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Prostate cancer stem cell biology

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Abstract

The cancer stem cell (CSC) model provides insights into pathophysiology of cancers and their therapeutic response. The CSC model has been both controversial, yet provides a foundation to explore cancer biology. In this review, we provide an overview of CSC concepts, biology and potential therapeutic avenues. We then focus on prostate CSC including (1) their purported origin as either basal-derived or luminal-derived cells; (2) markers used for prostate CSC identification; (3) alterations of signaling pathways in prostate CSCs (4) involvement of prostate CSCs in metastasis of PCa and (5) microRNA-mediated regulation of prostate CSCs. Although definitive evidence for the identification and characterization of prostate CSCs still remains unclear, future directions pursuing therapeutic targets of CSCs may provide novel insights for the treatment of PCa.

Keywords

Cancer stem cell; prostate cancer; cell-of-origin; marker; signaling pathway; microRNA regulation; Wnt; marker; metastatsis; microRNA

Introduction

In recent years, cancer stem cells (CSCs) have attracted considerable attention due to their potential role to help to elucidate a series of vital unexplained phenomena in cancer research, including the resistance to chemotherapy and radiation therapy, tumor recurrence and metastasis. The concept of CSC was introduced more than 50 years ago when it was recognized that only a small proportion of cells (0.01%–1%) in tumor isolates are clonogenic and extensively proliferative *in vitro* and *in vivo* (1, 2), indicating that these cells might represent tumor stem cells. The CSC hypothesis has recently been revitalized as the development of novel methods for identification, purification and characterization of normal stem cells. Although no consensus definition of a CSC exists, a general descriptor is “a cell within a tumor that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor” (3).

When CSCs are mentioned, it is important to pay note a lack of clarity in the literature between CSC and another term, tumor initiating cell (TIC). Many investigators regard these two concepts as same. Others define CSCs that have been strictly defined by their position and function within the cellular hierarchy as rare TICs and strict criteria have been proposed to distinguish them functionally (4, 5). A CSC is purified by lineage selection, possesses certain intrinsic biological properties normally associated with stem cells, and is solely able

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to reconstitute, in a recipient animal, a tumor that is identical to the parental tumor from which it was derived and can be serially xenotransplanted indefinitely; whereas, a TIC is able to regrow the tumor from which it was isolated, and it's not necessarily rare, which means the majority of cells within a tumor could potentially possess TIC properties, besides, the identification of TIC does not by itself imply a hierarchical organization of a tumor.

Prostate cancer (PCa) is the second leading malignancy in American men with an estimated 217,730 new cases and 32,050 deaths in 2010 (6). Most patients with advanced PCa respond to androgen deprivation therapy (ADT) at the beginning of the treatment period, since the majority of prostate cancer cells are androgen-dependent. However, many patients eventually present androgen-independent cancers and subsequently widespread metastasis may develop (7). Over 90% of PCa related mortality results from systemic dissemination and metastasis (8). PCa research has now focused on the CSC to get a better understanding of the mechanisms of the tumor initiation, progression and metastasis, which will eventually help to treat the PCa patients more effectively.

In this review, we discuss the origin, identification, alterations in signaling pathways and microRNA regulation of CSCs. We also review the current status of studies to identify CSCs in prostate malignancy and present evidence for the surface marker and therapeutic targets of prostate CSCs.

1. CSC characteristics

1.1 Origin of CSCs

CSCs share similar properties with normal stem cells, such as long lifespan, induction of angiogenesis, resistance to apoptosis, ability for self-renewal and differentiation, and expression of Oct4 (9) etc. These similarities suggest CSCs might originate from adult stem cells. Besides adult stem cells, there are other 3 possible origins of CSCs as reviewed by Soltanian et al (10). The second probable origin of CSCs is a population of more differentiated transit-amplifying/progenitor cells. There have been strong evidences supporting the concept that a committed progenitor can be the cancer-initiating cell as a result of oncogenic transformation. CSCs also might originate from embryonic stem cell-like cells that are abnormally left in the tissues during ontogenesis. The last possible origin is that tumor-initiating mutations in mature progenitor or in terminally differentiated cells may produce CSCs. Although the origin of CSCs has not been precisely defined, characteristics of CSCs can be determined.

1.2 Identification of CSCs

CSCs are a subpopulation of cells within a tumor that can initiate tumorigenesis by undergoing self-renewal and differentiation; whereas, other tumor cells lack these properties. Although there are still controversial opinions regarding CSCs, a large number of studies have been performed to identify CSCs in many human cancers. However, to date there is still no gold-standard to define and identify CSCs. Traditionally, CSCs are identified *in vitro* mainly utilizing spheroid formation in cell culture with Matrigel or extra-low attachment conditions. For in vivo evaluation cells are isolated using fluorescence activated cell sorting (FACS) by detecting particular surface markers and then serial transplanted into immune-comprised animal models to test their ability to form tumors (1) from small numbers of cells and (2) that recapitulate the cell distribution of the original tumor. The resultant tumor should present the phenotypic heterogeneity of the original tumor and also contain CSCs with their self-renewal and differentiation capacity in the following serial transplantations. Most studies use xenotransplantation of CSCs subpopulation into mice with a compromised immune system (such as non-obese diabetic/severe combined immunocompromised (NOD/SCID) mice). However, the use of the immunocompromised

animals presents a deficiency in the model as they do not reflect the true micro-environment of CSCs in human beings. In recent years, genetically defined mouse models which replicate essential features of human cancers such as molecular and histological characteristics, the process of cancer initiation and progression, and response to therapeutics have been developed for further illustration of CSCs in carcinogenesis in human.

Researchers have isolated CSCs by identifying cell surface markers, usually a specific molecule or combination of molecules, or other properties, some of which have been known to be shared by normal stem cells, in different cancers including leukemia (11), breast cancer (9, 12–17), brain tumor (18), lung cancer (19), colon cancer (20–22), melanoma (23), pancreatic cancer (24), prostate cancer (25, 26), head and neck cancer (27), ovarian cancer (28), and lung cancer (29).

The first strong evidence for the existence of CSCs was reported by Bonnet and Dick during their research on leukemia (11, 30). They isolated a subpopulation of leukemic cells that expressed the CD34 surface marker, but lacked the CD38 marker. This $CD34^+/CD38^-$ subpopulation was highly enriched for leukemia-initiating activity after transplantation; whereas, both of the $CD34^+/CD38^+$ and the $CD34^-$ subpopulations could not initiate leukemia (11, 30). However, $CD34^+/CD38^+$ subpopulation have since been shown to initiate and maintain the leukemic process when grafted in NOD/SCID mice (31). Meanwhile, there are leukemia-initiating cells (LICs) within $CD34^-$ fraction, whereas the $CD34^+$ fraction contained normal multi-lineage hematopoietic repopulating cells (32), indicating that the phenotype of LICs is more heterogeneous than previously realized and can vary even within a single sample, which may make LICs particularly difficult to eradicate using therapies targeted against surface antigens.

Compared with the hematopoietic tumors, the properties of CSCs in solid tumors remained relatively undefined until recently. The first solid CSCs were identified in breast cancer by Al-Hajj et al (12) in 2003. From then on, a series of surface markers of breast CSCs were distinguished, including $CD44^+/CD24^{-/low}$ (12, 15), $CD44^+/CD24^{-/low}$/mammosphere signature with claudin-low subtype (14), aldehyde dehydrogenase ($ALDH1^+$) (16), $CD24^{high}/CD49f^{high}/\Delta$-notch-like EGF repeat-containing transmembrane $(DNER)^{high}$ (17), $CD24^{high}/CD49f^{high}/\Delta$-like-1$(DLL1)^{high}$ (17), $CD49f^+/DLL1^{high}/DNER^{high}$ and cancer cells with low proteasome activity(17, 33). All of these markers have been tested and verified through implantation of a very rare subpopulation of the corresponding sorted cells with FACS into the mammary fat pad of NOD/SCID mice to evaluate their self-renewal and differentiation properties. For instance, $Lin^-/CD44^+/CD24^{-/low}$ ESA^+ subpopulation were capable of forming tumors in NOD/SCID mice, even in low concentrations (12). They exhibited invasive properties and demonstrated expression of genes associated with the aggressive behavior of cancer (34), which raises the possibility of $CD44^+/CD24^-$ cells corresponding to cells that have undergone epithelial-mesenchymal transition (EMT). Although the individual markers were reported by different studies, there are relations among different markers within the CSCs of one type of tumor. For example, there is overlap between $ALDH^+$ cell populations with $CD44^+/CD24^-$ cells; $CD44^+/CD24^-/ALDH^+$ cells show higher level of tumorigenic phenotype than $CD44^+/CD24^-$ or $ALDH^+$ cells, and is considered as demonstrating the most prominent tumor-initiating activity in breast cancers (16).

