**STEM CELLS IN PROSTATE CANCER**

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**Summary.-** Tumors constitute complex ecosystems with multiple interactions among neoplastic cells displaying various phenotypes and functions and where the tumoral niche is built with an active participation of the host environment that also impacts the malignant progression of the tumor cells. Irrespective of the cell of origin of prostate adenocarcinoma, mounting evidences support the existence of a hierarchy within neoplastic prostate cells that contributes to the heterogeneity of these tumors. At the origin of this hierarchy are small populations of tumor cells with high self-renewal potential and also capable of generating progeny tumor cells that lose self-renewal properties as they acquire more differentiated phenotypes. These cancer stem cells (CSC) depend on active gene networks that confer them with their self-renewal capacity through symmetrical divisions whereas they can also undergo asymmetrical division and differentiation either as stochastic events or in response to environmental cues. Although new experimental evidences indicate that this is can be a reversible process, thus blurring the distinction between CSCs and non-CSCs, the former are considered as the drivers of tumor growth and evolution, and thus a prime target for therapeutic intervention. Of particular importance in prostate cancer, CSCs may constitute the repository population of androgen-insensitive and chemotherapy-resistant tumor cells responsible for castration-resistant and chemotherapy-resistant tumors, thus their identification and quantification in primary and metastatic neoplasms could play important roles in the management of this disease.

**Keywords:** Cancer stem cell. Tumor heterogeneity. Tumor evolution. Prostate cancer. Castration resistance. Molecular biology.

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The cancer stem cell model implies that tumors are organized as a hierarchy, with CSCs endowed with indefinite self-renewal potential (symmetrical division) and giving rise to more differentiated and less tumorigenic non-CSC cells through asymmetrical division in response to epigenetic mechanisms (2, 3). These descendant cells may form the great bulk of many tumors. This lineal hierarchy would mirror that of lineage differentiation originated from normal stem cells (Figure 1A and B). 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.

Two properties shared with normal stem cells, namely self-renewal and differentiation into a range of heterogeneous tumor cell types, define cancer stem cells. The CSC concept must be distinguished from several other related designations, including tumor-initiating cells (TICs), tumor-propagating cells (TPCs) and cells of origin of tumors. In turn, the term TIC can overlap with that of cell of origin when it refers to the cell that suffers the oncogenic event that eventually results in neoplastic growth. However, TIC is more frequently used to designate those cancer cells, often isolated from established cell lines, that initiate and propagate the growth of tumors when implanted in immunodeficient mice. The same operational definition corresponds to the term tumor-propagating cells, and, indeed, the use of TPCs might be more rigorous and less confusing. Used in this sense, TICs (or TPCs) refer only to the in vivo tumorigenic phenotypes of cancer cells, and do not imply the generation of progeny cells with heterogeneous phenotypes. Finally, the term “cell of origin of a tumor” refers to the normal cell hit by the oncogenic events that eventually gives rise to the neoplasm. This definition overlaps with one of the two definitions of TIC, and thus it may be advisable not to use this latter term.

Several tests are used to measure CSC attributes. In vitro, self-renewal and symmetrical division is most often assessed as the capacity of cells for clonal growth under anchorage-independent conditions, and to indefinitely replicate such growth upon serial transfer. The most widely used in vivo test to assess self-renewal potential is the capacity of limited numbers of cells to form tumors when implanted in immunodeficient mice, and to maintain their tumorigenicity upon serial transplantation. To test their performance, subpopulations of tumor cells can be isolated by several methods, most often by fluorescence-activated cell sorting with the aid of antibodies specific for certain cell surface proteins.

