaling has been shown to transform mouse intestinal stem cells to give rise to adenocarcinomas (Barker *et al*., 2009; Zhu *et al*., 2009). Notably, although normal tissue stem cells could represent a cell of origin for cancer and may acquire the properties of TICs after transformation, such transformed cells might not correspond to CSCs and their existence might be compatible with either a CSC or clonal evolution model.

Below, we discuss the current status of the CSC model as applied to prostate cancer. We also review current controversies with regards to the identification of normal prostate epithelial stem cells and cell types of origin, in both human prostate tumors as well as in mouse models.

Studies of prospective human prostate cancer stem cells

In human and mouse, the normal prostate gland epithelium contains three primary differentiated cell types (Abate-Shen and Shen, 2000; Shen and Abate-Shen, 2010). Luminal cells are columnar epithelial cells that express secretory proteins as well as markers such as cytokeratin 8 (CK8), CK18, Nkx3.1, prostate-specific antigen and high levels of androgen receptor (AR). Basal cells are localized beneath the luminal layer and express markers including CK5, CK14 and p63, but express low levels of AR. A rare third type of cells termed neuroendocrine cells express endocrine markers such as synaptophysin and chromogranin A, but do not express AR.

Prostatic intraepithelial neoplasia (PIN) is often considered a precursor of prostate cancer, and is characterized histologically by luminal epithelial hyperplasia and a progressive loss of basal cells (Abate-Shen and Shen, 2000; Shen and Abate-Shen, 2010). As the disease progresses to adenocarcinoma, the loss of basal cells becomes complete resulting in the strong luminal phenotype of prostate cancer. Initially, human prostate cancers rely on androgens for survival and thus treatment of prostate cancer often utilizes androgen-deprivation therapy. However, the disease will almost certainly recur in a castration-resistant form (formerly referred to as androgen-independent), which is essentially incurable (Chen *et al*., 2008; Attard *et al*., 2009). This has led to the notion that CSCs in advanced prostate cancer should be castration resistant.

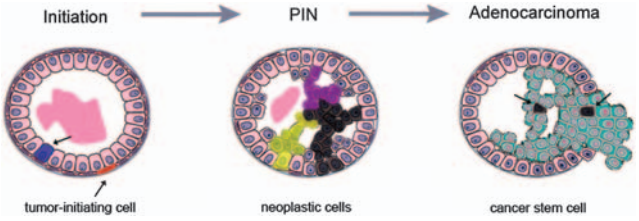

Figure 1 A CSC model for prostate cancer progression. Cancer initiation may occur in genetically mutated basal (orange) or luminal (blue) cells. As neoplastic cells (yellow, purple and black) accumulate more mutations in PIN lesions, some of their progeny may become selected to acquire CSC properties (brown). Such CSCs would give rise to the bulk of tumor cells with less proliferative potential (green). Note that the prostate CSCs may not have any features of the original cell of origin in this model.

Similar to other solid tumors, it is presently unclear whether prostate cancers are organized hierarchically and follow the CSC model. In principle, a CSC model of prostate cancer progression can be conceived, which also incorporates features of clonal evolution (Figure 1). In this model, cancer-initiating mutations could occur in different cells of origin within the prostate epithelium. During prostate cancer progression, clonal progeny of the resulting TICs are constantly under selection, including responses to treatment. Some of these clones may gain additional growth and survival advantages and become the driving force of tumor malignancy, acquiring the properties of CSCs. Below, we consider the published experimental data that are crucial for the evaluation of this and other theoretical models.

Identification of putative human prostate CSCs

A variety of studies have reported the identification of CSCs in prostate cancer, using a range of functional assays. Studies that have investigated human prostate CSCs have either used primary tumor cells for cell culture assays of stem cell properties, or instead have used established cell lines or xenografts in cell culture and grafting assays in immunodeficient mice (Figure 2). One notable study from the Maitland and Collins laboratories has used flow cytometry to isolate $CD44^+/\alpha2\beta1^{hi}/CD133^+$ cells from primary human tumors, and showed that this population displayed high proliferative potential in colony-forming assays, as well as the ability to differentiate to a luminal phenotype in culture (Collins *et al*., 2005). A subsequent study performed gene expression profiling to identify markers specific for the related $\alpha2\beta1^{hi}/CD133^+$ population (Birnie *et al*., 2008). Notably, however, it is unknown whether these $CD44^+/\alpha2\beta1^{hi}/CD133^+$ cells can initiate tumors following xenotransplantation, or if such tumors would display luminal phenotypes.

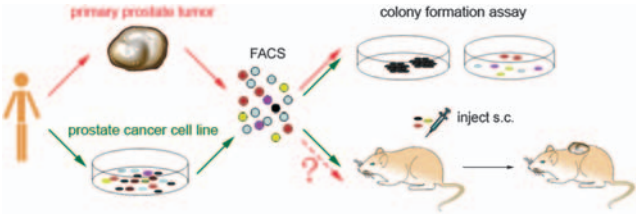

Figure 2 Strategies for identification of human prostate CSCs using different functional assays. In some studies, dissociated cells from primary tumors have been flow sorted using cell-surface markers and CSC (black) properties analyzed by colony formation assays in culture (red arrows). In other studies, CSCs were isolated from cultured prostate cancer cell lines or xenografted tumors and analyzed by colony formation assays and tumor growth following subcutaneous injection into mice (green arrows). However, tumor initiation from specific cell populations isolated from primary human prostate tumors has not been demonstrated to date (red dashed arrow).

Other studies have reported the isolation of putative prostate CSCs from established human prostate cancer cell lines, using similar combinations of cell-surface markers. For example, $CD44^+/\alpha2\beta1^{hi}/CD133^+$ cells were isolated from the DU145 cell line (Wei *et al*., 2007), whereas $CD133^{hi}$ cells were identified from human telomerase reverse transcriptase-immortalized primary tumor-derived prostate epithelial cell lines (Miki *et al*., 2007), and $CD44^+CD24^-$ cells were isolated from the LNCaP cell line (Hurt *et al*., 2008). In addition, the Pten/PI3K/Akt pathway has been shown to be critical for the tumorigenicity of $CD44^+CD133^+$ cells in the PC3 and DU145 cell lines (Dubrovska *et al*., 2009). The CSC properties of these cell populations were primarily demonstrated by colony formation assays and tumor initiation following subcutaneous injection. Other studies have demonstrated tumor formation from sub-populations of human prostate cell lines using renal grafting assays (Gu *et al*., 2007). Perhaps most interestingly, holoclones from the PC3 prostate cancer cell line were shown to contain cells expressing high levels of CD44, α2β1 and β-catenin, and could initiate serially transplantable tumors after subcutaneous injection (Li *et al*., 2008).

Finally, these approaches have been used to identify putative CSCs from prostate tumor xenografts that have been established from primary tumors, either by passaging through cell culture or through serial passaging in immunodeficient mice. Most notably, the Tang laboratory has identified $CD44^+/\alpha2\beta1^{hi}$ cells from human LAPC9 xenografts as candidate prostate CSCs, based on colony formation assays and tumor initiation following subcutaneous or orthotopic injection (Patrawala *et al*., 2005, 2006, 2007).