Notwithstanding the significant diversity of CSCs markers in different solid tumors, some tumors share same CSCs markers. For instance, CD133 (prominin 1), an apical plasma membrane protein predominantly on embryonic epithelial structures, has been reported to be an important CSC marker in a number of solid malignancies, including brain tumor (18), prostate carcinoma (25), hepatocellular carcinoma (35), ovarian carcinoma (36), colorectal

cancer (20, 22) and lung cancer (29). Interestingly, CD133 is shown to be a temporary marker of CSCs in small cell lung cancer, but not in non-small cell lung cancer (37). Besides breast cancer, CD44 is also enriched in the tumorigenic CSCs of colon cancer (38), ovarian carcinoma (39), head and neck squamous cell carcinoma (27), and prostate cancer (25). Meanwhile, $CD44^+/CD24^+/ESA^+$ pancreas cancer cells exhibited the stem cell properties of self-renewal, the ability to produce differentiated progeny, and increased expression of the developmental signaling molecule sonic hedgehog (40). Although CD133 and CD44 have been investigated to identify tumor cells with self-renewal CSCs capacity within a large number of solid malignancies, there is still ongoing debate with regard to how universal a marker can be in the solid tumor CSCs study. Contrary to the presence of CD133 and CD44 expression as a marker of CSC, ALDH1 expression is significantly reduced in malignant ovarian tumors compared to normal ovaries and benign tumors (41), although it has been confirmed to identify the tumorigenic cell fraction that is capable of self-renewal and of generating tumors that recapitulate the heterogeneity of the parental breast carcinoma as well as being significantly correlated with the shorter survival of breast cancer patients (16). ALDH is also enriched in bladder TICs and associated with progression of bladder cancer (42). Furthermore, ALDH1 is not co-expressed with the CSCs markers CD44 and CD133 in ovarian tumor, whereas $ALDH^{hi}CD44^+CD133^+$ cells showed enhanced tumorigenicity and metastasis relative to $ALDH^{low}CD44^{low/-}$ cells in breast cancers (43). Taken together, these findings suggest there are functional differences of the CSCs marker in different cancers. Furthermore, they suggest there could be temporal expression of various markers in CSC.

1.3 Altered signaling pathways and other possible therapeutic targets in CSCs

The Wnt, Notch and Hedgehog pathways, which are key mediators of normal embryonic development, were found to regulate CSC biology in different cancers, indicating they may become potential candidate targets for future cancer therapies. For example, Yeung et al demonstrated that activation of the Wnt/β-catenin pathway plays an important role for the establishment and drug-resistant properties of mixed lineage leukemia (MLL) leukemic stem cells (LSCs) (44). Suppression of β-catenin reversed LSCs to a pre-LSC-like stage and significantly reduced the growth of human MLL leukemic cells. Conditional deletion of β-catenin completely abolished the oncogenic potential of MLL-transformed cells. In addition, established MLL LSCs that have acquired resistance against GSK3 inhibitors could be re-sensitized by suppression of β-catenin expression (44). Wnt pathway (45–47) and Notch pathway (48–50) have been implicated in regulating CSCs in T-cell acute lymphoblastic leukemia (ALL), Chronic myeloid leukemia (CML), medulloblastoma, pancreatic cancer (51) and lung adenocarcinoma (52). Alterations in the Hedgehog pathway, either by mis-expression of components of that pathway or by changes in the expression of other cellular components that interfere with the Hedgehog signaling system, may trigger the development of cancer or become essential for maintenance of CSCs of different cancer types, such as colon cancer (53), myeloid leukemia (54) and breast cancer CSCs (55). Inhibition of aberrant Hedgehog signaling can limit clonogenic growth in multiple myeloma, CML, pancreatic cancer and brain tumors (46, 56–59).

Another potential therapeutic target involved in the regulation of CSCs is telomerase, the enzyme which maintains telomeres at the linear ends of chromosomes and plays a critical role in the maintenance of normal stem cells. In cancers, telomerase activity is increased; whereas, the inhibition of telomerase activity limits the self-renewal property of CSCs in multiple myeloma (60). Bmi-1 also exerts an important role in regulating the self-renewal process of stem cells and CSCs (61). In acute myeloid leukemia (AML), small molecules like parthenolide and its derivatives that inhibit NF-κB signaling pathway may primarily inhibit CSC rather than normal stem cells (62, 63). Thus, there are multiple signaling pathways that can be used to potentially target CSC.

1.4 MicroRNA regulation of CSCs

MicroRNA are 21- to 25-nucleotide (nt)–long, noncoding RNAs that induce the target mRNA degradation or repress mRNA translation by imperfect binding to their 30-untranslated region (64). MiRNAs have been demonstrated to control the self-renewal and differentiation of embryonic stem cells (ESC), and aberrant expression and/or functions of miRNAs are implicated in tumorigenesis (65). In recent years miRNAs have been observed to regulate CSCs.

A variety of studies have shown that miRNA expression is altered in CSC compared to normal tissues or non-CSC tumor tissues. In breast CSC studies, a number of miRNAs such as let-7, miR-16, miR-107 and miR-128 were expressed at a much lower level in CSC-enriched cells which contain a high percentage of $CD44^{+}CD24^{-/lo}$ subpopulation than the parental cells and the in vitro differentiated progeny (66). Three miRNA clusters including miR-200c-141, miR-200b-200a-429, and miR-183-96-182, were significantly down-regulated in the $CD44^{+}CD24^{-/lo}$ subpopulation (67). It is noteworthy that these miRNAs were also significantly reduced in normal mammary stem and/or progenitor cells. MiR-451, miR-486, miR-425, miR-16, miR-107, and miR-185, were found to be decreased in the $CD133^{+}$ population in glioblastoma multiforme (GBM) (68). In hepatocellular carcinoma (HCC), $EpCAM^{+}AFP^{+}$ CSCs expressed a unique miRNA signature with upregulation of miR-181 family members and several miR-17-92 cluster members (69). The altered miRNA expression in CSC leads to functional impact on the CSC.

Functional regulation of CSCs by miRNA has been demonstrated in multiple cancer types. In breast CSC, the Lentiviral-mediated overexpression of let-7a inhibited cell proliferation, mammosphere formation, tumor formation, and metastasis in NOD/SCID mice and reduced the proportion of undifferentiated cells *in vitro*. Antagonizing let-7 by antisense oligonucleotides improved *in vitro* propagation of non-CSCs (66). Overexpression of miR-30 in breast CSCs diminished their self-renewal ability and reduced anoikis resistance and increased apoptosis through targeting ubiquitin-conjugating enzyme 9 (UBC9) and integrin b3 (ITGB3). Additionally, knocking down endogenous miR-30 with antagomirs enhanced self-renewal, tumor regeneration, and metastasis in differentiated breast cancer cells. Notably, introduction of both let-7 and miR-30 resulted in greater inhibition of self-renewal and mammospheres formation in breast CSCs than either let-7 or miR-30 alone (70), indicating miRNAs might regulate CSC properties distinctively or synergistically. miRNA expression profiling also revealed that miR-205 and miR-22 were most abundant; whereas, let-7 family members and miR-93 were depleted in $ALDH^{+}$, $Sca\text{-}1^{+}$mouse mammary epithelial cells (71) suggesting that these miRNAs may play a role in the function of ALDH expression. In a study of brain tumor CSCs, miR-34a was found to be down-regulated in human glioblastoma multiforme (GBM) (72). Transfection of miR-34a into bulk GBM cells or GBM CSCs induced cell-cycle arrest or apoptosis and also inhibited xenograft growth, mediated by down-regulation of multiple oncogenic targets like c-MET, Notch-1/2, and CDK6 (72). miR-128 was shown to inhibit glioma stem cell proliferation *in vitro* and glioma xenograft growth *in vivo*, furthermore, overexpression of miR-128 significantly blocked glioma CSCs self-renewal by directly targeting BMI-1 (73). Although the regulation function of miRNAs on CSCs still needs further investigation, these data provide strong evidence that miRNA regulation of CSC will identify important aspects of CSC biology and will provide fresh insight in developing new strategies in the treatment of human cancer.