Using such assays, in addition to hematopoietic neoplasms, rare tumor cells with properties of CSCs...
Figure 1. Hierarchical and stochastic models of CSC. A) In normal tissues, stem cells give rise to cells with progressively limited replication potential with consequent enhanced differentiated traits or terminal attrition (including apoptosis). B) In the hierarchical model of cancer cell evolution, cancer stem cells (CSCs) constitute a subset of tumor cells with the potential both for self-renewal (through symmetrical divisions) and to produce daughter cells with reduced proliferative capacity (through asymmetrical divisions). In this model, the differentiating divisions produce non-CSCs in a linear irreversible mode. This model implies that CSCs can be isolated as distinct subpopulations, with properties different from non-CSC subpopulations. Breast cancers are the tumor types in which more evidence has accumulated in support of this model. C) In the stochastic model, every cancer cell within a tumor has the potential to function as a CSC, and thus no distinct population can be isolated with self-renewal or differentiation properties that distinguish one population from another. The stochastic model can mimic a hierarchy when only a proportion of cells at any given moment displays CSC properties or produce progeny with diminished proliferative potential. Melanomas appear to follow a stochastic mode because any cell population isolated, regardless of the phenotypic markers used, is capable of generating tumors in appropriate animal modelant to support serial propagation.
have been described in solid neoplasms, including breast, colorectal, lung or prostate cancers or glioblastoma (4). Cell surface markers used to separate subpopulations of human tumors with such putative CSC phenotypes include expression of CD44 and low levels of CD24 in breast cancer CSCs, or CD133 in colon and brain cancers.

Nevertheless, whether CSCs are a distinct phenotypic subclass of neoplastic cells is currently under debate (5). Indeed, it is plausible that the phenotypic plasticity operating within tumors may produce bidirectional interconversion between CSCs and non-CSCs, resulting in dynamic variation in the relative abundance of CSCs (6, 7). Such plasticity could complicate definitive measurements of their prevalence.

Furthermore, assays used to detect tumor cells with CSC properties can be flawed. Two dimensional colony formation assays in culture may selectively favor cells that better adapt 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 microenvironments mimicking the original human tumor site. Finally, the specificity of the cell-surface markers utilized to isolate presumptive CSCs by FACS is unclear and their biological function is often unknown. Although CD44 and CD133 have been implicated as CSC markers in a variety of tissues, their specificity for the identification of CSC populations has been questioned (8-11). Overall, the available evidence supporting the identification of CSCs in solid tumors has been less convincing than in hematopoietic neoplasms, perhaps with the remarkable exception of breast cancer, at least in part because solid tumor cells exist in a complex microenvironment that is not readily modeled by xenotransplantation.

Recent evidence has suggested that CSC traits can be acquired upon engagement of the epithelial-mesenchymal transition program (12). In certain model systems, induction of EMT is accompanied with an enhancement of many of the defining features of stem cells, including self-renewal and the expression of cell surface markers frequently associated with both normal and cancer stem cells. This suggests that the EMT program enables not only invasion and dissemination of cancer cells at their primary sites but also their self-renewal capability that is crucial to their subsequent clonal expansion at sites of metastatic dissemination.

However, and in contrast to the original proposal (12) and subsequent evidences that engagement of the EMT program in epithelial tumor cells may be a universal path to generating CSCs, more recent evidences (13-15) suggest the contrary, namely that, while EMT is important for cancer cells to acquire motility and a mesenchymal mode of migration during local invasion, the same cells need to revert to an epithelial program in order for them to display self-renewal and metastatic properties.

The phenotypic plasticity implicit in the CSC state may also enable the generation of functionally distinct subpopulations within a tumor that support overall tumor growth in various ways. For example, an EMT can convert epithelial carcinoma cells into mesenchymal, fibroblast-like cancer cells that may mimic functions associated with cancer-associated fibroblasts (CAF) in some tumors (13). In another example of remarkable cancer cell plasticity, it has been shown that glioblastoma cells can give rise to endothelial like cells that actively participate in tumor-associated neoangiogenesis (16-19). These are examples of generation of functionally heterogeneous subpopulations of cancer cells through the imposition of epigenetic clues on presumptive CSCs, followed by symbiotic (mutualist or commensalist) interactions among the resulting subpopulations (20, 21), resulting in enhanced fitness and performance of the population of cancer cells taken as a whole.

