REVIEW

Cellular interactions in prostate cancer genesis and dissemination. Looking beyond the obvious

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Abstract

Similar to normal organs arising from normal stem cells, cancers can be viewed as organs composed of heterogeneous cellular populations arising from cancer cells with indefinite proliferation abilities. The continuous malignant progression is maintained by the proliferation of cancer stem cells and not the progeny that undergo limited proliferation before terminally differentiating. Effective therapy must eradicate malignant cells with unlimited clonogenic expansion within the primary tumor bulk. Thus, resolving both the specific cell of origin for prostate cancer and the interactions between the cells and the surrounding microenvironment within the cancer stem cell niche are crucial to appropriately define rational targets for therapeutic intervention and cure prostate cancer.

***Keywords*:** prostate cancer stem cells, stem cell niche, cell plasticity.

Epidemiology and current management

Prostate cancer is one of the most commonly diagnosed cancers and the second leading cause of cancer-related death in European and American men, with approximately 220 000 new cases of prostate cancer and 30 000 deaths estimated to arise in the US alone in 2010 [1, 2]. The increase in absolute incidence can be ascribed to the combination of an aging male population and the use of early testing, based on more sophisticated measurement of serum levels for not only prostate-specific antigen (PSA), but also newer serum based markers such as PCA3 [3], and general proteomic markers [4].

Organ confined, low PSA (under 10 ng/mL) and low Gleason score (GS<7) prostate cancer can be managed effectively by radical surgical intervention, radiotherapy or even active monitoring, but the prognosis for patients with high-Gleason grade tumors detected either by screening or incidentally as a result of urinary tract symptoms remains bleak. In this case, most patients are cured by local-regional treatment in which radiotherapy has a prominent role and is used either alone or with hormonal treatment [5]. In metastatic disease, mainly in bones, the androgen-modulation therapy results in immediate and rapid decrease in pain and undetectable levels of PSA [6], but approximately 30–40% of prostate cancer patients reveal failure after treatment and the time to death remains stubbornly consistent, even with improved regimens of chemotherapy [7, 8].

Research strategies have focused on refining the delivery of radical therapies using laparoscopic or robotic surgery and intensity-modulated radiation therapy, which have met little success to reduce toxicity because treatment aimed at the whole gland results in damage to surrounding structures such as bladder neck, neurovascular bundles, external bladder sphincter, sigmoid or rectum. The answer to developing a more biologically adapted treatment might therefore be found in the specific architecture of the prostate epithelium and its patterning during ontogenic development and oncogenesis.

Stem cells in embryology and pathology

The prostate is a small, walnut shaped and sized gland, located below the bladder. It surrounds the urethra and has a fibromuscular function which acts to restrict urine flow, but its main function is to produce essential proteins for the functioning of sperm, such as acid phosphatase, citric acid and bioavailable zinc. It also produces some of the highest amounts of polyamines, which regulate the pH of sperm and preserves a mildly alkaline environment for the sperm within the acidic female cervix [9].

The human prostate develops from the urogenital sinus in response to testosterone stimulation and initially consists of a multilayered epithelium surrounded by mesenchyma. During ductal budding, which starts at 10 weeks of gestation, multiple epithelial outgrowths

invade the mesenchyma and form ducts that elongate and branch out from the urethra, terminating into acini [10]. From the 20th weeks of gestation up to puberty, the immature prostatic acini and ducts are lined with multiple layers of progenitor cells, with round nuclei and scant cytoplasm. Postnatal development includes periods of growth during the first year and during puberty because of testosterone surge, separated by a period of quiescence during childhood. After having received androgen stimulation, the immature multilayered epithelium differentiates into a two-layered epithelium consisting of peripheral flattened to cuboidal basal cells and inner secretory cylindrical epithelial cells [11].

Both the epithelial and stromal components of the adult prostate cell structure express the receptor for testosteron, called the androgen receptor (AR). In the absence of AR, the prostate cannot develop and shrinks or involutes after castration, but will regenerate after restoration of normal androgen levels. The castration resistant fraction of normal prostate epithelium, called prostate epithelial stem cells, has been proposed to reside within the basal epithelial compartment by Collins AT and Maitland NJ [12], but the key stimulus for regrowth lies within the key AR.