2. CSCs in PCa

2.1 Origin of Prostate CSCs

Prostatic epithelium contains 3 distinct epithelial cell populations including secretory luminal, basal and neuroendocrine (NE) cells. Luminal cells express prostate-specific antigen (PSA), prostatic acid phosphatase (PAP), androgen receptor (AR), and cytokeratin (CK) 8 and 18. Basal cells are localized beneath the luminal layer and express CK5 and CK14, which has been proved to be the prostatic stem cell niche, but express low levels of AR and no PSA or PAP (74, 75). NE cells are quiescent and express NE-specific markers like chromogranin A and do not express AR or PSA (76).

As the origin of CSCs still remains unclear, the origin of prostate CSCs is controversial as well and is even more complicated. The cell of origin for PCa is highly relevant for the prostate CSCs study. Different cells of origin may generate clinically relevant subtypes with different prognosis and outcome. In breast cancer, different tumor subtypes have been proposed to originate through transformation of different progenitors within the mammary epithelial lineage hierarchy (77). There are 2 possible cell-of-origin resources in PCa; basal cell-of-origin and luminal cell-of-origin. Evidence for derivation of CSC of both of these cell types have been published.

2.1.1 Basal cell-of-origin—The basal-cell layer of the prostate has been traditionally hypothesized to harbor stem cells (74, 78), since basal cells not only express many molecules that regulate stem cell self-renewal and survival, such as p63, hTERT and Bcl-2 (5, 79), but also express stem cell markers including CD44, CD49f, CD117, CD133, Tert and p63 (80).

In recent years, prevailing evidence has arisen to support the basal cell-of-origin theory in PCa. For example, basal prostate CSCs with a $CD44^{+}\alpha2\beta1integrin^{high}CD133^{+}$ phenotype were successfully isolated from human PCa biopsies in 2005 (26). Mouse $Lin^{-}Sca\text{-}1^{+}CD49f^{high}$cells correspond to a predominantly basal population, and can differentiate into luminal cells in grafts (81). Basal $Lin^{-}Sca\text{-}1^{+}CD49f^{high}$ cells have the capacity to form tumor-like spheroids in vitro and grafts in vivo (82). Deletion of *Pten* in *Pten*-null mice is associated with an increase in $p63^{+}$ basal cell numbers and the expansion of a prostate stem/progenitor-like subpopulation and consequent tumor initiation (83). The strongest tumor-initiating fraction where fewer than 100 cells are required to initiate new tumor growth in immune-compromised mice has been confirmed to have a basal phenotype (84). Study with FACS-sorted primary cells showed that basal cells but not luminal cells are the cell of origin for PCa (85, 86). Basal cells from primary benign human prostate tissue can initiate prostate cancer in immunodeficient mice (85). The recurrent gene fusions of the 5′ un-translated region of TMPRSS2 to ERG or ETV1 in PCa tissues with outlier expression was identified by Tomlins et al (87), TMPRSS2–ERG is expressed in $CD44^{+}\alpha2\beta1integrin^{high}CD133^{+}$ cells from prostate tumors (88), which supports the hypothesis that the cell-of-origin of PCa is a basal stem cell (84). Recently Rajasekhar et al identified a small population of $TRA\text{-}1\text{-}60^{+}/CD151^{+}/CD166^{+}$ TICs isolated from human prostate xenograft tumor. These stem-like sphere cells do not express AR, PSA, CK18, and are of basal epithelial-like cell type based on the expression of E-cadherin, CK5 and SOX9 and lack of expression of markers of myoepithelial cells (smooth muscle actin), mesenchemal cells (vimentin) and neuroendocrine cells (synaptophysin). However, these sphere cells also lack detectable expression of basal cell enriched p63 and its polarity associated zonula occludens-1(ZO-1) (89). Thus, there is a strong line of evidence that many prostate CSC are derived from basal cells. However, evidence has also accumulated for luminal-cell-of-origin prostate CSC.

2.1.2 Luminal cell-of-origin—Recent studies have provided support for the luminal cell-of-origin theory for prostate CSC. For instance, in *Pten* knockout mice, single $pAkt^+$ cells in the luminal epithelial cell layer overexpressed CK8, Sca-1, Tacstd2 and Clu; whereas basal epithelial cells were always $pAkt^-$. Importantly, $Clu^+Tacstd2^+Sca\text{-}1^+$ progenitor cells, which are candidate TICs, were detected in the luminal epithelial cell layer of normal prostates (90). The initial hyperplastic cells were all luminal as well (90). Genetic lineage-marking demonstrates that rare luminal cells that express Nkx3.1(androgen/AR-regulated transcriptional co-activator) in the absence of testicular androgens (castration-resistant Nkx3-1-expressing cells, CARNs) are bipotential and can self-renew in vivo, and single-cell transplantation assays show that CARNs can reconstitute prostate ducts in renal grafts. Functional assays of Nkx3.1 mutant mice in serial prostate regeneration suggest that Nkx3.1 is required for stem cell maintenance. Furthermore, targeted deletion of *Pten* in CARNs leads to high-grade PIN and rapid carcinoma formation after androgen-mediated regeneration. These observations indicate that CARNs represent a new luminal stem cell population that is an efficient target for oncogenic transformation in prostate cancer (91).

The origin of PCa and the cell type of origin remains controversial in part because distinct functional assays were employed. Furthermore, as PCa is a very heterogenous disease, it is plausible that different PCas are derived from different originating cell types.

2.2 Identification of prostate CSCs

In the research of tumorigenicity or organgenesis for CSCs or normal stem cells, human primary cells are the optimal tool to mimic and represent the original characteristics of tissues. It has been suggested that primary cancer cells rather than cancer cell lines should be chosen to perform CSCs studies (92). However, in prostate CSCs study, it's quite difficult to get primary cell cultures due to limited access. Whether cell lines can serve as *in vitro* models for CSCs study still remains controversial. There are several disadvantages in utilization of this *in vitro* model. Firstly, it cannot replicate exact *in vivo* conditions; secondly, during the long-term culture process, some cell property changes might take place like gene alterations; thirdly, the *in vitro* cultured cells often lose their original differentiated function, and cannot stably maintain the exact properties of the original organ. Regardless of these disadvantages, the cell lines still contain heterogeneous and hierarchical sub-populations (93). Therefore, primary PCa cells, established PCa cell lines, xenografts and animal models have been utilized to identify prostate CSCs with different surface markers.

2.2.1 Putative markers of prostate CSCs—Prostate CSCs express a number of same markers as prostate stem cells, such as CD44, CD133, intergrins, breast cancer resistance protein (BCRP) and Sca-1, all of which have been utilized to identify Prostate CSCs or prostate stem cells. CD44 has been proven to be a candidate marker for normal prostatic epithelium stem cell and prostate CSCs (5). $CD44^+$ PCa cell population is enriched in tumorigenic and metastatic progenitor cells.

$CD44^+$ PCa cells are more proliferative, clonogenic, tumorigenic, and metastatic than the isogenic $CD44^-$ PCa cells (94). $CD44^+$ PCa cells have been evaluated with a series of characteristics (94): possess certain intrinsic properties of progenitor cells; co-localize with a population of intermediate label-retaining cells; express higher mRNA levels of several 'stemness' genes including Oct-3/4, Bmi, β-catenin, and SMO; generate $CD44^-$ cells in vitro and in vivo. $CD44^+$ PCa cells, which are androgen receptor $(AR)^-$, can differentiate into AR^+ tumor cells. A very small percentage of $CD44^+$ PCa cells appear to undergo asymmetric cell division in clonal analyses (94). $CD44^+/CD24^-$ LNCaP cells could form prostaspheres in vitro (95). $CD44^+/CD24^-$cells form colonies in soft agar and form tumors in NOD/SCID mice when as few as 100 cells are injected (95). Interestingly, expression of

CD44 is associated with cells of NE phenotype, which is of significance in therapy resistance and tumor recurrence (76). Long-term maintained sphere-propagating DU145 cells with stem-like properties are also enriched with CD44, CD24 and integrinα2β1 (96). All of the above evidence confirms that CD44 is a prospective marker for prostate CSCs.