Limitations of current approaches

Despite these promising studies, there are several key experimental limitations to current methods for identification of prostate CSCs. First, to date, tumor-initiation has not yet been demonstrated for flow-sorted sub-populations isolated from primary prostate tumors. This fundamental limitation may be due to the relative indolence of many prostate cancers, and/or due to the difficulty in culturing prostate cells with luminal phenotypes, even for a limited time. As a consequence, most work has utilized established prostate cancer cell lines or xenografts. However, after establishment in culture followed by extensive passaging, the genetic properties of cell lines are likely to be different from human primary tumors, as it is extremely difficult to culture primary prostate cancer cells (Peehl, 2005). Established xenografts may be more similar to primary tumors, but recent studies of melanomas have suggested that xenografts display significantly higher frequencies of TICs relative to primary tumors (Boiko *et al*., 2010).

Secondly, assays utilized for demonstration of prostate CSC properties can be flawed. In particular, two-dimensional colony formation assays in culture may selectively favor cells that better adapted to the culture conditions, and may not accurately model microenvironmental conditions *in vivo*. Similar concerns apply to *in vivo* assays, as several studies employ subcutaneous injection to assess tumor-initiation, and thus are not conducted within a prostate microenvironment. Orthotopic injection of human cells into immunodeficient mice alleviate this concern, but also face the issue of cross-species compatibility, possibly resulting in variable outcomes and underestimation of tumorigenicity. Notably, technical improvements in xenotransplantation methodology can significantly increase the efficiency of tumor-initiation assays, with up to 25% of melanoma cells displaying TIC properties (Quintana *et al*., 2008). Thus, the use of more highly immunodeficient mice, orthotopic xenotransplantation and tissue reconstitution with prostate stromal cells may all improve the efficiency and specificity of tumor-initiation assays. However, it remains unclear whether these technical improvements will substantially affect tumor-initiation frequency in many cancers (Ishizawa *et al*., 2010).

Third, the specificity of the cell-surface markers utilized in these studies is unclear and their biological function is often unknown. Although CD44 and CD133 have been implicated as CSC markers in a variety of tissues (Uchida *et al*., 2000; Al-Hajj *et al*., 2003; Richardson *et al*., 2004; Singh *et al*., 2004), their specificity for identification of CSC populations has been questioned. For example, the expression pattern of CD44 in both benign and malignant human prostate lesions varies in different reports, and its relationship with tumor grade is controversial (Patrawala *et al*., 2006; Ugolkov *et al*., 2010). Furthermore, CD133 is broadly expressed in luminal cells of colon, lung and pancreas, and appears to display little specificity for colon cancer-initiating cells (Shmelkov *et al*., 2008). Moreover, the apparent expression pattern of CD133 may vary greatly according to the staining conditions used, as the most commonly used antibody recognizes a glycosylated epitope (Shmelkov *et al*., 2008). One recent study found that $CD133^+$ cells from the DU145 cell line were not more clonogenic than $CD133^-$ cells, while CD133 was not expressed in other prostate cell lines examined (Pfeiffer and Schalken, 2010). Finally, another study has shown that mouse CD133 is widely expressed

by prostate luminal cells, whereas human CD133 is less broadly expressed in benign tissue, but is upregulated in regions of inflammation within tumors, suggesting a lack of association with CSCs (Missol-Kolka *et al*., 2010). Thus, the utility of putative CSC markers for identification of prostate CSCs or for clinical prognosis remains unclear.

Over time, the CSC nomenclature has become increasingly non-specific in the published literature. As it remains difficult to evaluate the hierarchical organization of solid tumors, the strict *in vivo* definition of CSCs has not yet been experimentally demonstrated in prostate cancer. Instead, current efforts in the field have been focused on the identification of rare TICs using transplantation assays in a heterologous microenvironment. Even by this standard, however, prostate CSCs have not been satisfactorily demonstrated, as they have not been isolated from primary human tumors and unequivocally shown to have higher tumor-initiating capability than non-CSC populations in xenotransplantation assays. Consequently, at present there is relatively little evidence that human prostate cancer adheres to a CSC model.

Studies of prospective mouse prostate cancer stem cells

Given the technical issues associated with direct identification of human prostate CSCs/TICs, mouse models represent an important alternative to investigate the CSC model in prostate cancer. Mouse models are advantageous as they can largely circumvent the cross-species transplantation issue and as candidate CSCs can be analyzed from genetically defined tumors *in vivo*. For example, several studies have utilized flow cytometry approaches to identify candidate CSCs in mouse models of breast cancer, including the *MMTV-Wnt-1*, *MMTV-erbB2* and *p53* null models (Cho *et al*., 2008; Vaillant *et al*., 2008; Zhang *et al*., 2008). However, to what extent the CSCs in these mouse models recapitulate human cancers is not clear. For example, distinct genetic models of breast cancer have yielded different outcomes with respect to conforming to the CSC model (Vaillant *et al*., 2008).

To date, mouse prostate cancer models have not been extensively evaluated with respect to tumor-initiation assays and the identification of candidate CSCs. Notably, one study has shown that flow-sorted Lin^- $Sca\text{-}1^+CD49f^{high}$ cells from a *Pten* null mouse model could display TIC properties using both sphere-forming assays in culture and following renal grafting *in vivo*, which gave rise to carcinoma lesions in the resulting grafts (Mulholland *et al*., 2009). A similar approach has been recently used to isolate $Lin^-Sca\text{-}1^+$ cells from tumors in a conditional *Pten* deletion model, and showed that cancer-associated fibroblasts can significantly potentiate the stemness and growth-properties of these candidate CSCs in renal grafts (Liao *et al*., 2010).

Although these studies avoid the technical issues associated with cross-species transplantation, the extent to which renal grafting assays can recapitulate the endogenous prostate cancer microenvironment remains an issue for their interpretation. Moreover, the markers used for flow sorting of putative CSCs require further validation, both in mouse and in human prostate. For example, one recent study found that the proportion of $Sca\text{-}1^+$ cells first increases and then decreases in tumors of TRAMP (transgenic adenocarcinoma mouse prostate) mice after castration, perhaps in agreement with a role for $Sca\text{-}1^+$ CSCs in castration-resistant cancer progression (Tang *et al*., 2009). Nevertheless, it is already apparent that mouse models will be of tremendous utility for mechanistic investigations of putative CSCs in prostate cancer.

Identification of normal prostate epithelial stem cells in mice

As a CSC may originate from oncogenic transformation of a normal tissue stem cell, many studies have focused on the identification of normal prostate epithelial stem cells as a starting point for subsequent studies to determine whether genetic alterations of these stem cells may confer tumor-initiating properties. However, studies from different laboratories have used distinct experimental methodologies, and have identified potentially non-overlapping candidate stem cell populations. The identification of these candidate stem cells has led to examination of whether these populations in the mouse or analogous populations in human can serve as cells of origin for prostate cancer, and has engendered similar controversies.