Secretory cells typically express high levels of AR, prostate specific antigen (PSA) and low molecular weight keratins (CK8 and CK18). In contrast, basal cells show either low or undetectable levels of AR, expressing high molecular keratins (CK5, CK14 and CK34β E12) and the basal cell marker p63 [13]. The progenitor cells are phenotypically intermediate between basal and secretory cells and represent the transient amplifying cell population, expressing CK19. Using an immunocytochemical approach, Wang Y *et al.* [14] have demonstrated that most cells in the urogenital sinus co-express secretory and basal cell markers, as well as CK19, CD133, α2β1 or Sca-1. These data proposed the idea that the intermediate double positive basal cell population represents prostate stem cells, able to differentiate into both mature secretory and basal cells. A third type of cells, neuroendocrine cells are scarce and express chromogranin A and synaptophysin but lack PSA and AR and induce proliferation of adjacent cells through paracrine secretion of neuropeptides, as proposed by Bonkhoff H *et al.* [15] (Figure 1).

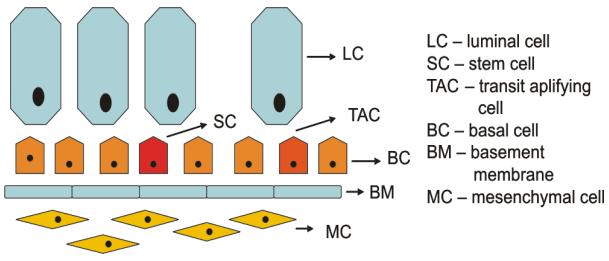

Figure 1 – ***The basic histology of the prostate gland.***

With regards to prostate cancer, it has remained a long-standing conundrum that prostate carcinoma is effectively diagnosed by a complete absence of basal cells [16], whereas prostate epithelial stem cells have been thought to reside in the basal layer. Prostate adenocarcinoma usually proceeds through a series of defined stages, from prostatic intraepithelial neoplasia (PIN), to prostatic cancer *in situ*, to invasive and metastatic cancer. Tumor cells are usually AR+ and PSA+, mimicking ontogenesis, and initially respond to androgen depletion. After a remission of up to several years, cancer will become resistant to therapy and eventually progress despite low levels of androgen and chemoradiotherapy. Most investigators link prostate carcinogenesis to the "cancer stem cell model", which explains most symptoms and pathology examinations.

Cancer stem cells and resistance to conventional therapy

As therapy-resistant, prostate cancer is the second most common cause of cancer death in men, there is an urgent need for the development of alternative targeted therapies. The most compelling evidence of the existence of prostate stem cells in the basal cell compartment is derived from the mouse castration model, where androgen withdrawal results in glandular involution and apoptosis in more than 90% of epithelial cells, but leaves the basal cell layer intact [17]. As slow-cycling cells-retaining bromodeoxyuridine (BrdU) labeling following androgen withdrawal or replacement experiments have been identified in both basal and luminal cell compartments, Tsujimura A *et al.* imply that prostate stem cells are not restricted to one epithelial cell layer [18].

It is possible that cancer arises in a cell at any stage of differentiation, from the most primitive stem cells to the most differentiated tissue-specific cell. Early events are most likely to occur in normal stem cells, as only these cells live long enough to accumulate the several genetic changes required for an invasive cancer to develop. Once one or more initiating genetic changes have occurred in the progenitor, all the downstream cells will contain this change, in which case it is possible that one of the daughter cells acquire not only the properties of a stem cell, but also the additional genetic changes that allows the cancer to progress to the next step and invade the surrounding tissues.

Cancer stem cells (CSC) were first isolated in acute myeloid leukemia as $CD34^{+}$ $CD38^{--}$ cells, and after just a few years, from solid tumors, including hepatocellular carcinoma and brain cancer [19, 20]. Prostate cancer stem cells were also isolated by a variety of methods, including isolation of the "side population" based on the exclusion of different dyes, on their ability to form tumor spheres (prostatospheres) under serum-free non-attachment conditions and on the basis of CD44, CD133, Sca-1, CD49f or integrin α2β1 surface marker expression [21–23].