**Clonal evolution and intratumoral heterogeneity**

The discovery of CSCs and biological plasticity in tumors indicates that a single, genetically homogeneous population of cells within a tumor may nevertheless be phenotypically heterogeneous due to the presence of cells in distinct states of differentiation. In addition to epigenetic mechanisms, an equally important source of phenotypic variability may derive from the genetic heterogeneity within a tumor that accumulates as cancer progression proceeds (22). Thus, high genetic instability occurring in later stages of tumor progression may produce rich genetic diversification (23) that outpaces the process of Darwinian selection, generating genetically distinct subpopulations more rapidly than they can be eliminated (24).

The clonal evolution model (25) proposes that most of the cancer cells within a tumor display tumorigenic potential and their phenotypes evolve through the stochastic acquisition of different genetic or epigenetic properties (Figure 1C). In this model, different clones would have distinct growth advantages and/or therapeutic resistance, and thereby drive the malignant evolution of the tumor. A neoplasm can be viewed from an evolutionary perspective as a genetically and epigenetically heterogeneous population of individual cells. Genetic and epigenetic alterations that are beneficial to a neoplastic clone,
enabling it to expand, are generally deleterious to the host, ultimately causing death to both the host and the neoplasm, similar to the suicidal collapse of a population owed to resource exhaustion from over exploitation. Because these somatic abnormalities have differing, heritable effects on the fitness of neoplastic cells, mutant clones might expand or contract in the neoplasm by natural selection and genetic drift, regardless of any negative effects on the organism. The fitness of a neoplastic cell is shaped by its interactions with cells and other factors in its microenvironment, including therapeutic interventions. Clonal evolution generally selects for increased proliferation and survival, and might lead to invasion, metastasis and therapeutic resistance (21).

Single-cell genome sequencing and hierarchical reconstruction analyses support that transformation and metastases are probably clonal because they are derived from single cells. Furthermore, individual metastatic lesions are clonal in origin and genetically unique, yet have a clonal ancestry traceable to the primary tumor (26). In addition, the metastatic cells emerge from a main advanced expansion and not from an earlier intermediate or a completely differentiated subpopulation (27), suggesting that they arise late in tumor development (28, 29). The classic model of clonal evolution suggests the occurrence of a sequential acquisition of mutations followed by subclonal dominance. Single-cell mutational analysis in serial samples have provided evidence that the evolutionary trajectories are complex and branching and not linear (26, 30, 31), as originally proposed by Nowell (25) and mirroring Darwin’s evolutionary speciation tree.

Stem cells in the normal adult prostate gland

The prostate is a glandular epithelium that originates from the urogenital sinus during embryonic development. After birth, the prostate keeps developing until the end of puberty. The normal adult prostate epithelium remains in a growth-quiescent state because prostate cells do not need to divide continuously to maintain tissue homeostasis, which might imply that prostate stem cells do not exist. However, in the context of androgen deprivation, the prostate epithelium regresses and it will regenerate to its initial size when physiological levels of androgens are restored. This phenomenon indicates that prostate epithelial stem cells exist in the regressed state, perhaps behaving similarly to quiescent stem cells in other tissues that function in tissue regeneration.

The normal prostate gland epithelium contains three morphologically distinct cell types that express distinct markers (32).

1) 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 (PSA), prostatic acid phosphatase (PAP), and high levels of androgen receptor (AR).

2) Basal cells are localized beneath the luminal layer and express CK5, CK14 and p63, low levels of AR and no PSA or PAP.

3) Neuroendocrine cells are scarce and quiescent, and express synaptophysin in and chromogranin A, but do not express AR or PSA.

In addition, a population of “intermediate” or “transit-amplifying” prostate epithelial cells has been reported that co-express markers of both basal and luminal cells. These observations prompted a model in which basal stem cells give rise to intermediate “transit-amplifying” cells that are progenitors for luminal cells (33). However, this model lacked experimental verification. Following androgen ablation, 90% of the luminal cells, but only a small percentage of basal cells will undergo apoptosis during the process of regression. Consequently, early studies favored a basal localization of stem cells, as most basal cells are castration resistant. However, some evidence has also supported the existence of luminal epithelial stem cells. Thus, although p63 is a marker of basal cells and p63 null mutant mice fail to form prostate (10, 34, 35), grafting of urogenital rudiments from p63 null cells could rescue tissue with prostatic ductal structures which, despite lacking basal cells, could undergo multiple rounds of regression/regeneration (36).