Adult prostate epithelial stem cells are unlikely to divide continuously to maintain tissue homeostasis, unlike stem cell populations in the epidermis and intestine. Consequently, the growth-quiescence of the normal adult prostate epithelium might imply that prostate stem cells do not exist. However, it is very likely that adult prostate epithelial stem cells function in the context of androgen-mediated prostate regeneration. Although the prostate regresses following androgen-deprivation treatment, it will regenerate back to its hormonally-intact size when physiological levels of androgen are restored, and this process of serial regression and regeneration can be repeated for many cycles (Isaacs, 1985; Tsujimura *et al*., 2002). This phenomenon indicates that adult prostate epithelial stem cells exist in the regressed state, perhaps behaving similarly to quiescent stem cells in other tissues that respond to wound repair and function in tissue regeneration.

Following androgen ablation, 90% of the luminal cells, but only a small percentage of basal cells will undergo apoptosis during the process of regression (English *et al*., 1987; Evans and Chandler, 1987). Consequently, early studies favored a basal localization of stem cells, as most basal cells are castration resistant. Moreover, immunostaining analyses of gene expression identified 'intermediate' cells in the basal layer, which coexpress both basal-specific and luminal-specific CKs, as well as the luminal marker prostate-specific antigen

(Verhagen *et al*., 1988; Bonkhoff *et al*., 1994; van Leenders *et al*., 2000). These observations suggested a model in which basal stem cells give rise to intermediate 'transit-amplifying' cells that are progenitors for luminal cells (Bonkhoff and Remberger, 1996), although this lineage relationship lacked experimental verification.

Some evidence has also supported the existence of luminal epithelial stem cells. Most notably, although *p63* is a marker of basal cells, and *p63* null mutant mice fail to form prostate (Mills *et al*., 1999; Yang *et al*., 1999; Signoretti *et al*., 2000), grafting of urogenital rudiments from *p63* null cells could rescue tissue with prostatic ductal structures (Kurita *et al*., 2004). Although these prostate grafts lacked basal cells, they nonetheless could undergo multiple rounds of regression/regeneration (Kurita *et al*., 2004).

As discussed below, more recent studies have adopted two distinct experimental approaches for the identification of prostate epithelial stem cells. Notably, flow cytometry and tissue reconstitution approaches have identified basal stem cells, whereas a genetic lineage-marking approach has identified luminal stem cells.

Flow cytometry-based approaches

One major approach to identify normal prostate epithelial stem cells has been to use cell-surface markers for isolation of cell populations by flow cytometry, followed by assaying their stem cell properties by tissue reconstitution in renal grafts. This tissue reconstitution assay is based on the pioneering work of (Cunha and Lung, 1978) who showed that combination of normally quiescent adult prostate tissue with embryonic urogenital mesenchyme (UGM) in renal grafts could stimulate formation of new prostatic ducts. This assay has been further developed by the Witte group, who used dissociated prostate epithelial cells together with rat UGM for tissue reconstitution in renal grafts (Xin *et al*., 2003).

Using this assay, the Wilson and Witte laboratories showed that Sca-1$^+$ cells were more efficient in generating prostatic tissues than Sca-1$^-$ cells, and thus were enriched for candidate prostate stem cells (Burger *et al*., 2005; Xin *et al*., 2005). Further studies using prostate colony- and sphere-forming assays in culture as well as tissue reconstitution assays have shown that prostate stem cell populations could be further enriched in combination with markers such as CD49f and Trop2 (Lawson *et al*., 2007; Goldstein *et al*., 2008). Importantly, these Lin$^-$Sca-1$^+$ CD49f$^+$ populations express basal markers such as CK5 and CK14, but express low levels of luminal markers such as CK8, CK18 and AR (Lawson *et al*., 2007). Other studies have employed cell-surface markers such as CD44 and CD133 for enrichment of prostate stem cell populations. Notably, 10% of single Lin$^-$Sca-1$^+$ CD133$^+$CD44$^+$CD117$^+$ cells were reported to be capable of prostate reconstitution in the renal grafting assay (Leong *et al*., 2008). However, it is less clear whether the responsible cells are basal or luminal in this study, as Lin$^-$Sca-1$^+$CD133$^+$CD44$^+$CD117$^+$ cells are both basal and luminal in the mouse prostate epithelium, but exclusively basal in the human prostate epithelium (Leong *et al*., 2008); a further complication arises from a previous report that CD117 (c-kit) is not expressed within the prostate epithelium (Simak *et al*., 2000). Overall, these different studies in the literature have used distinct marker combinations and thus raise the possibility that they are not defining similar cell populations.

Although it represents a powerful methodology, the renal graft tissue reconstitution assay may have limitations for identification of adult prostate stem cells. As noted above, adult prostate epithelial stem cells are likely to correspond to quiescent cells that can drive the process of regeneration during androgen restoration to the regressed state. However, it is unclear whether the renal graft tissue reconstitution assay accurately models the process of prostate epithelial regeneration. In particular, this approach relies upon the use of a heterologous stromal component (embryonic UGM) that promotes epithelial proliferation and differentiation, and may alter the properties of adult prostate epithelial progenitors (Risbridger and Taylor, 2008). Thus, the epithelial cells used for tissue reconstitution in renal grafts are removed from their original microenvironment, perhaps comprising a stem cell niche, and are instead placed in the context of embryonic mesenchyme, which may reprogram their fates. Thus, the ability of isolated cells to reconstitute prostate ducts in this assay may not represent definitive evidence that they correspond to stem cells *in vivo*, as conceivably non-stem cells could be reprogrammed to yield similar outcomes in this assay. Furthermore, the removal of epithelial cells from their normal microenvironment may lead to a failure to survive and/or proliferate, whereas UGM might not always represent a permissive environment to display stem cell properties.

In addition to the use of mice, several studies have utilized flow cytometry-based approaches to isolate candidate prostate epithelial stem cells directly from primary human tissue, and have shown that these stem cells are basal. In particular, human prostate epithelial cells expressing high levels of $\alpha_2\beta_1$-integrin performed better than the total basal population in colony formation assays, as well as in prostate duct reconstitution after subcutaneous injection (Collins *et al*., 2001). A subsequent study showed that further enrichment of prospective human stem cells can be carried out using the marker CD133 (Richardson *et al*., 2004). Similarly, the Trop2 marker can be used in combination with CD49f to enrich sphere-forming cells from the human prostate (Goldstein *et al*., 2008). Furthermore, the Witte group has shown that human prostate sphere-forming cells are predominantly basal, and can reconstitute prostatic tissue after recombination with UGM and subcutaneous injection into immunodeficient SCID mice (Garraway *et al*., 2010). As is the case with the analogous studies carried out in the mouse, it remains unclear whether these isolated cell populations truly behave as adult stem cells in the human prostate.