Current therapies are not yet curative as CSC may escape through both increased efflux of chemotherapeutic agents due to the ABCB1 (MDR, P-gp) and ABCG2 cell membrane proteins, and through increased DNA-repair. Several proteins of the ABC transporter superfamily are overexpressed by stem cells and make up the so-called "side population", having the capability for accelerated efflux from the cells of fluorescent dyes Hoechst 33342 and Rhodamine 123, transported by the very same ABC family proteins [24, 25]. Metastatic

prostate cancer may be treated with androgen deprivation therapy by either surgical castration or medical castration with gonadotropin-releasing hormone agonists. The AR is expressed in almost all prostate adenocarcinoma cells and dictates the response to therapy. The initial response is transient and the tumor will progress to castrate-resistant prostate cancer because of AR gene amplification resulting in reactivation of androgen-responsive genes involved in growth and survival [26].

Radiotherapy plays and important role in the management of prostate carcinoma, but the radioresistance of tumor cells limits the outcome of ionizing radiation. The developed resistance is related to global changes in expression of proteins interfering with various intracellular pathways. Such is the case of over-expressed pre-mRNA-processing factor 19 (PRPF19) and programmed cell death 6-interacting protein (PDCD6IP) that reduce the levels of apoptosis in cells exposed to stress or DNA-damaging factors [27]. Other changes in the proteomic profile, such as up-regulation of glyceraldehyde-3-phosphate (GAPDH) or phospho-glycerate kinase 1 (PGK1), also influence cancer cell aggressiveness and the capacity for invasion and metastasis formation, being implicated in the behavior of other types of cancer as well (as PGK1, that influences HER-2/neu signaling in breast cancer) [28].

Because it is much easier to kill downstream cells than CSC, we may explain why the vast majority of metastatic cancers respond for relatively short periods to drugs or hormonal therapy, until the stem cell' pool recovers and resumes its inexorable growth.

The stem cell niche

An important consideration for cancerogenesis is the influence of tissue microenvironment, which has a deep effect on the behavior of cells in a tumor and their ability to metastasize. It is well known that *in vivo* cell–cell and cell–matrix interactions play an important role in how different cells respond to specific stimuli. In a normal tissue, these interactions are critical in cell function and are important for the concept of "stem cell niche". The niche is a cell environment that provides critical stem cell maintenance signals to support the undifferentiated phenotype of progenitor cells. Such relevant signals include the Hedgehog, Wnt or Notch pathways, all of them important in early ontogenesis and control of cell differentiation and proliferation. In cancer, the cell-cell and cell-matrix interactions are overlaid on top of other features of tumor physiopathology microenvironment, including the presence of hypoxia, low pH and nutrient deprivation. Fluctuations of these parameters have profound effects on the activity of cancer stem cells and their potential niche.

Hypoxia is an important feature of the niche because is intrinsically linked to the formation of neovasculature, regulating the production of proangiogenic factors. According to Hill RP *et al.* [29], 1% to 1.5% of the genome is transcriptionally regulated by hypoxia and many of these genes are controlled by hypoxia-inducible factor-1 (HIF-1). Under hypoxic conditions (3–5% O_2), gene expression may be altered toward an immature phenotype, promoting de-dedifferentiation of prostate tumour cells into more "stem-like" ones. This hypothesis has recently been confirmed by a neuroblastoma cell line [30]. The hypoxic cells expressed higher levels of the embryonic stem-ness gene OCT-4 due to the interactions between HIF-transcription factors (HIF-1α and HIF-2α). OCT-4 is a direct target of HIF-2α, the induction of whom could contribute to the formation and maintenance of cancer stem cells. Hypoxia also modulates the activity of Notch signaling, as well as Wnt by the down-regulation of E-cadherin, which leads to increased levels of β-catenin, a Wnt intracellular messenger. Also, both HIF-α subunits control the activity of the well-known oncogene c-Myc through the antagonization of its activity. It competes for binding with the c-Myc partner Sp-1, resulting in cell cycle arrest at low O_2 levels [31]. These observations may have a significant role in cell signaling within prostate neoplasia containing regions of hypoxia, considering the multiple properties of the Myc protein. It should also be pointed out that Myc and OCT-4 were two of the four genes shown to be capable of inducing fibroblasts to revert to a stem cell phenotype [32].