CD133 has been proposed to be a putative surface marker in a number of tumors as mentioned above. Collins et al. found only tumor-derived $CD133^+$ cells were capable of self-renewal and extensive proliferation (26). $CD133^+$ cells, enriched in the $CD44^+$ integrinα2β1^{high} basal population and representing about 0.75% of basal cells, were shown to possess a high in vitro proliferative potential and are able to reconstitute prostatic-like acini in ~20% recipient nude mice (97). However, the $CD133^-$ cell population also contained clonogenic cells and the prostatic-like acini were not very typical structures (97). In DU145 cells, the clones formed by $CD44^+$ integrinα2β1$^{high}CD133^+$ subpopulation are remarkably different morphologically and quantitatively from those formed by integrinα2β1$^{-/low}$ $CD133^-$ cells, and $CD133^+$ cells have the capacity of self-renewal, extensive differentiation potential and high proliferative and tumorigenic potential (98). Within a series of AR^+ human PCa cell lines including LAPC-4, LNCaP and CWR22Rv1 cells, $CD133^+$ cells are present at a low frequency, self-renew, express AR, generate phenotypically heterogeneous progeny negative for CD133, and possess an unlimited proliferative capacity (99). However, other investigators found that CD133 was only expressed in DU145 cells except for DuCaP, LAPC-4, CWR22Rv1, LNCaP and PC3 cells, and considered CD133 selection does not enrich for stem-like cells in PCa cell lines (100). This variance may be caused by the application of different antibodies to CD133.

ALDH is an enzyme involved in intracellular retinoic acid production (101). In prostate CSCs studies, the high expression of ALDH1A1, a member of ALDH family, was found to be positively correlated with Gleason score and pathologic stage, and inversely associated with overall survival and cancer-specific survival of the patients, indicating ALDH1A1 could be a potential prostate CSC-related marker (102). The $ALDH^{hi}$ cells have greater in vitro proliferative potential than cells with low ALDH activity and high levels of ALDH activity might be a functional marker of murine prostate stem/progenitor cells (103). Van den Hoogen et al successfully used high ALDH activity to identify tumor initiating PCa cells and metastasis (104): $ALDH^{hi}$ PCa cells not only display enhanced clonogenicity and migration *in vitro*, but also show enhanced tumorigenicity and metastatic ability *in vivo*. These cells demonstrate increased metastatic ability *in vivo* as well (104). We have shown that ALDH activity indicates increased tumorigenicity, but not a CSC phenotype, in PCa cell lines (105). We found that $ALDH^{hi}$ $CD44^+$ cells exhibit a higher proliferative, clonogenic and metastatic capacity *in vitro* and demonstrate higher tumorigenicity capacity *in vivo* than $ALDH^{lo}$ CD44-cells. However, $ALDH^{lo}$ $CD44^-$ cells were able to develop tumors, albeit with longer latency periods (105). This might be caused by different cell lines utilized in different groups and by the complexity and diversity of PCa cell lines. Clonally derived holoclones are thought to contain self-renewing stem cells whereas meroclones and paraclones consist of transit amplifying cells (106). Isolation of $ALDH^{hi}$ PC3 cells enriches for the most primitive holoclone population (107). Therefore ALDH activity is a promising surface marker for prostate CSCs in clinically-derived tissues.

2.2.2 A novel prospective marker of prostate CSCs: TRA-1-60$^+$/CD151$^+$/CD166$^+$—Rajasekhar et al (89) recently performed a thorough investigation on prostate CSCs and identified 2 noteworthy new features of prostate CSCs: expression of TRA-1-60, CD151 and CD166; and elevated NF-κB signaling.

Firstly, a very small percentage of primary cells isolated from the orthotopic (OT, injection into prostate) CWR22 tumors were found to form spheroids, which were called “primary

spheres". The tumor-initiation with primary sphere-cells was at least 100-times more efficient than the tumor-initiation with the total tumor cells. The spheres express distinct characteristics and are multipotent as the sphere-cell-derived tumors reconstituted histopathological features and immunophenotypes of the parent CWR22 tumor, and closely mimicked the features of freshly obtained patient prostate primary tumor specimens. These stem-like sphere cells do not express key markers of prostate cancer, including AR, PSA, CK18 and Nkx3.1, on the contrary, cancer stem cell and cell-proliferation-associated markers such as Met receptor kinase, Musashi-1, inhibitor of differentiation 1, phosphor-histone 3 and Ji67 are selectively enriched in these spheres relative to tumors.

Secondly, a further investigation of expression of novel cell surface marker that could facilitate prospective isolation of the TICs was undertaken in the human prostate CWR22 OT-tumors. The authors confirmed the expression of a set of markers known to associate with stem-like tumor cells in other epithelial cancers including human prostate tumors, such as epithelial cellular adhesion molecule (EpCAM), CD44 and integrins (α2-integrin,α6-intergrin andβ4-intergrin). Prostate tumor cells expressing these markers displayed increased sphere-formation capacity as compared with unsorted total tumor cells or cells expressingβ4-intergrin. Except for CD44, the percentage of cells expressing these markers (EpCAM,α2-integrin for example) was consistently enriched over sequential passages of sphere cells.

Thirdly, a novel marker was determined in the sphere cells, i.e. the tumor rejection antigen, TRA-1-60, a cell surface epitope of human embryonic, embryonal germline and teratocarcinoma stem cells (108). It turned out that TRA-1-60, particularly when co-expressed with CD166 and CD151, significantly enriched the prostate CSCs. CD166 and CD151 have been known to be associated with colon epithelial CSCs (21) and other stem-like cells in tumor stroma, respectively, and during prostate cancer progression (109). The triple-marker-positive (TRA-1-60$^+$/CD151$^+$/CD166$^+$) subset had considerably higher capacity of *in vitro* sphere formation and *in vivo* tumor generation than the single or double positives and triple negatives, and were capable of both self-renewal and differentiation by recapitulating a cellular hierarchy of the original parental tumor.

Fourthly, the triple-marker expression and the association with tumor-initiation were consistent in additional human prostate cancer cell line-derived xenograft tumor models, namely, androgen-independent metastatic prostate cancer cell line-derived DU-145 (brain metastasis), PC3 (bone metastasis) and VCaP (vertebral metastasis) xenograft tumors, and also in another androgen-dependent and human patient-derived primary OT-xenograft tumor (PC-82). Strikingly, all the resulting tumors demonstrated their expected immunohistochemical characteristics such as cytosolic localization of AR in androgen-insensitive DU-145 and PC3 tumors, or nuclear localization in androgen-sensitive VCaP and PC-82 tumors. All these tumors contained a set of triple-marker-positive cells. Tumors derived from triple-marker-positive DU-145 tumor cells were sequentially passaged *in vivo* over multiple transplantation cycles. The marker-positive cells from the DU-145 tumor recapitulated the original parent tumor heterogeneity by hierarchically differentiating *in vivo* into both marker-positive and -negative tumor cells. Marker expression and sphere-forming/tumor-initiating abilities were also correlated in the DU-145 tumor cells, although the marker negative cells have considerably diminished sphere-forming and tumor-initiating abilities. The TRA-1-60$^+$/CD151$^+$/CD166$^+$ subset represented a rare population (0.1%–0.5%) in all the above pCa models. It was also detected in human prostate clinical tumors from radical prostatectomy with a population of ~2.5%.

Finally, the gene expression profiles and signaling status of the TRA-1-60+/CD151+/CD166+ prostate CSCs was also investigated. IL-6, NF-κB signaling, Met,

PKCαphosphorylation and anti-apoptotic Bcl-2 family member MCL-1 displayed differentiated expression/activity between CSCs and non-CSCs. The elevation of IL-6 was consistent with another study indicating IL-6 mediated a dynamic equilibrium between CSCs and non-stem cancer cells and could convert non-stem cancer cells to CSCs in mammary and PCa models (110). Taken together, these findings provide strong evidence that TRA-1-60+/CD151+/CD166+ can identify the CSC phenotype.