Genetic lineage marking

An alternative method for identification of adult stem cells involves genetic lineage marking of progenitor cells,

followed by analysis of progeny differentiation *in vivo*, as has initially been demonstrated for stem cells in the *Drosophila* ovary, testis and intestine (Spradling *et al*., 2008). Analogous studies in the mouse have used genetic lineage marking to identify stem cell populations in the intestine, stomach and hair follicle (Barker *et al*., 2007, 2010; Snippert *et al*., 2010). In the mouse, this methodology generally utilizes a cell type-specific inducible Cre recombinase to drive genetic recombination events in cell types of interest. Typically, Cre recombinase can be made inducible through fusion with truncated-steroid hormone receptors, such as the estrogen receptor (ER), which have been engineered to be unresponsive to endogenous estradiol and instead can be activated by tamoxifen (Feil *et al*., 1997; Indra *et al*., 1999). In combination with an appropriate Cre reporter allele, induction of recombination results in cell marking through the expression of a reporter such as yellow fluorescent protein (YFP), so that these specific cells and their progeny can be indelibly marked and followed *in vivo*. This method also allows the simultaneous deletion or activation of genes of interest (such as oncogenes), so that the targeted cells can both be marked and mutated. Thus, this methodology is highly suitable for the investigation of normal adult stem cells, as well as TICs, as organ development and tumor formation occur within an intact *in vivo* environment, rather than in heterologous graft sites.

However, there are also several potential caveats for the genetic lineage-marking approach. One important issue is the specificity of the lineage-marking methodology, which requires careful control to ensure that activation of the inducible Cre recombinase takes place only in the cells of interest, and not in ectopic cell types, as is sometimes observed. Another limitation is that transient administration of tamoxifen may perturb normal cell behavior, which is a particular concern in hormonally responsive tissues such as the prostate. Finally, the inducible Cre allele itself may alter cellular properties, as gene targeting to place Cre recombinase under the control of a specific promoter may also alter the function of the endogenous locus. Ideally, these limitations in the identification of stem cells by lineage marking should be circumvented by validation using additional experimental approaches.

Our laboratory has recently applied this lineage-marking methodology to identify a rare luminal epithelial population with stem cell properties during prostate regeneration (Wang *et al*., 2009). These cells, which we term CARNs (Castration-resistant Nkx3.1-expressing cells) can be marked using a genetically engineered mouse line in which the activity of an inducible Cre^{ERT2} recombinase is under the control of the endogenous promoter for *Nkx3.1*. Interestingly, *Nkx3.1* encodes a homeodomain transcription factor that represents the earliest specific marker for the prostate epithelium in organogenesis, and is also frequently downregulated during prostate cancer initiation (Abate-Shen *et al*., 2008). Although all luminal cells as well as a small percentage of basal cells in the hormonally intact adult prostate express Nkx3.1, only rare luminal CARNs express Nkx3.1 in the regressed epithelium (Wang *et al*., 2009).

Thus, after Cre activation by tamoxifen treatment in $Nkx3.1^{CreERT2/+}$, *R26R-YFP*/+ castrated males, we observed yellow fluorescent protein expression in rare luminal epithelial cells, corresponding to lineage-marked CARNs. Following androgen-mediated prostate regeneration, we observed that lineage-marked YFP^+ progeny was present in both luminal ($CK18^+$) and basal ($CK5^+$, $p63^+$) compartments, indicating that the CARNs population contains a bipotential progenitor. Furthermore, evidence for short-term and long-term self-renewal was obtained from BrdU-labeling experiments during prostate regeneration, as well as from the persistence of lineaged-marked cells after multiple rounds of serial regression/regeneration. In further studies, we showed that single-cell transplantation of lineage-marked CARNs resulted in formation of prostatic ducts in renal grafts that contained all three prostate epithelial cell types, indicating multipotency of CARNs. Taken together, these data provide strong evidence for the stem cell properties of CARNs in prostate regeneration, using both *in vivo* lineage marking as well as tissue reconstitution assays.

These findings suggest two possible models for the function of CARNs in the prostate epithelium (Wang *et al*., 2009). One model is that basal and luminal stem cell populations may coexist within the prostate epithelium, and that CARNs serve as luminal stem cells. Alternatively, CARNs might correspond to facultative stem cells, corresponding to luminal progenitors that acquire stem cell properties following androgen deprivation. In this second model, CARNs would be functionally indistinguishable from other adult prostate stem cells, and would represent the stem cells that drive prostate epithelial regeneration, which can be considered analogous to a wound-healing response. These two models have different implications for interpreting the role of CARNs as cells of origin for prostate cancer, as discussed below.

In summary, flow cytometry-based approaches have identified distinct prostate basal cell populations, whereas a genetic lineage-marking approach has identified a luminal stem cell population (CARNs). Notably, these putative stem cell populations have been assayed for their functional properties using different experimental assays. As CARNs have been shown to display stem cell properties *in vivo,* they may be particularly relevant for understanding the origin of prostate cancer.

Cell of origin and TICs

The identification of normal cells that can serve as a cell of origin for prostate cancer is highly relevant for understanding the applicability of a CSC model, and is currently under intense investigation. The cell of origin may also have clinical significance, as in the case of breast cancer, distinct tumor subtypes have been proposed to originate through transformation of different

progenitors within the mammary epithelial lineage hierarchy (Lim *et al*., 2009; Visvader, 2009; Molyneux *et al*., 2010). Thus, it is conceivable that there may be distinct cells of origin for other epithelial cancers, and different cells of origin may give rise to clinically relevant subtypes that differ in their prognosis and treatment outcome.

Basal cells as a cell of origin

Although prostate tumors display a strongly luminal phenotype, this does not exclude the possibility that basal cells could be a cell of origin for prostate cancer. In particular, it is possible that transformed basal cells could differentiate to generate large numbers of luminal cancer cells. For example, prostate-specific conditional deletion of *Pten* by a probasin-Cre driver allele has been shown to result in a basal cell expansion accompanied by increased number of intermediate cells, suggesting a basal cell of origin (Wang *et al*., 2006). Other studies investigating the capabilities of basal cells have primarily utilized flow –cytometry-based approaches (Figure 3a). In particular, mouse $Lin^{-}Sca\text{-}1^{+}CD49f^{high}$ cells correspond to a predominantly basal population, yet can differentiate into luminal cells in grafts (Lawson *et al*., 2007). Furthermore, grafting of $Lin^{-}Sca\text{-}1^{+}CD49f^{high}$ cells together with FGF10-expressing UGM produced multifocal glandular carcinoma similar to the small glandular structures observed in human prostate cancer (Lawson *et al*., 2010). Similarly, lentiviral overexpression of ERG1 in $Lin^{-}Sca\text{-}1^{+}CD49f^{high}$ cells resulted in a PIN phenotype, while co-activation of Akt and AR signaling resulted in adenocarcinoma. However, these mouse tumors do not always recapitulate the luminal features of human prostate cancer, as expression of the basal marker p63 is frequently observed in these tumors (Lawson *et al*., 2010).

An important recent study from the Witte laboratory has used similar approaches with primary human prostate tissues to show that basal cells are a cell of origin for human prostate cancer (Goldstein *et al*., 2010). Similar to the mouse studies, cell-surface markers were used for flow sorting of dissociated-luminal ($CD49f^{lo}Trop2^{hi}$) and -basal ($CD49f^{hi}Trop2^{hi}$) cells. After combination with mouse UGM and subcutaneous injection into highly immunodeficient NOD-SCID-IL-$2R\gamma^{-/-}$ mice, only basal cells formed prostatic ducts after 16 weeks of growth. Furthermore, lentiviral overexpression of activated Akt and ERG in $CD49f^{hi}Trop2^{hi}$ cells resulted in high-grade PIN, and coexpression of these two genes together with AR resulted in adenocarcinoma with strong resemblance to clinical prostate cancer.