More recently, the issues of the properties, localization (luminal or basal) of prostate stem cells and their hierarchical relationships with presumptive progeny cells have been addressed through two general experimental approaches, flow-cytometry followed by tissue reconstitution assays or genetic lineage marking.

Flow cytometry-based approaches use cell-surface markers to isolate cell populations from the prostate. Subsequently, the isolated cells are combined with embryonically derived urogenital synus mesenchymal cells (UGSM) and grafted under the renal capsules of immunodeficient host mice. Using this assay, several groups have identified Sca-1, α2β1-integrin, CD49f, CD133 and Trop2 as cell-surface markers specific for prostate stem cells (9, 37-40). Interestingly, Lin-Sca-1-CD49f+ populations express basal markers such as CK5 and CK14, but express low levels of CK8, CK18 and AR (39). Other properties proposed in the past to identify candidate prostate epithelial stem cells are expression of CD44
or the ability to efflux the fluorescent dye exclusion of Hoechst 33342 detected by flow cytometry as a "side population" (41). Such studies have concluded that candidate prostate epithelial stem cells are basal. Furthermore, it has been shown that human prostate sphere-forming cells are predominantly basal and can reconstitute prostatic tissue after recombination with UGSM and subcutaneous injection in SCID mice (42). Of these markers, Sca-1 is not expressed in the human prostate, while α2β1-integrin, CD49f, CD133 and Trop2 are expressed in presumptive human prostate cancer stem cells. Nevertheless, it remains unclear whether these isolated cell populations truly behave as adult stem cells in the human prostate.

An alternative method to identify adult stem cells consists in the genetic lineage marking of progenitor cells followed by analysis of progeny differentiation in vivo. Using this methodology, a luminal population with stem cell properties was identified during prostate regeneration (43, 44). This study utilized a mouse strain that expresses the tamoxifen-responding CreERT2 under the promoter of Nkx3.1, a gene that encodes a transcription factor that represents the earliest specific marker for prostate epithelium during organogenesis. Using a fluorescence reporter, Wang et al. (43) showed that in castrated Nkx3.1-CreERT2 mice, Nkx3.1 is only expressed in a small fraction of luminal cells termed castration-resistant Nkx3.1-expressing (CARN) cells. Following androgen-mediated prostate regeneration, the lineage progeny was found to be present in both luminal and basal compartments, indicating that the CARNs population is composed by bipotent progenitors.

In conclusion, studies using flow cytometry-based approaches have mainly identified the basal compartment as the source of adult stem cells in the prostate, whereas genetic lineage marking studies uncovered the presence of stem cells in the luminal compartment. However, a recent study (45) that uses inducible genetic lineage marking suggests that postnatal development is mediated by basal multipotent stem cells that differentiate into basal, luminal and neuroendocrine cells, as well as by unipotent basal and luminal progenitors. In contrast, adult prostate regeneration is mediated by distinct pools of unipotent basal and luminal progenitors, as also shown by Lu et al. (46) and in agreement with prior work (44). These studies suggest that during postnatal development multipotent basal stem cells contribute to both basal and luminal expansion, whereas in adult prostate regeneration following androgen ablation and reconstitution, both basal and luminal unipotent stem cells mediate prostate regeneration. The switch from multipotent progenitors to unipotent basal and luminal cells in the adult prostate epithelium is reminiscent of the situation found in the mammary gland, although this switch occurs much more precociously in the mammary gland and postnatal mammary gland development is ensured by unipotent progenitors (47).

Cell of origin of prostate cancer: luminal, basal or both?

CSCs are more likely to originate from a normal tissue stem cell because of their indefinite proliferative nature, and thus 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 examine 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.

A further reason why the identification of the cell (or cells) of origin for prostate cancer is relevant is the possibility that different subtypes of tumors may originate through transformation of different progenitors, as in the case of breast cancer (48). Thus, understanding the cellular origin of prostate cancer can help improve disease prevention and therapeutics because different subtypes of tumor, such as acinar ductal, small basal, basal cell or sarcomatoid carcinomas, may differ in their prognosis and response to treatment (49-52).