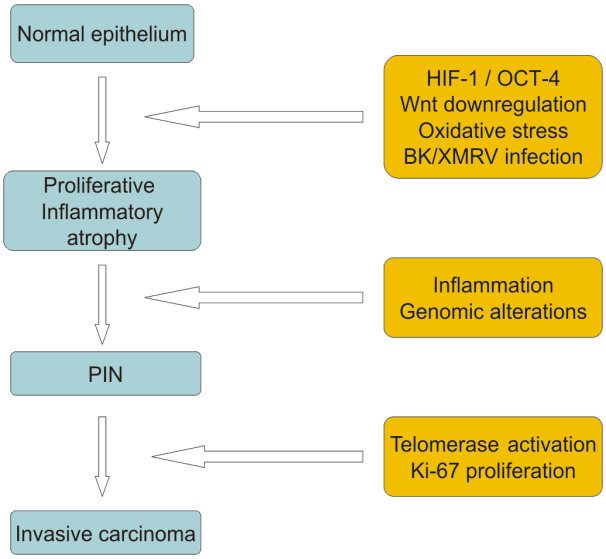

Figure 2 – ***A model for prostate oncogenesis. The normal epithelium (under oxidative stress, Wnt down-regulation or human gammaretrovirus Xenotrophic MuLV-related virus XMRV infection) will have an increased proliferative potential and eventually lead to the appearance of the prostate intraepithelial neoplasia (PIN). The high proliferative potential and telomerase activation determines the evolution from PIN to invasive, metastatic and treatment-resistant carcinoma.***

Inflammation is a common occurrence in the human prostate and is emerging as a strong candidate for the primary etiological event of the tumor. The origin of the inflammatory infiltrates can be related to infectious agents or chemical damage [33]. Since the results of zur Hausen in cervical epithelial cancer viral infection plays a very important role in urogenital malignant progression. A number of potentially carcinogenic viruses have been detected in human prostatic tissues, such

as the oncogenic human papovavirus BK or the human gammaretrovirus Xenotrophic MuLV-related virus (XMRV) in premalignant lesions [34, 35]. These ideas have improved the hypothesis of prostate oncogenesis, where PIN is preceded by an inflammatory atrophy with prostatic epithelial cells showing an increased Ki-67-marked proliferation (Figure 2).

Zipori suggests that stem-ness could be "a transient and reversible trait that almost any cell can assume given the correct trigger (niche) and that is characterized by having many potential outcomes but no specialization" [36]. Even if in his article, he refers to normal stem multi-lineage differentiation, it raises the hypothesis that the same normal stem cells, circulating into the blood stream and guided by chemokine signaling, may interact with differentiated cells from a "metastatic niche" that provides appropriate stimuli (i.e., hypoxia or chronic inflammation due to infection) for de-differentiation or cell fusion. Can this be the birth of the prostate cancer stem cell?

Cell behavior and plasticity

In the extrinsic pathways, it remains uncertain whether hypoxia or chronic inflammation is enough for carcinogenesis, but these factors are certainly the main triggers. Inflammatory cells and mediators can destabilize the cell genome by inducing DNA-damage or affecting the cell cycle checkpoints and repair systems. Genetic instability, through accelerated somatic evolutions, leads to a genomically heterogeneous population of expanding cells selected for their ability to proliferate, invade distant tissues and evade host defense.

Inflammation affects first of all the mismatch repair (MMR) family members, whose mutation or epigenetic silencing is associated with microsatellite instability. Microsatellite instability show increased rates of DNA-replication errors throughout the genome, affecting especially genes that contain in their coding regions microsatellites intrinsically unstable and therefore prone to be copied incorrectly during replication. MMR is downregulated by a variety of mechanisms (including HIF-1α, TNF or IL-1β) and leads to chromosomal instability, that in turn results in abnormal segregation of chromosomes and aneuploidy [37, 38]. Chromosomal instability is also associated with both inactivation of p53 that normally protects cells from transformation by inducing apoptosis upon DNA-damage, and with matrix metalloproteinase dysregulation that act as oncogenes in this case.

Bone marrow-derived mesenchymal stem cells do not only constitute only the hematopoietic microenvironment, but are also responsible for the regeneration of most tissues from the human body because of their capacity to give rise to multiple mesenchymal lineage cells and even endodermal or ectodermal lineage cells. Because chronic inflammation can destabilize a cell's differentiation program, it is possible that prostate cancer may emerge as a consequence of the interplay between the microenvironment already prone for oncogenesis and the plasticity of a circulating normal stem cell, attracted to this inflammation site in the first place through chemotaxis. This is a highly controversial point of view that was already confirmed by Houghton J *et al.* in gastric cancer. Her team proved that chronic gastric inflammation consecutive to *Helicobacter pylori* infection, who leads over time to repetitive injury and repair resulting in hyperproliferation and increased rate of mitotic error, is linked to homing and engraftment in peripheral tissue by bone marrow-derived stem cells. These cells posses a very high degree of plasticity and their signals for cell growth and differentiation are unexpectedly sensitive. Thus, gastric cancer may originate from bone marrow-derived cells [39].