In contrast with basal cells, no prostate ducts formed using either infected or uninfected luminal cells in this study (Goldstein *et al*., 2010). However, the failure of luminal cells to form prostate ducts when combined with UGM and matrigel may indicate that luminal cell outgrowth is disfavored in this heterologous environment. As luminal cells do not grow under these conditions, it remains possible that basal stem cells can give rise to luminal cells that are the actual target of tumor initiation in reconstituted tissue within these grafts. Moreover, it is conceivable that the overexpression of AR may actually drive basal cells toward a luminal fate and thereby facilitate their transformation. Nonetheless, the work of Goldstein and colleagues provides the first strong evidence that oncogenic transformation of basal cells can result in tumors with the luminal features of human prostate cancer.

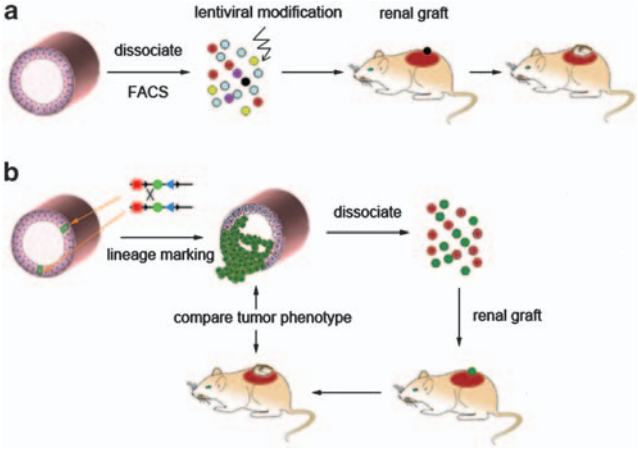

Figure 3 Analysis of prostate TICs in mouse models. (**a**) Identification of mouse prostate TICs using a renal grafting tissue reconstitution assay. Dissociated mouse prostate cells are flow sorted using cell-surface markers, followed by lentiviral infection for expression of oncogenes to induce transformation. (**b**) Lineage analysis for identification of prostate TICs. Genetic lineage marking is used to induce transformation of specific cell types followed by tumor growth in the prostate *in vivo*. Tumor cells can be dissociated and analyzed by renal grafting to compare phenotypes of tumors generated using these two different approaches.

Luminal cells as a cell of origin

Other studies have provided evidence that luminal cells can serve as cells of origin for prostate cancer. For example, pathological analysis of high-grade PIN samples, which still retain basal cells, suggest that molecular events associated with human prostate cancer initiation such as upregulation of c-MYC and shortening of telomere length occur exclusively in luminal cells but not their basal neighbors (Meeker *et al*., 2002; Gurel *et al*., 2008). In mouse models, a recent study using a prostate-specific antigen-*Cre, Pten*$^{loxP/loxP}$ prostate cancer model reported that the initial hyperplastic cells were all luminal (Korsten *et al*., 2009). Finally, our laboratory has shown that targeted deletion of *Pten* in CARNs resulted in high-grade PIN and carcinoma, indicating that CARNs are a cell of origin (Wang *et al*., 2009). At present, however, it is unknown whether CARNs exist in the hormonally intact prostate epithelium, and if so, whether these cells can serve as cells of origin. Indeed, if CARNs correspond to facultative stem cells, as discussed above, they may correspond to a cell state that is only acquired in the regressed epithelium. Therefore, as CARNs can currently only be visualized in

the regressed prostate, the identification of other molecular markers for CARNs will be invaluable in addressing this central question.

In principle, the approach of genetic lineage marking can be extended to other cell types and prostate cancer models. The advantage of this methodology is that the tumor-initiating capability of cells can be readily tested by introducing genetically defined mutations *in vivo*, allowing growth and assessment of tumors in their natural environment (Figure 3b). To test the CSC model, tumors derived from these genetic models can be analyzed for any features of hierarchical heterogeneity and compared with human disease samples. In addition, lineage-marked TICs may also be transplanted in renal graft assays to compare tumors derived using these two different methods (Figure 3b). Given the complexity of human prostate cancers, it is likely that distinct mouse models may only recapitulate properties of specific subtypes of human prostate cancer, not all of which may follow a CSC model.

As in the case of the normal prostate epithelial stem cell, it is important to note that these studies on the cell of origin for prostate cancer are not mutually inconsistent, in part because they employ distinct functional assays. On balance, we favor genetic lineage marking and inducible tumorigenesis *in vivo* as evidence for a cell of origin as tumor formation occurs in the endogenous site without disruption of putative niche interactions, whereas transplantation assays may not recapitulate an authentic prostate tissue microenvironment. However, there may also be multiple cells of origin for prostate cancer. By analogy with breast cancer (Visvader, 2009), it may be the case that distinct cells of origin give rise to prostate cancer that display different subtypes. Such subtypes might correspond to rare pathological variants that in total account for less than 5–10% of disease cases (Mazzucchelli *et al*., 2008), or to molecular subtypes that are now being defined through comprehensive oncogenomic analyses (Taylor *et al*., 2010). Importantly, as such subtypes might differ in their prognosis and/or response to treatment, the investigation of cells of origin for prostate cancer might have important clinical implications.

Role of AR

The functional role of AR in prostate TICs and CSCs has received relatively little attention in the literature. Expression of AR is observed throughout human prostate cancer progression and remains at high-levels in castration-resistant tumors (Attard *et al*., 2009; Taylor *et al*., 2010). Indeed, despite the wealth of molecular mechanisms that have been documented to result in castration resistance, all of these mechanisms result in upregulation of AR signaling activity (Chen *et al*., 2008; Attard *et al*., 2009).

In this context, it is perhaps unexpected that the existing literature has suggested that prostate CSCs are AR-negative. For example, $CD133^+$ cells in clinical prostate specimens and $CD44^+$ cells from human prostate cancer cell lines have been reported to be AR^- (Patrawala *et al*., 2006; Miki *et al*., 2007). These findings appear to be inconsistent with the documented function of AR in prostate cancer progression and mechanisms of castration resistance, as has been noted previously (Sharifi *et al*., 2006). Instead, the upregulation of AR activity through cancer progression is consistent with selection for a castration-resistant AR^+ CSC that can generate tumor cells that are themselves castration resistant. In contrast, it is unclear how castration resistance involving upregulation of AR activity could be selected for in non-AR-expressing CSCs. Moreover, the ability of putative AR^- CSCs to differentiate into AR^+ cancer cells in sphere formation or renal graft assays does not readily resolve this issue, as such AR^+ progeny presumably lack CSC properties, and thus should not be capable of propagating the tumor. One possibility is that these AR^+ progeny could dedifferentiate into AR^- CSCs, but this would suggest that such AR^+ cells should be identifiable in appropriate assays as having CSC properties.