Historically, luminal cells were considered to be the cells of origin of prostate cancer because they are mainly composed by cells that display a luminal phenotype. In addition, pathological analysis of high-grade PIN samples, which 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 (53, 54).

Other studies have provided evidence of luminal cells as cells of origin of prostate cancer. A recent study (55) generated a prostate-specific antigen-Cre, Ptenfl/flouP mouse prostate cancer model and reported that the initial hyperplastic cells were all luminal. Another study (43) utilized a mouse strain that expresses the tamoxifen-responding CreERT2 under the Nkx3.1 promoter and discovered a small fraction of luminal cells that resists androgen depletion, which they termed castration-resistant Nkx3.1-expressing (CARN) cells. Targeted deletion of the Pten tumor-suppressor gene in CARN cells resulted in prostate carcinoma, indicating that CARNs are a cell of origin...
of such tumors. 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 in human prostate cancer. Indeed, if CARNs correspond to facultative stem cells, they may correspond to a cell state that is only acquired in the regressed epithelium.

On the other hand, experimental evidences also support a basal cell of origin of prostate cancer. Some of those studies are based on the dissociated prostate cell regeneration assay mentioned above, which involves dissociating single prostate epithelial cells, combining them with embryonically derived urogenital sinus mesenchymal cells (UGSM) and graft the cells under the renal capsules of immunodeficient host mice. UGSM cells are capable of stimulating proliferation and differentiation of prostate stem cells to generate glandular structures de novo. Using this approach, mouse LinSca1+CD49Fhigh cells were sorted, that correspond to a predominantly basal population. Those basal cells have been demonstrated to be capable of differentiating into luminal cells in grafts (39). Grafting those cells together with FGF10-expressing UGSM produced multifocal glandular carcinoma similar to the small glandular structures observed in human prostate cancer (56). Also, lentiviral overexpression of activated Akt and ERG, and coexpression of these two genes with AR in basal and luminal cells resulted in adenocarcinoma only in the case of basal cells. These studies demonstrate that basal cells are efficient targets of transformation in prostate cancer.

Finally, two very recent studies (46, 57) in which Pten was knocked out specifically in basal or luminal cells in intact adult mice have provided evidence that both basal and luminal cells can serve as the cell of origin of prostate cancer. The study by Choi et al. (57) revealed that luminal cells rapidly developed prostatic intraepithelial neoplasia lesions after inducible Pten deletion, whereas basal cells were more indolent to oncogenic insults and mice only developed tumors after a long latency. In contrast, the study by Lu et al. (46) found that tumors that originated from luminal cells after deletion of Pten exhibited slower disease progression compared to basal-derived tumors. Both studies were concordant, though, in providing evidence that trans-differentiation of basal cells into luminal cells in the context of oncogenic signaling is a limiting step in disease initiation because as soon as trans-differentiation took place, the derived luminal cells proliferated in response to the pre-existing oncogenic insults.

**The elusive identity of human prostate cancer stem cells**

Experimental evidence has shown that human prostate cancers harbor cells displaying properties of CSCs, including in vitro self-renewal and in vivo reconstitution of tumors. However, no definitive consensus has been reached regarding the molecular profiles that may define prostate CSCs. This is not surprising, given the caveats indicated above about the use of experimental models and cell surface markers or the inherent plasticity of cancer stem cells.