2.3 Therapeutic targets of prostate CSCs

2.3.1 Prostate CSCs and metastasis—Human PCa encompasses multiple processes including oncogenesis, local invasion and metastasis and development of androgen independence (111, 112). CSCs may play a role in all of these processes. Thus defining how CSCs contributes to these processes may lead to therapeutic interventions. A large effort has focused on the role of CSC in metastasis.

Eaton et al detected the distribution of putative stem cell markers like CD133, CD44, α2β1 integrin, CXCR4 etc. by immunohistochemistry and stain intensity with matched (primary and bone metastasis) specimens from PCa patients, and found in established metastases, phenotypically positive ($CD44^+$) prostate CSCs was shown to be more frequent in metastasis samples than in primary cancers, although no single or combination of marker expression profiles identify the established metastatic phenotype (113). Besides, a subpopulation of $CD44^+$ CSC-like cells invade Matrigel through epithelial-mesenchymal transition, while in contrast, $CD44^-$ cells are non-invasive (114). Colombel et al also confirmed that percentage of stem cell-like PCa cells has a prognostic impact especially on the risk of metastatic bone progression, which favors the hypothesis that bone metastasis from PCa is the end result of prostate CSCs dissemination from the primary tumors (115). Other evidence from experiments verifies this hypothesis as well (116). ALDH7A1, a member of ALDH family, has been shown to be functionally involved in the formation of bone metastases (104). The knockdown of ALDH7A1 resulted in decreased intra-bone growth and inhibited experimentally induced bone metastasis (104), indicating the possible connection of stem/progenitor cell with bone metastasis.

2.3.2 Altered signaling pathways—Alterations of signaling pathways may account for the tumorigenic potential of CSCs. Therefore, understanding the signaling status of prostate CSCs may provide potential therapeutic targets. For instance, treatment with galiellalactone, a potent and specific inhibitor of STAT3 signaling, decreased the proportion and induced the apoptosis of $ALDH^+$ PCa cells (117). Furthermore, the gene expression of ALDH1A1 was downregulated in vivo in galiellalactone-treated DU145 xenografts, indicating that targeting the STAT3 pathway in prostate cancer cells, including PCa stem cell-like cells, is a promising therapeutic approach (117). The observation that that components of the JAK-STAT pathway are over-expressed in prostate CSCs (88) provides further support for this approach.

Rajasekhar et al. revealed a specifically enhanced and functional NF-κB signaling in the prospectively purified naïve stem-like human prostatic TICs for the first time (89). When treated with small molecule inhibitors targeting NF-κB or Met, both secondary sphere-formation *in vitro* and tumor-initiation *in vivo* were blocked (89). This is consistent with the strong correlation between positive NF-κB nuclear staining in patients' radical prostatectomy specimens with positive margin PCa and the presence of a several-fold increase in their risk for biochemical recurrence (118).

Components of Wnt signaling are over-represented in CSCs and they play a central role in modulating the delicate balance between stemness and differentiation in several adult stem cell niches as reviewed in (119). PCa cells with stem cell characteristics were identified in

human PCa cell lines by their ability to form single cells self-renewing prostaspheres in non-adherent cultures. Prostaspheres exhibited heterogeneous expression of proliferation, differentiation and stem cell-associated makers CD44, ABCG2 and CD133 (120). Bisson et al found that treatment with WNT inhibitors reduced both prostasphere size and self-renewal (120). Whereas addition of Wnt3a increased prostasphere size and self-renewal, which was associated with a significant increase in nuclear beta-catenin, keratin 18, CD133 and CD44 expression (120). Inhibition of WNT signaling therefore has the potential to reduce the self-renewal of prostate cancer cells with stem cell characteristics and improve the therapeutic outcome.

2.3.3 Prostate CSCs and AR—Androgen deprivation therapy is often used for treatment of advance PCa (121). The response toward ADT in the metastatic PCa is transient and the tumor progresses to castrate-resistant prostate cancer (CRPC). In CRPC, AR is reactivated by a variety of mechanisms. Evaluation of the AR expression in PCa is of great significance in PCa. Studies has shown prostate CSCs do not express AR (26, 89, 94). $CD133^+$ PCa cells were originally reported to be AR^- (97); however, other studies suggest $CD133^+$ cells responsible for tumor propagation and progression are AR^+ and there for are direct target for androgen stimulation (99). $CD44^+$ cells from human PCa cell lines are also AR^- (24). There is no evidence yet explaining how castration resistance with upregulation of AR could be selected for AR^- CSCs. Continued studies are required to clearly define a role of AR and androgens in prostate CSC.

2.4 MicroRNA regulation of prostate CSCs

As described in the overview above, studies of dysregulation of miRNAs have been investigated in tumor development and regulation of CSC. This has extended to studies of prostate CSC. For example, Liu et al. were the first to profile miRNA expression in prostate CSC and/or progenitor cells (122). In their study, through an unbiased miRNA expression profiling in 5 prostate CSCs and/or progenitor cell populations purified from PCa xenografts, including 3 $CD44^+$ populations from the LAPC9, LAPC4, and Du145 tumors, $CD133^+$ cells from LAPC4 tumors, and $\alpha2\beta1^+$ cells from Du145 tumors, they identified miR-34a, together with let-7b, to be commonly under-expressed in all marker-positive cell populations (122). The underexpression of miR-34a was subsequently corroborated in $CD44^+$ PCa cells purified from 20 patient prostate tumors. Overexpression of miR-34a in bulk PCa cells or purified $CD44^+$ cells by transfecting with mature oligonucleotide mimics or infecting with lentiviral vectors encoding pre–miR-34a exerted pronounced inhibitory effects on tumor growth and metastasis *in vivo*. In contrast, neutralizing endogenous miR-34a using antagomirs in bulk or $CD44^-$ prostate cancer cells promoted tumor regeneration and metastasis. Interestingly, delivery of miR-34a oligos systemically through tail vein inhibited metastasis to the lung and other organs and prolonged the survival of animals bearing orthotopic human PCa, indicating the therapeutic potential of this miRNA. Mechanistically, miR-34a suppressed prostate CSCs properties as it inhibited prostasphere establishment, migration and invasiveness of $CD44^+$ PCa cells, and serial prostasphere passaging and serial tumor transplantation. It's of great significance that CD44 itself represented a direct and relevant downstream target of miR-34a. The CD44 protein levels decreased in cells overexpressing miR-34a, and knocking down of CD44 functionally phenocopied the miR-34a effects in inhibiting tumor development and metastasis. Thus these studies provide proof of concept regarding the important and potential therapeutic potential of miRNA in PC CSC.

3. Conclusions and future perspectives

Although there are a large number of unknowns and controversies regarding prostate CSCs the bulk of research on CSCs studies has provided evidence to clarify important matters such as existence and identification of CSCs. Additionally, insight into the mechanisms and function of CSCs development, self-renewal, survival and differentiation have been elucidated. These achievements will impact how PCa as a whole is investigated and therapeutically targeted. Events involved in prostate CSC development and the contribution of signaling pathways and microRNA in regulation of prostate CSCs will provide novel cancer therapeutic strategies.