Overall, these considerations suggest that prostate CSCs, if they exist, should be AR^+, at least in more advanced stages of cancer progression. However, an earlier role for AR is also suggested by recent studies of the mechanisms for formation of *TMPRSS2–ERG* fusions, a chromosomal rearrangement that is found in nearly 50% of prostate adenocarcinomas, and may represent an initiating event in prostate carcinogenesis (Clark *et al*., 2008; Mosquera *et al*., 2008, 2009). Studies in AR-expressing prostate cancer cell lines have shown that this chromosomal rearrangement can arise due to induced-chromosomal proximity due to AR binding to the *TMPRSS2* and *ERG* genomic loci, followed by DNA damage and/or binding of topoisomerase II (Lin *et al*., 2009; Mani *et al*., 2009; Haffner *et al*., 2010). These findings strongly suggest that *TMPRSS2–ERG* fusion occurs in cells expressing high levels of AR, namely luminal cells. Given the early occurrence of this rearrangement in prostate carcinogenesis, these results are also consistent with a luminal cell of origin.

Conclusions and perspectives

The frequent ineffectiveness of conventional cancer therapeutics may be explained by the CSC model, which suggests that rare therapy-resistant CSCs can repopulate and sustain tumor growth after treatment. However, similar to several other solid tumors, it is still open to debate as to whether CSCs exist in some or all prostate cancers. Even though xenotransplantation of primary human cancer cells into immunodeficient mice may significantly underestimate their tumorigenic potential, prostate CSCs have not been identified to date from human primary tumors using this assay. Furthermore, much work on isolation of prostate CSCs has neglected the importance of the prostate *in vivo* structures and stromal microenvironment, which might provide a niche for normal stem cells or for CSCs.

In particular, the analysis of mouse models using lineage marking and/or flow cytometry approaches has great promise for the identification of normal adult prostate stem cells and for testing their tumor-initiating capability under transformed conditions. Although the identification of mouse TICs may not necessarily support a CSC model, such approaches would allow investigation of the potential hierarchical organization of mouse prostate tumors, followed by validation in human cancers. Such studies should be instrumental in understanding prostate cancer etiology, with the ultimate hope of identifying rational drug targets.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We would like to thank Cory Abate-Shen for comments on the manuscript. We apologize for our inability to cite many relevant papers due to length constraints. This work was supported by grants to MMS from the NIH and the DOD Prostate Cancer Research Program. MMS is a member of the NCI Mouse Models of Human Cancer Consortium.

References

Abate-Shen C, Shen MM. (2000). Molecular genetics of prostate cancer. *Genes Dev* **14**: 2410–2434.

Abate-Shen C, Shen MM, Gelmann E. (2008). Integrating differentiation and cancer: the Nkx3.1 homeobox gene in prostate organogenesis and carcinogenesis. *Differentiation* **76**: 717–727.

Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. (2003). Prospective identification of tumorigenic breast cancer cells. **100**: 3983–3988.

Attard G, Cooper CS, de Bono JS. (2009). Steroid hormone receptors in prostate cancer: a hard habit to break? *Cancer Cell* **16**: 458–462.

Barabe F, Kennedy JA, Hope KJ, Dick JE. (2007). Modeling the initiation and progression of human acute leukemia in mice. *Science* **316**: 600–604.

Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M *et al*. (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* **449**: 1003–1007.

Barker N, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M *et al*. (2009). Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature* **457**: 608–611.

Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH *et al*. (2010). Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units *in vitro*. *Cell Stem Cell* **6**: 25–36.

Birnie R, Bryce SD, Roome C, Dussupt V, Droop A, Lang SH *et al*. (2008). Gene expression profiling of human prostate cancer stem cells reveals a pro-inflammatory phenotype and the importance of extracellular matrix interactions. *Genome Biol* **9**: R83.

Boiko AD, Razorenova OV, van de Rijn M, Swetter SM, Johnson DL, Ly DP *et al*. (2010). Human melanoma-initiating cells express neural crest nerve growth factor receptor CD271. *Nature* **466**: 133–137.

Bonkhoff H, Stein U, Remberger K. (1994). The proliferative function of basal cells in the normal and hyperplastic human prostate. *Prostate* **24**: 114–118.

Bonkhoff H, Remberger K. (1996). Differentiation pathways and histogenetic aspects of normal and abnormal prostatic growth: a stem cell model. *Prostate* **28**: 98–106.

Bonnet D, Dick JE. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* **3**: 730–737.

Burger PE, Xiong X, Coetzee S, Salm SN, Moscatelli D, Goto K *et al*. (2005). Sca-1 expression identifies stem cells in the proximal region of prostatic ducts with high capacity to reconstitute prostatic tissue. **102**: 7180–7185.

Chen Y, Sawyers CL, Scher HI. (2008). Targeting the androgen receptor pathway in prostate cancer. *Curr Opin Pharmacol* **8**: 440–448.

Cho RW, Wang X, Diehn M, Shedden K, Chen GY, Sherlock G *et al*. (2008). Isolation and molecular characterization of cancer stem cells in MMTV-Wnt-1 murine breast tumors. *Stem Cells* **26**: 364–371.

Clark J, Attard G, Jhavar S, Flohr P, Reid A, De-Bono J *et al*. (2008). Complex patterns of ETS gene alteration arise during cancer development in the human prostate. *Oncogene* **27**: 1993–2003.

Collins AT, Habib FK, Maitland NJ, Neal DE. (2001). Identification and isolation of human prostate epithelial stem cells based on alpha(2)beta(1)-integrin expression. *J Cell Sci* **114**: 3865–3872.

Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. (2005). Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* **65**: 10946–10951.

Cozzio A, Passegue E, Ayton PM, Karsunky H, Cleary ML, Weissman IL. (2003). Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. *Genes Dev* **17**: 3029–3035.

Cunha GR, Lung B. (1978). The possible influence of temporal factors in androgenic responsiveness of urogenital tissue recombinants from wild-type and androgen-insensitive (Tfm) mice. *J Exp Zool* **205**: 181–193.

Dubrovska A, Kim S, Salamone RJ, Walker JR, Maira SM, Garcia-Echeverria C *et al*. (2009). The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations. **106**: 268–273.

English HF, Santen RJ, Isaacs JT. (1987). Response of glandular versus basal rat ventral prostatic epithelial cells to androgen withdrawal and replacement. *Prostate* **11**: 229–242.

Evans GS, Chandler JA. (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.

Feil R, Wagner J, Metzger D, Chambon P. (1997). Regulation of Cre recombinase activity by mutated estrogen receptor ligand-binding domains. *Biochem Biophys Res Commun* **237**: 752–757.

Garraway IP, Sun W, Tran CP, Perner S, Zhang B, Goldstein AS *et al*. (2010). Human prostate sphere-forming cells represent a subset of basal epithelial cells capable of glandular regeneration *in vivo*. *Prostate* **70**: 491–501.

Goldstein AS, Lawson DA, Cheng D, Sun W, Garraway IP, Witte ON. (2008). Trop2 identifies a subpopulation of murine and human prostate basal cells with stem cell characteristics. **105**: 20882–20887.