We have established that the differentiation status of a tumor cell is determined by the expression of a large number of genes and their products and that the cell is in close contact with it's surrounding microenvironment, which can secrete a large amount of growth factors and cytokines, in order to regulate the complex biology of tumor cells. The niche is known to accelerate the differentiation of cells, to sustain cells in G0 state for long periods of time or to induce apoptosis and also recently emerging data have proven that the tumor microenvironment may down-regulate specific markers, changing a previous phenotype. The extrinsic signals may enhance the cell's drug resistance and promote its longevity, causing it even to de-differentiate into a cancer stem cell. This theory has also been confirmed by Dezorella N *et al.* in a multiple myeloma model, proving that mesenchymal stromal cells can revert a myeloma cell to a less differentiated state by the combined effects of interleukin-6 and extracellular matrix interactions [40].

As unconventional the two theories mentioned above might be, a true breakthrough in fundamental oncology is the idea that clinically significant cancer evolves from transient mutated or aneuploid neoplasia by cell fusion to form unstable syncytia. Viruses, recently detected in human prostatic tissues, induce the host cell to express adhesion molecules that effects joining the cytoplasms of two cells. Then viral Bcl-2 or over-expressed cellular Bcl-2 prevents the syncytia from undergoing p53-dependent apoptosis or p53-independent death through mitotic catastrophe and as a direct result, new aneuploid cells survive. Virus-catalyzed cell fusion of tumor cells with normal cells in adjacent tissues appears to be a pathway to invasion of the normal tissue by the tumor [41].

The stated hypothesis is demonstrated by Human Papilloma Virus (HPV), the etiological agent for cervical cancer. HPV-infection begins by engaging in cell-to-cell transmission resulting in many stable binuclear syncytia observed in low-grade squamous intraepithelial lesions. Chromosomal aberrations then accumulate in polyploid cells leading to high-grade squamous intraepithelial lesions [42].

The result of the fusion and tumor growth is prevented physiologically by the attachment of cells to the intercellular matrix through the help of binding to different components of the matrix such as hyaluronic acid with different surface adhesion molecules, such as CD44. Usually, this system does the job, but sometimes the arrangement may be disrupted. One way is the

hyperglycosylation of glycoproteins like CD44 to an extent that they cannot bind to the matrix. The intervention of other ligands, facilitated by polyamines such as putresceine, spermidine or spermine, may affects the normal tissue architecture and last but equally as important, the surface adhesion molecules can be cleaved and broken down such as cells are no longer held in place. The last process appears to be facilitated by over-expression of matrix metalloproteinases and proteins with a disintegrin and metalloproteinase domain (ADAMs) [43].

The recent association between human pathogens and prostate cell plasticity, leading either to dedifferentiation, transdifferentiation or even to cell fusion, raise possibilities of undiscovered functions and therapeutics targeting the cancer stem cell niche for intraprostatic cancers.

The circulating CSC – early metastatic site interplay

Through clonal proliferation, a prostate CSC originating in the primary lesion and dispersed into circulation will arrest in the capillary bed of distant organs, which provides either pro or anti-metastatic stimuli regulating the onset of distant colonization by the tumor cell. If the surrounding environment is just right, this moment marks the turning point from a localized, potentially curable disease, to a system disorder. The progression of prostate cancer is not random, but a highly orchestrated, multi-step process, governed by basic cancer immunology. Once a clone capable of metastazing is formed, the complex network of events leading to distant tumor formation is still a matter of debate between two main theories despite all recent breakthroughs in fundamental oncology. This debate started when James Ewing challenged Stephen Paget's 'seed and soil' concept in the early 1930's [44, 45], but both hypothesis are not mutually exclusive.