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References

1. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001; 414:105–11. [PubMed: 11689955]
2. Huntly BJ, Gilliland DG. Leukaemia stem cells and the evolution of cancer-stem-cell research. Nat Rev Cancer. 2005; 5:311–21. [PubMed: 15803157]
3. Clarke M. Cancer Stem Cells—Perspectives on Current Status and Future Directions: AACR Workshop on Cancer Stem Cells. Cancer Res. 2006; 66:9339–44. [PubMed: 16990346]
4. Wang ZA, Shen MM. Revisiting the concept of cancer stem cells in prostate cancer. Oncogene. 2011; 30:1261–71. [PubMed: 21119602]
5. Tang DG, Patrawala L, Calhoun T, Bhatia B, Choy G, Schneider-Broussard R, et al. Prostate cancer stem/progenitor cells: identification, characterization, and implications. Mol Carcinog. 2007; 46:1–14. [PubMed: 16921491]
6. Jemal A SR, Ward E, et al. Cancer statistics. CA Cancer J Clin. 2010; 60:277–300. [PubMed: 20610543]
7. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer. 2001; 1:34–45. [PubMed: 11900250]
8. Man YG, Gardner WA. Bad seeds produce bad crops: a single stage-process of prostate tumor invasion. Int J Biol Sci. 2008; 4:246–58. [PubMed: 18725981]
9. Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, et al. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. Cancer Res. 2005; 65:5506–11. [PubMed: 15994920]
10. Soltanian S. Cancer stem cells and cancer therapy. Tumor biol. 2011:32.
11. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997; 3:730–7. [PubMed: 9212098]
12. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A. 2003; 100:3983–8. [PubMed: 12629218]
13. Charafe-Jauffret E, Ginestier C, Iovino F, Wicinski J, Cervera N, Finetti P, et al. Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature. Cancer Res. 2009; 69:1302–13. [PubMed: 19190339]
14. Creighton CJ, Li X, Landis M, Dixon JM, Neumeister VM, Sjolund A, et al. Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. Proc Natl Acad Sci U S A. 2009; 106:13820–5. [PubMed: 19666588]
15. Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, et al. Molecular definition of breast tumor heterogeneity. Cancer Cell. 2007; 11:259–73. [PubMed: 17349583]

16. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell. 2007; 1:555–67. [PubMed: 18371393]
17. Pece S, Tosoni D, Confalonieri S, Mazzarol G, Vecchi M, Ronzoni S, et al. Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. Cell. 2010; 140:62–73. [PubMed: 20074520]
18. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res. 2004; 64:7011–21. [PubMed: 15466194]
19. Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, et al. Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell. 2005; 121:823–35. [PubMed: 15960971]
20. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, et al. Identification and expansion of human colon-cancer-initiating cells. Nature. 2007; 445:111–5. [PubMed: 17122771]
21. Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, et al. Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci U S A. 2007; 104:10158–63. [PubMed: 17548814]
22. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature. 2007; 445:106–10. [PubMed: 17122772]
23. Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, et al. Identification of cells initiating human melanomas. Nature. 2008; 451:345–9. [PubMed: 18202660]
24. Miki J, Furusato B, Li H, Gu Y, Takahashi H, Egawa S, et al. Identification of putative stem cell markers, CD133 and CXCR4, in hTERT-immortalized primary nonmalignant and malignant tumor-derived human prostate epithelial cell lines and in prostate cancer specimens. Cancer Res. 2007; 67:3153–61. [PubMed: 17409422]
25. Patrawala L, Calhoun-Davis T, Schneider-Broussard R, Tang DG. Hierarchical organization of prostate cancer cells in xenograft tumors: the CD44+alpha2beta1+ cell population is enriched in tumor-initiating cells. Cancer Res. 2007; 67:6796–805. [PubMed: 17638891]
26. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res. 2005; 65:10946–51. [PubMed: 16322242]
27. Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proc Natl Acad Sci U S A. 2007; 104:973–8. [PubMed: 17210912]
28. Szotek PP, Pieretti-Vanmarcke R, Masiakos PT, Dinulescu DM, Connolly D, Foster R, et al. Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian Inhibiting Substance responsiveness. Proc Natl Acad Sci U S A. 2006; 103:11154–9. [PubMed: 16849428]
29. Eramo A, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio A, et al. Identification and expansion of the tumorigenic lung cancer stem cell population. Cell Death Differ. 2008; 15:504–14. [PubMed: 18049477]
30. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994; 367:645–8. [PubMed: 7509044]
31. Taussig DC, Miraki-Moud F, Anjos-Afonso F, Pearce DJ, Allen K, Ridler C, et al. Anti-CD38 antibody-mediated clearance of human repopulating cells masks the heterogeneity of leukemia-initiating cells. Blood. 2008; 112:568–75. [PubMed: 18523148]
32. Taussig DC, Vargaftig J, Miraki-Moud F, Griessinger E, Sharrock K, Luke T, et al. Leukemia-initiating cells from some acute myeloid leukemia patients with mutated nucleophosmin reside in the CD34(−) fraction. Blood. 2010; 115:1976–84. [PubMed: 20053758]
33. Vlashi E, Kim K, Lagadec C, Donna LD, McDonald JT, Eghbali M, et al. In vivo imaging, tracking, and targeting of cancer stem cells. J Natl Cancer Inst. 2009; 101:350–9. [PubMed: 19244169]

34. Sheridan C, Kishimoto H, Fuchs RK, Mehrotra S, Bhat-Nakshatri P, Turner CH, et al. CD44+/CD24-breast cancer cells exhibit enhanced invasive properties: an early step necessary for metastasis. Breast Cancer Res. 2006; 8:R59. [PubMed: 17062128]
35. Ma S, Chan KW, Hu L, Lee TK, Wo JY, Ng IO, et al. Identification and characterization of tumorigenic liver cancer stem/progenitor cells. Gastroenterology. 2007; 132:2542–56. [PubMed: 17570225]
36. Baba T, Convery PA, Matsumura N, Whitaker RS, Kondoh E, Perry T, et al. Epigenetic regulation of CD133 and tumorigenicity of CD133+ ovarian cancer cells. Oncogene. 2009; 28:209–18. [PubMed: 18836486]
37. Cui F, Wang J, Chen D, Chen YJ. CD133 is a temporary marker of cancer stem cells in small cell lung cancer, but not in non-small cell lung cancer. Oncol Rep. 2011; 25:701–8. [PubMed: 21174061]
38. Chu P, Clanton DJ, Snipas TS, Lee J, Mitchell E, Nguyen ML, et al. Characterization of a subpopulation of colon cancer cells with stem cell-like properties. Int J Cancer. 2009; 124:1312–21. [PubMed: 19072981]
39. Shi MF, Jiao J, Lu WG, Ye F, Ma D, Dong QG, et al. Identification of cancer stem cell-like cells from human epithelial ovarian carcinoma cell line. Cell Mol Life Sci. 2010; 67:3915–25. [PubMed: 20549538]
40. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, et al. Identification of pancreatic cancer stem cells. Cancer Res. 2007; 67:1030–7. [PubMed: 17283135]
41. Penumatsa K, Edassery SL, Barua A, Bradaric MJ, Luborsky JL. Differential expression of aldehyde dehydrogenase 1a1 (ALDH1) in normal ovary and serous ovarian tumors. J Ovarian Res. 2010; 3:28. [PubMed: 21176222]
42. Su Y, Qiu Q, Zhang X, Jiang Z, Leng Q, Liu Z, et al. Aldehyde dehydrogenase 1 A1-positive cell population is enriched in tumor-initiating cells and associated with progression of bladder cancer. Cancer Epidemiol Biomarkers Prev. 2010; 19:327–37. [PubMed: 20142235]
43. Croker AK, Goodale D, Chu J, Postenka C, Hedley BD, Hess DA, et al. High aldehyde dehydrogenase and expression of cancer stem cell markers selects for breast cancer cells with enhanced malignant and metastatic ability. J Cell Mol Med. 2009; 13:2236–52. [PubMed: 18681906]
44. Yeung J, Esposito MT, Gandillet A, Zeisig BB, Griessinger E, Bonnet D, et al. beta-Catenin mediates the establishment and drug resistance of MLL leukemic stem cells. Cancer Cell. 2010; 18:606–18. [PubMed: 21156284]
45. Jamieson CH, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N Engl J Med. 2004; 351:657–67. [PubMed: 15306667]
46. Dierks C, Beigi R, Guo GR, Zirlik K, Stegert MR, Manley P, et al. Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. Cancer Cell. 2008; 14:238–49. [PubMed: 18772113]
47. Zhao C, Blum J, Chen A, Kwon HY, Jung SH, Cook JM, et al. Loss of beta-catenin impairs the renewal of normal and CML stem cells in vivo. Cancer Cell. 2007; 12:528–41. [PubMed: 18068630]
48. Armstrong F, Brunet de la Grange P, Gerby B, Rouyez MC, Calvo J, Fontenay M, et al. NOTCH is a key regulator of human T-cell acute leukemia initiating cell activity. Blood. 2009; 113:1730–40. [PubMed: 18984862]
49. Fan X, Matsui W, Khaki L, Stearns D, Chun J, Li YM, et al. Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. Cancer Res. 2006; 66:7445–52. [PubMed: 16885340]
50. Farnie G, Clarke RB, Spence K, Pinnock N, Brennan K, Anderson NG, et al. Novel cell culture technique for primary ductal carcinoma in situ: role of Notch and epidermal growth factor receptor signaling pathways. J Natl Cancer Inst. 2007; 99:616–27. [PubMed: 17440163]
51. Wang Z, Ahmad A, Li Y, Azmi AS, Miele L, Sarkar FH. Targeting notch to eradicate pancreatic cancer stem cells for cancer therapy. Anticancer Res. 2011; 31:1105–13. [PubMed: 21508353]