Goldstein AS, Huang J, Guo C, Garraway IP, Witte ON. (2010). Identification of a cell of origin for human prostate cancer. *Science* **329**: 568–571.

Gu G, Yuan J, Wills M, Kasper S. (2007). Prostate cancer cells with stem cell characteristics reconstitute the original human tumor *in vivo*. *Cancer Res* **67**: 4807–4815.

Gurel B, Iwata T, Koh CM, Jenkins RB, Lan F, Van Dang C *et al*. (2008). Nuclear MYC protein overexpression is an early alteration in human prostate carcinogenesis. *Mod Pathol* **21**: 1156–1167.

Haffner MC, Aryee MJ, Toubaji A, Esopi DM, Albadine R, Gurel B *et al*. (2010). Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. *Nat Genet* **42**: 668–675.

Hill RP. (2006). Identifying cancer stem cells in solid tumors: case not proven. *Cancer Res* **66**: 1891–1895, discussion 1890.

Huntly BJ, Shigematsu H, Deguchi K, Lee BH, Mizuno S, Duclos N *et al*. (2004). MOZ-TIF2, but not BCR-ABL, confers properties of leukemic stem cells to committed murine hematopoietic progenitors. *Cancer Cell* **6**: 587–596.

Hurt EM, Kawasaki BT, Klarmann GJ, Thomas SB, Farrar WL. (2008). CD44+ CD24(−) prostate cells are early cancer progenitor/stem cells that provide a model for patients with poor prognosis. *Br J Cancer* **98**: 756–765.

Indra AK, Warot X, Brocard J, Bornert JM, Xiao JH, Chambon P *et al*. (1999). Temporally-controlled site-specific mutagenesis in the basal layer of the epidermis: comparison of the recombinase activity of the tamoxifen-inducible Cre-ER(T) and Cre-ER(T2) recombinases. *Nucleic Acids Res* **27**: 4324–4327.

Isaacs JT. (1985). Control of cell proliferation and cell death in the normal and neoplastic prostate: a stem cell model. In: Rodgers CH, Coffey DS, Cunha G, Grayshack JT, Henman R, Horton R (eds). *Benign Prostatic Hyperplasia*. Department of Health and Human Services: Washington, DC, pp 85–94.

Ishizawa K, Rasheed ZA, Karisch R, Wang Q, Kowalski J, Susky E *et al*. (2010). Tumor-initiating cells are rare in many human tumors. *Cell Stem Cell* **7**: 279–282.

Korsten H, Ziel-van der Made A, Ma X, van der Kwast T, Trapman J. (2009). Accumulating progenitor cells in the luminal epithelial cell layer are candidate tumor initiating cells in a Pten knockout mouse prostate cancer model. *PLoS One* **4**: e5662.

Kurita T, Medina RT, Mills AA, Cunha GR. (2004). Role of p63 and basal cells in the prostate. *Development* **131**: 4955–4964.

Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J *et al*. (1994). A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* **367**: 645–648.

Lawson DA, Xin L, Lukacs RU, Cheng D, Witte ON. (2007). Isolation and functional characterization of murine prostate stem cells. **104**: 181–186.

Lawson DA, Zong Y, Memarzadeh S, Xin L, Huang J, Witte ON. (2010). Basal epithelial stem cells are efficient targets for prostate cancer initiation. **107**: 2610–2615.

Leong KG, Wang BE, Johnson L, Gao WQ. (2008). Generation of a prostate from a single adult stem cell. *Nature* **456**: 804–808.

Li H, Chen X, Calhoun-Davis T, Claypool K, Tang DG. (2008). PC3 human prostate carcinoma cell holoclones contain self-renewing tumor-initiating cells. *Cancer Res* **68**: 1820–1825.

Liao CP, Adisetiyo H, Liang M, Roy-Burman P. (2010). Cancer-associated fibroblasts enhance the gland-forming capability of prostate cancer stem cells. *Cancer Res* **70**: 7294–7303.

Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH *et al*. (2009). Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. *Nat Med* **15**: 907–913.

Lin C, Yang L, Tanasa B, Hutt K, Ju BG, Ohgi K *et al*. (2009). Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer. *Cell* **139**: 1069–1083.

Mani RS, Tomlins SA, Callahan K, Ghosh A, Nyati MK, Varambally S *et al*. (2009). Induced chromosomal proximity and gene fusions in prostate cancer. *Science* **326**: 1230.

Marotta LL, Polyak K. (2009). Cancer stem cells: a model in the making. *Curr Opin Genet Dev* **19**: 44–50.

Mazzucchelli R, Lopez-Beltran A, Cheng L, Scarpelli M, Kirkali Z, Montironi R. (2008). Rare and unusual histological variants of prostatic carcinoma: clinical significance. *BJU Int* **102**: 1369–1374.

Meeker AK, Hicks JL, Platz EA, March GE, Bennett CJ, Delannoy MJ *et al*. (2002). Telomere shortening is an early somatic DNA alteration in human prostate tumorigenesis. *Cancer Res* **62**: 6405–6409.

Miki J, Furusato B, Li H, Gu Y, Takahashi H, Egawa S *et al*. (2007). 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* **67**: 3153–3161.

Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR, Bradley A. (1999). p63 is a p53 homologue required for limb and epidermal morphogenesis. *Nature* **398**: 708–713.

Missol-Kolka E, Karbanova J, Janich P, Haase M, Fargeas CA, Huttner WB *et al*. (2010). Prominin-1 (CD133) is not restricted to stem cells located in the basal compartment of murine and human prostate. *Prostate* (doi:10.1002/pros.21239).

Molyneux G, Geyer FC, Magnay FA, McCarthy A, Kendrick H, Natrajan R *et al*. (2010). BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells. *Cell Stem Cell* **7**: 403–417.

Mosquera JM, Perner S, Genega EM, Sanda M, Hofer MD, Mertz KD *et al*. (2008). Characterization of TMPRSS2-ERG fusion high-grade prostatic intraepithelial neoplasia and potential clinical implications. *Clin Cancer Res* **14**: 3380–3385.

Mosquera JM, Mehra R, Regan MM, Perner S, Genega EM, Bueti G *et al*. (2009). Prevalence of TMPRSS2-ERG fusion prostate cancer among men undergoing prostate biopsy in the United States. *Clin Cancer Res* **15**: 4706–4711.

Mulholland DJ, Xin L, Morim A, Lawson D, Witte O, Wu H. (2009). Lin-Sca-1+CD49f high stem/progenitors are tumor-initiating cells in the Pten-null prostate cancer model. *Cancer Res* **69**: 8555–8562.

O'Brien CA, Pollett A, Gallinger S, Dick JE. (2007). A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* **445**: 106–110.

Passegue E, Wagner EF, Weissman IL. (2004). JunB deficiency leads to a myeloproliferative disorder arising from hematopoietic stem cells. *Cell* **119**: 431–443.

Patrawala L, Calhoun T, Schneider-Broussard R, Zhou J, Claypool K, Tang DG. (2005). Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2- cancer cells are similarly tumorigenic. *Cancer Res* **65**: 6207–6219.

Patrawala L, Calhoun T, Schneider-Broussard R, Li H, Bhatia B, Tang S *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.