Once having left the primary tumor, the prostatospheres (binded prostate CSC) are subjected to intense mechanical stress by shear forces caused by blood flow. These tumor spheres soon disintegrate into solitary prostate CSC in narrow capillaries, including the microvasculature of contracting skeletal and heart muscles where the cells require sphere-to-cylinder shape transformation [46]. The lack of high plasticity is lethal to the majority of tumor cells. Nevertheless, this is just the first step of the Darwinian selection of the cells capable of disseminating. The organization and structure of cytoskeletal components such as actin can be modified by external forces and since integrins appear to be directly involved in the early steps of metastasis, cell signaling and regulatory processes modulate their affinity may influence CSC adhesion or migration into host organs [47]. As circulating tumor cells are usually adhesion-dependent, anoikis limits the available circulation time and the cells' resistance to this special form of apoptosis. Thus, the most effective way for CSC is the establishment of adhesive interactions within metastatic target organs via the integrin–cadherin interplay.

Pathology examinations of target organ microvasculature often show tumor cells closely associated with platelets, as well as leukocytes or the coagulation system because specific adhesive systems provide selective mechanisms for these interactions. The altered surface glycosylation is a common feature of carcinoma cells, including prostate adenocarcinoma, with high expression of sialyl Lewis$^{a/x}$ as selectin ligands is association with poor prognosis in various cancers [48, 49] suggesting a potential role of such cell adhesion molecules in the process of metastasis. The aggregation of platelets around CSC may also involve thrombin and fibrin, but in experimental settings, platelet inhibition reduces the number of metastatic lesions and does not affect organ distribution of size of metastatic foci. Borsig L *et al.* report that the anti-metastatic effect of platelet inhibition using heparin is limited to the initial five hours after tumor cell inoculation [50], which indicates that platelets are able to interfere with early events of organ colonization.

The role of platelets in the metastatic network is not, however, limited to pro-adhesive processes as aggregating platelets and fibrin meshworks can form a potent shield around CSC that seems to prevent the contact with Natural Killer (NK) cells. In addition, for arrest in the capillary bed of target organs, prostate CSC may be associated with polymorphonuclear neutrophils (PMN) to enhance the colonization capacity. This is possible because of the expression of inter-cellular adhesion molecule-1 (ICAM-1), that enables tumor cells to adhere to PMN during their presence in the blood stream. If CSC were to interact with NK cells, programmed cell death would be induced by the perforin/granizyme pathway following granule exocytosis or by the Fas/Fas-L pathway within a few hours [51].

The CSC–endothelial cell interactions are equally as important as the previous step. Because blood vessels are generally lined with endothelial cells, circulating CSC are similar to leukocytes in using endothelial cell specific adhesion molecules, such as selectins or ICAMs to interact the same endothelial cells before they touch the underlying basement membrane in the course of extravasation. Endothelial cell surface molecules play a role in organ specific settlement of prostate CSC and the inhibition of tumor cell adhesion by anti-TF (Thomsen–Friedenreich factor) results in increased survival without impairing tumor cell proliferation, in a mouse model for spontaneous breast cancer metastasis [52].

CSC may also induce apoptosis of endothelial cells or increase their E-selectin surface expression, facilitating further the adhesion to the endothelium. The last, but not least of the major interactions a cancer stem cells must overcome to colonize a distant organ is that with the extracellular matrix (ECM). Among the most important molecules are again the integrins, the expression of whom may be altered in comparison with normal tissue cells. Certain integrins, such as $\alpha5\beta1$ [53], can change the growth behavior, neoangiogenesis and anchorage independent survival of normal cells and act as oncogenes or tumor suppressor genes. Integrins interfere with metastasis through their dual role in impaired cell adhesion at the primary site and by allowing tumor-

initiating cells to settle in tissue with an ECM composition different to their home tissue [54].

As CSC posses an altered expression of cell adhesion molecules and the stromal ECM of metastatic sites differ from one of the primary tumour or from that of normal tissues, the adhesive interactions are particularly sensitive in prostate cancer dissemination. Additionally, soluble matrix proteins, such as osteopontin, hyaluronectin or sialoprotein may also influence these interactions. The stroma also contains various paracrine factors, such as growth factors, cytokines or hormones that act with ECM components and regulate the metastatic niche availability in a very specific manner for each type of primary neoplasia [55–57]. Such is the case of prostate circulating stem cells, where the bone marrow derived ECM molecule osteonectin acts chemotactically for the primary tumor cells. Prostate adenocarcinoma will therefore disseminate preferentially to bones.