52. Sullivan JP, Spinola M, Dodge M, Raso MG, Behrens C, Gao B, et al. Aldehyde dehydrogenase activity selects for lung adenocarcinoma stem cells dependent on notch signaling. Cancer Res. 2010; 70:9937–48. [PubMed: 21118965]
53. Gulino A, Ferretti E, De Smaele E. Hedgehog signalling in colon cancer and stem cells. EMBO Mol Med. 2009; 1:300–2. [PubMed: 20049733]
54. Zhao C, Chen A, Jamieson CH, Fereshteh M, Abrahamsson A, Blum J, et al. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. Nature. 2009; 458:776–9. [PubMed: 19169242]
55. O'Toole SA, Swarbrick A, Sutherland RL. The Hedgehog signalling pathway as a therapeutic target in early breast cancer development. Expert Opin Ther Targets. 2009; 13:1095–103. [PubMed: 19659449]
56. Peacock CD, Wang Q, Gesell GS, Corcoran-Schwartz IM, Jones E, Kim J, et al. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. Proc Natl Acad Sci U S A. 2007; 104:4048–53. [PubMed: 17360475]
57. Feldmann G, Dhara S, Fendrich V, Bedja D, Beaty R, Mullendore M, et al. Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers. Cancer Res. 2007; 67:2187–96. [PubMed: 17332349]
58. Bar EE, Chaudhry A, Lin A, Fan X, Schreck K, Matsui W, et al. Cyclopamine-mediated hedgehog pathway inhibition depletes stem-like cancer cells in glioblastoma. Stem Cells. 2007; 25:2524–33. [PubMed: 17628016]
59. Liu S, Dontu G, Mantle ID, Patel S, Ahn NS, Jackson KW, et al. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. Cancer Res. 2006; 66:6063–71. [PubMed: 16778178]
60. Matsui W. Cancer stem cell targeting in multiple myeloma by grn 163 1, a novel and potent telomerase inhibitor. Blood (ASH Annu Meet Abstr). 2006; 108:2540.
61. Park IK, Qian D, Kiel M, Becker MW, Pihalja M, Weissman IL, et al. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. Nature. 2003; 423:302–5. [PubMed: 12714971]
62. Guzman ML, Rossi RM, Neelakantan S, Li X, Corbett CA, Hassane DC, et al. An orally bioavailable parthenolide analog selectively eradicates acute myelogenous leukemia stem and progenitor cells. Blood. 2007; 110:4427–35. [PubMed: 17804695]
63. Guzman ML, Neering SJ, Upchurch D, Grimes B, Howard DS, Rizzieri DA, et al. Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells. Blood. 2001; 98:2301–7. [PubMed: 11588023]
64. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116:281–97. [PubMed: 14744438]
65. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer. 2006; 6:857–66. [PubMed: 17060945]
66. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, et al. let-7 regulates self renewal and tumorigenicity of breast cancer cells. Cell. 2007; 131:1109–23. [PubMed: 18083101]
67. Shimono Y, Zabala M, Cho RW, Lobo N, Dalerba P, Qian D, et al. Downregulation of miRNA-200c links breast cancer stem cells with normal stem cells. Cell. 2009; 138:592–603. [PubMed: 19665978]
68. Gal H, Pandi G, Kanner AA, Ram Z, Lithwick-Yanai G, Amariglio N, et al. MIR-451 and Imatinib mesylate inhibit tumor growth of Glioblastoma stem cells. Biochem Biophys Res Commun. 2008; 376:86–90. [PubMed: 18765229]
69. Ji J, Yamashita T, Budhu A, Forgues M, Jia HL, Li C, et al. Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells. Hepatology. 2009; 50:472–80. [PubMed: 19585654]
70. Yu F, Deng H, Yao H, Liu Q, Su F, Song E. Mir-30 reduction maintains self-renewal and inhibits apoptosis in breast tumor-initiating cells. Oncogene. 2010; 29:4194–204. [PubMed: 20498642]
71. Ibarra I, Erlich Y, Muthuswamy SK, Sachidanandam R, Hannon GJ. A role for microRNAs in maintenance of mouse mammary epithelial progenitor cells. Genes Dev. 2007; 21:3238–43. [PubMed: 18079172]

72. Li Y, Guessous F, Zhang Y, Dipierro C, Kefas B, Johnson E, et al. MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes. Cancer Res. 2009; 69:7569–76. [PubMed: 19773441]
73. Park SM, Gaur AB, Lengyel E, Peter ME. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev. 2008; 22:894–907. [PubMed: 18381893]
74. Lawson DA, Witte ON. Stem cells in prostate cancer initiation and progression. J Clin Invest. 2007; 117:2044–50. [PubMed: 17671638]
75. Abate-Shen C, Shen MM. Molecular genetics of prostate cancer. Genes Dev. 2000; 14:2410–34. [PubMed: 11018010]
76. Palapattu GS, Wu C, Silvers CR, Martin HB, Williams K, Salamone L, et al. Selective expression of CD44, a putative prostate cancer stem cell marker, in neuroendocrine tumor cells of human prostate cancer. Prostate. 2009; 69:787–98. [PubMed: 19189306]
77. Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, et al. Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. Nat Med. 2009; 15:907–13. [PubMed: 19648928]
78. English HF, Santen RJ, Isaacs JT. Response of glandular versus basal rat ventral prostatic epithelial cells to androgen withdrawal and replacement. Prostate. 1987; 11:229–42. [PubMed: 3684783]
79. Liu AY, True LD, LaTray L, Nelson PS, Ellis WJ, Vessella RL, et al. Cell-cell interaction in prostate gene regulation and cytodifferentiation. Proc Natl Acad Sci U S A. 1997; 94:10705–10. [PubMed: 9380699]
80. Tsujimura A, Koikawa Y, Salm S, Takao T, Coetzee S, Moscatelli D, et al. Proximal location of mouse prostate epithelial stem cells: a model of prostatic homeostasis. J Cell Biol. 2002; 157:1257–65. [PubMed: 12082083]
81. Lawson DA, Xin L, Lukacs RU, Cheng D, Witte ON. Isolation and functional characterization of murine prostate stem cells. Proc Natl Acad Sci U S A. 2007; 104:181–6. [PubMed: 17185413]
82. Liao CP, Adisetiyo H, Liang M, Roy-Burman P. Cancer-associated fibroblasts enhance the gland-forming capability of prostate cancer stem cells. Cancer Res. 2010; 70:7294–303. [PubMed: 20807814]
83. Wang S, Garcia AJ, Wu M, Lawson DA, Witte ON, Wu H. Pten deletion leads to the expansion of a prostatic stem/progenitor cell subpopulation and tumor initiation. Proc Natl Acad Sci U S A. 2006; 103:1480–5. [PubMed: 16432235]
84. Maitland NJ, Frame FM, Polson ES, Lewis JL, Collins AT. Prostate cancer stem cells: do they have a basal or luminal phenotype? Horm Cancer. 2011; 2:47–61. [PubMed: 21761340]
85. Goldstein AS, Huang J, Guo C, Garraway IP, Witte ON. Identification of a cell of origin for human prostate cancer. Science. 2010; 329:568–71. [PubMed: 20671189]
86. Lawson DA, Zong Y, Memarzadeh S, Xin L, Huang J, Witte ON. Basal epithelial stem cells are efficient targets for prostate cancer initiation. Proc Natl Acad Sci U S A. 2010; 107:2610–5. [PubMed: 20133806]
87. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science. 2005; 310:644–8. [PubMed: 16254181]
88. Birnie R, Bryce SD, Roome C, Dussupt V, Droop A, Lang SH, et al. Gene expression profiling of human prostate cancer stem cells reveals a pro-inflammatory phenotype and the importance of extracellular matrix interactions. Genome Biol. 2008; 9:R83. [PubMed: 18492237]
89. Rajasekhar VK, Studer L, Gerald W, Socci ND, Scher HI. Tumour-initiating stem-like cellsin human prostate cancer exhibit increased NF-kappaB signalling. Nat Commun. 2011; 2:162. [PubMed: 21245843]
90. Korsten H, Ziel-van der Made A, Ma X, van der Kwast T, Trapman J. Accumulating progenitor cells in the luminal epithelial cell layer are candidate tumor initiating cells in a Pten knockout mouse prostate cancer model. PLoS One. 2009; 4:e5662. [PubMed: 19461893]