A number of studies have reported the isolation of presumptive prostate CSCs from established human prostate cancer cell lines, using combinations of cell-surface markers. For example, CD44+/α2β1high/CD133+ cells were isolated from the DU145 and LAPC9 cell lines as candidate prostate CSCs, based on colony formation assays and tumor initiation (58, 59). CD133high cells were identified from human telomerase-immortalized primary tumor-derived prostate epithelial cell lines (60). Clones from the PC3 prostate cancer cell line grown in in vitro 3D cultures were shown to contain cells expressing high levels of CD44, α2β1 and β-catenin, and could initiate serially transplantable tumors after subcutaneous injection (61). Recently, the Tang laboratory has described that LNCaP prostate cancer cells spontaneously expressing low levels of (PSA-low) are quiescent and refractory to stresses including androgen deprivation, exhibit high clonogenic potential, and possess long-term tumor-propagating capacity (62). This subpopulation harbors highly tumorigenic castration-resistant cells that display a ALDH1+/CD44+/CD133high+/CD133low- phenotype, thus suggesting a direct link between prostate CSCs and development of castration-resistance. Further supporting that they represent genuine stem cells, subpopulations of cells enriched in presumptive prostate CSCs have been found to display preferential activation of the PI3K/AKT signaling pathway (59) or highly active gene networks shared with embryonic or adult stem cells (13, 63).

Flow cytometry has also been used to isolate cell subpopulations from primary human tumors, such as CD44+/α2β1high+/CD133+ cells (9, 64), with high proliferative potential in anchorage-independent growth assays, as well as the ability to differentiate to a luminal phenotype in vitro. However, tumor initiation in vivo has not yet been demonstrated for any flow-sorted sub-populations isolated from primary prostate tumors (2, 65). Several reasons may explain this failure to unequivocally identify prostate CSCs. First, it is extremely difficult to culture primary prostate cancer cells (65). To date, human prostate cancer cell lines have been established from only two primary prostate cancer patients, with the remaining established lines being derived from metastatic sites from fewer than 10 patients. Second, prostate CSCs, similar to normal prostate epithelial stem cells (66), engage in complex interactions with stromal cells and factors (67, 68) that may form a niche required for the maintenance of
stem cell characteristics. Depriving cancer cells of their niches by in vitro culturing or by transplantation in a foreign organism may prevent the full display of CSC phenotypes. Until these issues are not resolved, it will be unclear whether prostate cancers are organized hierarchically and follow the CSC model or the utility of putative CSC markers in clinical prognosis.

**Prostate cancer stem cells and therapeutic resistance**

Increasing evidence in a variety of tumor types suggests that cells with properties of CSCs are more resistant to various commonly used chemotherapeutic treatments (69). Their persistence may help to explain the almost inevitable disease recurrence following apparently successful debulking of human solid tumors by radiation and various forms of chemotherapy. Indeed, CSCs may well prove to underlie certain forms of tumor dormancy, whereby latent cancer cells persist for years or even decades after surgical resection or radio/chemotherapy, only to suddenly erupt and generate life-threatening disease. Hence, CSCs may represent a double threat, in that they are more resistant to therapeutic killing and, at the same time, endowed with the ability to regenerate a tumor once therapy has been halted (70).

In spite of the difficulties encountered for the unequivocal identification and scoring of CSCs in prostate cancer, experimental evidences suggest that the enrichment of prostate cancer cells with CSCs along tumor progression or in response to therapeutic pressure may significantly contribute to the development of therapeutic resistance. Reciprocally, therapy-resistant prostate cancer cells tend to display CSC properties. For example, docetaxel-resistant DU145 prostate cancer cells have been found to be more clonogenic and tumorigenic than bulk parental cells, and have active Notch and Hedgehog pathways (71), active PI3K pathway (72) or display traits of epithelial-mesenchymal transition (73). However, there is also evidence that not all drug-resistant prostate cancer cells are CSC, as found by Yan et al. (74), who observed that docetaxel-tolerant Du145 cells were less proliferative, clonogenic and tumorigenic than docetaxel-sensitive parental cells and were depleted of CD44+ cells.

In prostate cancer, resistance to chemotherapy is a most relevant issue in those cases that have developed highly aggressive androgen-resistant tumors. A pressing question that concerns a broader population of prostate cancer patients regards the molecular mechanisms that lead to unresponsiveness of tumors to hormonal therapy (castration-resistant prostate cancer) and, in particular, whether cells with CSC properties may contribute to CRPC. Unveiling the identity and properties of the cancer cells responsible for CRPC should facilitate the development of new therapeutic strategies targeted at that particular subpopulation.