Patrawala L, Calhoun-Davis T, Schneider-Broussard R, Tang DG. (2007). Hierarchical organization of prostate cancer cells in xenograft tumors: the CD44+alpha2beta1+ cell population is enriched in tumor-initiating cells. *Cancer Res* **67**: 6796–6805.

Peehl DM. (2005). Primary cell cultures as models of prostate cancer development. *Endocr Relat Cancer* **12**: 19–47.

Pfeiffer MJ, Schalken JA. (2010). Stem cell characteristics in prostate cancer cell lines. *Eur Urol* **57**: 246–254.

Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. (2008). Efficient tumour formation by single human melanoma cells. *Nature* **456**: 593–598.

Reya T, Morrison SJ, Clarke MF, Weissman IL. (2001). Stem cells, cancer, and cancer stem cells. *Nature* **414**: 105–111.

Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C *et al*. (2007). Identification and expansion of human colon-cancer-initiating cells. *Nature* **445**: 111–115.

Richardson GD, Robson CN, Lang SH, Neal DE, Maitland NJ, Collins AT. (2004). CD133, a novel marker for human prostatic epithelial stem cells. *J Cell Sci* **117**: 3539–3545.

Risbridger GP, Taylor RA. (2008). Minireview: regulation of prostatic stem cells by stromal niche in health and disease. *Endocrinology* **149**: 4303–4306.

Rosen JM, Jordan CT. (2009). The increasing complexity of the cancer stem cell paradigm. *Science* **324**: 1670–1673.

Shackleton M, Quintana E, Fearon ER, Morrison SJ. (2009). Heterogeneity in cancer: cancer stem cells versus clonal evolution. *Cell* **138**: 822–829.

Sharifi N, Kawasaki BT, Hurt EM, Farrar WL. (2006). Stem cells in prostate cancer: resolving the castrate-resistant conundrum and implications for hormonal therapy. *Cancer Biol Ther* **5**: 901–906.

Shen MM, Abate-Shen C. (2010). Molecular genetics of prostate cancer: new prospects for old challenges. *Genes Dev* **24**: 1967–2000.

Shmelkov SV, Butler JM, Hooper AT, Hormigo A, Kushner J, Milde T *et al*. (2008). CD133 expression is not restricted to stem cells, and both CD133+ and CD133- metastatic colon cancer cells initiate tumors. *J Clin Invest* **118**: 2111–2120.

Signoretti S, Waltregny D, Dilks J, Isaac B, Lin D, Garraway L *et al*. (2000). p63 is a prostate basal cell marker and is required for prostate development. *Am J Pathol* **157**: 1769–1775.

Simak R, Capodieci P, Cohen DW, Fair WR, Scher H, Melamed J *et al*. (2000). Expression of c-kit and kit-ligand in benign and malignant prostatic tissues. *Histol Histopathol* **15**: 365–374.

Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T *et al*. (2004). Identification of human brain tumour initiating cells. *Nature* **432**: 396–401.

Snippert HJ, Haegebarth A, Kasper M, Jaks V, van Es JH, Barker N *et al*. (2010). Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. *Science* **327**: 1385–1389.

Spradling AC, Nystul T, Lighthouse D, Morris L, Fox D, Cox R *et al*. (2008). Stem cells and their niches: integrated units that maintain Drosophila tissues. *Cold Spring Harb Symp Quant Biol* **73**: 49–57.

Tang Y, Hamburger AW, Wang L, Khan MA, Hussain A. (2009). Androgen deprivation and stem cell markers in prostate cancers. *Int J Clin Exp Pathol* **3**: 128–138.

Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS *et al*. (2010). Integrative genomic profiling of human prostate cancer. *Cancer Cell* **18**: 11–22.

Tsujimura A, Koikawa Y, Salm S, Takao T, Coetzee S, Moscatelli D *et al*. (2002). Proximal location of mouse prostate epithelial stem cells: a model of prostatic homeostasis. *J Cell Biol* **157**: 1257–1265.

Uchida N, Buck DW, He D, Reitsma MJ, Masek M, Phan TV *et al*. (2000). Direct isolation of human central nervous system stem cells. **97**: 14720–14725.

Ugolkov AV, Eisengart LJ, Luan C, Yang XJ. (2010). Expression analysis of putative stem cell markers in human benign and malignant prostate. *Prostate* (doi:10.1002/pros.21217).

Vaillant F, Asselin-Labat ML, Shackleton M, Forrest NC, Lindeman GJ, Visvader JE. (2008). The mammary progenitor marker CD61/beta3 integrin identifies cancer stem cells in mouse models of mammary tumorigenesis. *Cancer Res* **68**: 7711–7717.

van Leenders G, Dijkman H, Hulsbergen-van de Kaa C, Ruiter D, Schalken J. (2000). Demonstration of intermediate cells during human prostate epithelial differentiation in situ and *in vitro* using triple-staining confocal scanning microscopy. *Lab Invest* **80**: 1251–1258.

Verhagen AP, Aalders TW, Ramaekers FC, Debruyne FM, Schalken JA. (1988). Differential expression of keratins in the basal and luminal compartments of rat prostatic epithelium during degeneration and regeneration. *Prostate* **13**: 25–38.

Visvader JE. (2009). Keeping abreast of the mammary epithelial hierarchy and breast tumorigenesis. *Genes Dev* **23**: 2563–2577.

Wang JC, Dick JE. (2005). Cancer stem cells: lessons from leukemia. *Trends Cell Biol* **15**: 494–501.

Wang S, Garcia AJ, Wu M, Lawson DA, Witte ON, Wu H. (2006). Pten deletion leads to the expansion of a prostatic stem/progenitor cell subpopulation and tumor initiation. **103**: 1480–1485.

Wang X, Kruithof-de Julio M, Economides KD, Walker D, Yu H, Halili MV *et al*. (2009). A luminal epithelial stem cell that is a cell of origin for prostate cancer. *Nature* **461**: 495–500.

Wei C, Guomin W, Yujun L, Ruizhe Q. (2007). Cancer stem-like cells in human prostate carcinoma cells DU145: the seeds of the cell line? *Cancer Biol Ther* **6**: 763–768.

Xin L, Ide H, Kim Y, Dubey P, Witte ON. (2003). *in vivo* regeneration of murine prostate from dissociated cell populations of postnatal epithelia and urogenital sinus mesenchyme. **100**(Suppl 1): 11896–11903.

Xin L, Lawson DA, Witte ON. (2005). The Sca-1 cell surface marker enriches for a prostate-regenerating cell subpopulation that can initiate prostate tumorigenesis. **102**: 6942–6947.

Yang A, Schweitzer R, Sun D, Kaghad M, Walker N, Bronson RT *et al*. (1999). p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. *Nature* **398**: 714–718.

Zhang M, Behbod F, Atkinson RL, Landis MD, Kittrell F, Edwards D *et al*. (2008). Identification of tumor-initiating cells in a p53-null mouse model of breast cancer. *Cancer Res* **68**: 4674–4682.

Zhu L, Gibson P, Currle DS, Tong Y, Richardson RJ, Bayazitov IT *et al*. (2009). Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. *Nature* **457**: 603–607.