91. Wang X, Kruithof-de Julio M, Economides KD, Walker D, Yu H, Halili MV, et al. A luminal epithelial stem cell that is a cell of origin for prostate cancer. Nature. 2009; 461:495–500. [PubMed: 19741607]
92. Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. Nat Rev Cancer. 2003; 3:895–902. [PubMed: 14737120]
93. Miki J, Rhim JS. Prostate cell cultures as in vitro models for the study of normal stem cells and cancer stem cells. Prostate Cancer Prostatic Dis. 2008; 11:32–9. [PubMed: 17984999]
94. Patrawala L, Calhoun T, Schneider-Broussard R, Li H, Bhatia B, Tang S, et al. Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. Oncogene. 2006; 25:1696–708. [PubMed: 16449977]
95. Hurt EM, Kawasaki BT, Klarmann GJ, Thomas SB, Farrar WL. CD44+ CD24(−) prostate cells are early cancer progenitor/stem cells that provide a model for patients with poor prognosis. Br J Cancer. 2008; 98:756–65. [PubMed: 18268494]
96. Rybak AP, He L, Kapoor A, Cutz JC, Tang D. Characterization of sphere-propagating cells with stem-like properties from DU145 prostate cancer cells. Biochim Biophys Acta. 2011; 1813:683–94. [PubMed: 21277911]
97. Richardson GD, Robson CN, Lang SH, Neal DE, Maitland NJ, Collins AT. CD133, a novel marker for human prostatic epithelial stem cells. J Cell Sci. 2004; 117:3539–45. [PubMed: 15226377]
98. Wei C, Guomin W, Yujun L, Ruizhe Q. Cancer stem-like cells in human prostate carcinoma cells DU145: the seeds of the cell line? Cancer Biol Ther. 2007; 6:763–8. [PubMed: 17592251]
99. Vander Griend DJ, Karthaus WL, Dalrymple S, Meeker A, DeMarzo AM, Isaacs JT. The role of CD133 in normal human prostate stem cells and malignant cancer-initiating cells. Cancer Res. 2008; 68:9703–11. [PubMed: 19047148]
100. Pfeiffer MJ, Schalken JA. Stem cell characteristics in prostate cancer cell lines. Eur Urol. 2010; 57:246–54. [PubMed: 19200636]
101. Yoshida A, Hsu LC, Dave V. Retinal oxidation activity and biological role of human cytosolic aldehyde dehydrogenase. Enzyme. 1992; 46:239–44. [PubMed: 1292933]
102. Li T, Su Y, Mei Y, Leng Q, Leng B, Liu Z, et al. ALDH1A1 is a marker for malignant prostate stem cells and predictor of prostate cancer patients' outcome. Lab Invest. 2010; 90:234–44. [PubMed: 20010854]
103. Burger PE, Gupta R, Xiong X, Ontiveros CS, Salm SN, Moscatelli D, et al. High aldehyde dehydrogenase activity: a novel functional marker of murine prostate stem/progenitor cells. Stem Cells. 2009; 27:2220–8. [PubMed: 19544409]
104. van den Hoogen C, van der Horst G, Cheung H, Buijs JT, Lippitt JM, Guzman-Ramirez N, et al. High aldehyde dehydrogenase activity identifies tumor-initiating and metastasis-initiating cells in human prostate cancer. Cancer Res. 2010; 70:5163–73. [PubMed: 20516116]
105. Yu C, Yao Z, Dai J, Zhang H, Escara-Wilke J, Zhang X, et al. ALDH activity indicates increased tumorigenic cells, but not cancer stem cells, in prostate cancer cell lines. In Vivo. 2011; 25:69–76. [PubMed: 21282737]
106. Pellegrini G, Ranno R, Stracuzzi G, Bondanza S, Guerra L, Zambruno G, et al. The control of epidermal stem cells (holoclones) in the treatment of massive full-thickness burns with autologous keratinocytes cultured on fibrin. Transplantation. 1999; 68:868–79. [PubMed: 10515389]
107. Doherty RE, Haywood-Small SL, Sisley K, Cross NA. Aldehyde dehydrogenase activity selects for the holoclone phenotype in prostate cancer cells. Biochem Biophys Res Commun. 2011; 414:801–7. [PubMed: 22005464]
108. Draper JS, Pigott C, Thomson JA, Andrews PW. Surface antigens of human embryonic stem cells: changes upon differentiation in culture. J Anat. 2002; 200:249–58. [PubMed: 12033729]
109. Ang J, Lijovic M, Ashman LK, Kan K, Frauman AG. CD151 protein expression predicts the clinical outcome of low-grade primary prostate cancer better than histologic grading: a new prognostic indicator? Cancer Epidemiol Biomarkers Prev. 2004; 13:1717–21. [PubMed: 15533898]

110. Iliopoulos D, Hirsch HA, Wang G, Struhl K. Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. Proc Natl Acad Sci U S A. 2011; 108:1397–402. [PubMed: 21220315]
111. Nelson WG, De Marzo AM, Isaacs WB. Prostate cancer. N Engl J Med. 2003; 349:366–81. [PubMed: 12878745]
112. MAW. Neoplastic lesions of the prostate and seminal vesicles. Genitourinary Tract Pathology. 1993; 14:1–8.
113. Eaton CL, Colombel M, van der Pluijm G, Cecchini M, Wetterwald A, Lippitt J, et al. Evaluation of the frequency of putative prostate cancer stem cells in primary and metastatic prostate cancer. Prostate. 2010; 70:875–82. [PubMed: 20127735]
114. Klarmann GJ, Hurt EM, Mathews LA, Zhang X, Duhagon MA, Mistree T, et al. Invasive prostate cancer cells are tumor initiating cells that have a stem cell-like genomic signature. Clin Exp Metastasis. 2009; 26:433–46. [PubMed: 19221883]
115. Colombel M, Eaton CL, Hamdy F, Ricci E, van der Pluijm G, Cecchini M, et al. Increased expression of putative cancer stem cell markers in primary prostate cancer is associated with progression of bone metastases. Prostate. 2011
116. Guzman-Ramirez N, Voller M, Wetterwald A, Germann M, Cross NA, Rentsch CA, et al. In vitro propagation and characterization of neoplastic stem/progenitor-like cells from human prostate cancer tissue. Prostate. 2009; 69:1683–93. [PubMed: 19644960]
117. Hellsten R, Johansson M, Dahlman A, Sterner O, Bjartell A. Galiellalactone inhibits stem cell-like ALDH-positive prostate cancer cells. PLoS One. 2011; 6:e22118. [PubMed: 21779382]
118. Ross JS, Kallakury BV, Sheehan CE, Fisher HA, Kaufman RP Jr, Kaur P, et al. Expression of nuclear factor-kappa B and I kappa B alpha proteins in prostatic adenocarcinomas: correlation of nuclear factor-kappa B immunoreactivity with disease recurrence. Clin Cancer Res. 2004; 10:2466–72. [PubMed: 15073126]
119. Fodde R, Brabletz T. Wnt/beta-catenin signaling in cancer stemness and malignant behavior. Curr Opin Cell Biol. 2007; 19:150–8. [PubMed: 17306971]
120. Bisson I, Prowse DM. WNT signaling regulates self-renewal and differentiation of prostate cancer cells with stem cell characteristics. Cell Res. 2009; 19:683–97. [PubMed: 19365403]
121. Cooperberg MR, Grossfeld GD, Lubeck DP, Carroll PR. National practice patterns and time trends in androgen ablation for localized prostate cancer. J Natl Cancer Inst. 2003; 95:981–9. [PubMed: 12837834]
122. Liu C, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H, et al. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. Nat Med. 2011; 17:211–5. [PubMed: 21240262]