Androgen receptor (AR) is expressed throughout human prostate cancer progression and remains at high levels in CRPC (75). The maintenance or even upregulation of AR activity through cancer progression would be consistent with selection for castration-resistant AR+ CSCs that can generate tumor cells that are themselves castration resistant. AR is expressed in CRPC and may function in an androgen independent manner through autocrine signaling or crosstalk with other prosurvival and proliferative pathways (76). However, levels of AR are also heterogeneous and, in some instances, absent from late stage diseases (77).

Recent evidence suggests that loss of PTEN might provide a unifying mechanism linking CRPC, maintenance of AR expression and CSCs. PTEN loss occurs frequently during human prostate cancer progression, with up to 70% of late stage samples exhibiting loss of PTEN function or activation of the PI3K pathway (51). It has been shown that PTEN loss or activation of the PI3K/PI3K pathway leads to castration-resistant growth (78-81). Epithelial cells from Pten-null mice remain sensitive to androgen withdrawal or AR ablation. However, the activation of the PI3K/PI3K pathway that results from loss of Pten can counteract androgen/AR-signaling blockage, mobilize basal and transient-amplifying stem/progenitor cells, and promote cell proliferation. Therefore, in prostate cancer initiated by PTEN loss or PI3K activation, the overall outcome of cancer development, especially CRPC development, depends on the balance of androgen-dependent cell survival/differentiation and androgen-independent cell proliferation.

Mulholland et al. (82) have found that PTEN loss suppresses AR transcriptional factor activity and androgen-responsive gene expression in both murine models and human prostate samples. AR itself is not down-regulated in Pten-null prostate cancer cells, even after castration. This suppression of androgen-responsive gene transcription is accomplished through the activation of multiple coregulators that accompany loss of Pten, including EGR1, c-Jun and EZH2. Therefore, loss of Pten renders cells less dependent or completely independent of signaling provided by androgens, thus promoting CRPC growth. In addition to EGR1 and c-JUN, AR target gene expression is also inhibited by the ERG transcription factor, a member of the ETS family whose gene is frequently translocated to the AR-responding TMPRSS2 gene (83). Therefore, it is possible that some oncogenic events that drive prostate cancer initiation, such as the TMPRSS2-associated gene rearrangements, can also participate in progression to
later stages of the disease, including the acquisition of CRPC. For these molecular mechanisms to enhance androgen-independent growth, they might require to be engaged in CRPC-specific CSCs that acquire additional pathway abnormalities, such as activation of PI3K through loss of PTEN.

Conclusions and future perspectives

Stem cells and cancer stem cells constitute a very active field of research in which many questions are still open, including definitive markers and methods to reliably identify normal SC and CSC. In spite of that, evidences accumulate pointing to that support the importance of CSC identification and measurement for diagnostic, prognostic and therapeutic purposes. For instance, BMI1, a stem cell factor that plays a role in cell cycle, cell immortalization, and senescence, has been recently proposed as a new marker for prostate carcinoma which can be detected both in serum and in tissue after being secreted by tumor cells (84). More interesting is the possible application of the analysis of stemness traits in primary tumors for prognostic purposes. Thus, Colombel et al have recently proposed that immunohistochemical expression of c-Met, α2, and α6 integrin in prostate tumors is associated with the occurrence of bone metastasis (85). Similarly, we have observed that the immunohistochemical expression of the self-renewing populations marker SOX2 occurs at higher levels in prostate cancers with more advanced stage and is enhanced in some metastases when compared to primary tumors (13), therefore being a candidate prognostic marker. The potential of microarrays signatures for molecular prognostic classification of prostate carcinomas has also been explored to detect stemness signatures, that include the loss of expression of p53 and PTEN, which can be useful to classify tumors and to predict very poor survival outcome (86).

Finally, therapeutic applications of this new knowledge about SC and CSC are obvious if the resistance of CSCs to current systemic therapies are definitively confirmed and cells with stemness traits can be accurately detected in recurrent neoplasms. Indeed, new drugs or interventions specifically targeting molecules overexpressed or active in presumptive CSCs such as CD44 (87) or to counteract full stemness programs could prove fruitful in eliminating therapy-resistant neoplastic clones.

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