The mechanism for preferential dissemination of prostate tumor cells is more complex and it involves CXC chemokine interplay. The homeostatic chemokine stromal cell-derived factor-1 (CXCL12/SDF-1) regulates development, stem cell motility, neoangiogenesis and tumorigenesis. It binds to the widely expressed cell surface receptor CXCR4 and the involvement of this receptor–ligand interaction in the directed migration of cancer cells to metastatic sites has been proven not only in prostate adenocarcinoma, but also in breast, lung, ovarian, renal or brain tumors [58]. Up-regulated by HIF-1α or VEGF, CXCR4 receptor blocking using monoclonal antibodies should inhibit tumor growth and metastasis in head and neck cancers, primary brain tumors or acute lymphoblastic leukemia, apart from urology malignancies.

Targeting prostate cancer stem cells

It is well known that cancer stem cells are immortal due to the telomerase enzyme hTERT. Telomerase is present in all prostate cancers and has a very high activity. One small molecule shown to inhibit hTERT *in vitro* and *in vivo* is RHPS4, shown to stabilize the four-stranded G-quadruplex structure formed by the tracts of G-rich single-stranded DNA at the telomeres. Another promising agent with similar effects is the phosphoramidate oligonucleotide GRN163L, targeting the telomerase active site and inhibits binding to telomeres in $CD44^{hi}$ and $CD133^{+}$ cells [59, 60]. Phosphate and tension homolog (PTEN) is a lipid phosphatase known to play a vital role in the proliferation, motility, survival and metabolism of cells and interacts with many signaling pathways, including p53, Akt/PI3K and mTOR. In prostate neoplasia, the loss of PTEN occurs in 30–50% of cases and re-expression in various cell lines results in apoptosis. Re-expression is achieved by inhibition of PI3K with wortmannin and LY294002, but these compounds have broad specificity and have not been employed in the clinical setting [61].

The Wnt/β-catenin pathway controls self-renewal and proliferation of CSC, the members of the Wnt being generally secreted by other cells of the niche and bind to the seven transmembrane receptor Frizzled. Targeted molecular therapy is difficult to perform because of the high complexity of the pathway. Wnt antibodies proven to induce apoptosis are currently being developed in non-small cell lung carcinoma, melanoma and mesothelioma [62], but no results have been published prostate cancers.

Hedgehog (HH) signaling is a highly conserved developmental pathway and orchestrates body patterning, contributing to stem cell maintenance. HH is activated by three soluble molecules (Indian, Sonic and Desert) that act as paracrine or autocrine signals before binding to Patched (PTCH) receptors and in this way activating the transmembrane protein Smoothened (SMO). SMO triggers translocation of GLI1 and GLI2 (Glioma Associated Homolog) transcription signals to the nucleus and thus modulates cell proliferation, epithelial-to-mesenchymal transition or angiogenesis. During ductal morphogenesis, Sonic HH is expressed in sites of active epithelial growth and the loss of GLI1/2 function results in impaired ductal budding and stem cell depletion. HH is more active in prostate cancer in comparison with normal or hyperplastic tissue and PTCH and GLI expression is dramatically increased in metastatic lesion in comparison with primary tumors. The best-known HH inhibitor is Cyclopamine, that targets SMO and is shown to downregulate drug transporter expression in castration-resistant cancer, enhancing chemotherapy [63]. Cyclopamine drives androgen-independent growth in prostate cancer, synergistic with ErbB, but its employment in the clinic is impaired by low oral bioavailability and poor pharmacokinetics. A better pharmacokinetic profile has been shown by GDC-0449, a SMO-antagonist currently in phase II clinical trials for ovarian and colorectal carcinoma, as well as basal cell carcinoma. This molecule has also shown outstanding results in the treatment of medulloblastoma [64].

Another important pathway in the stem cell conundrum is Notch signaling, that regulates cell fate determination. Notch proteins are heterodimeric receptors that interact with the surface ligands Delta, Delta-like and Jagged from an adjacent cell. This bind will release the intracellular Notch domain through proteolysis by ADAMs and γ-secretase/presenilin. The effects will be either oncogenic or tumor-suppressor. Notch is necessary in prostate development for the proper branching morphogenesis and in the differentiation of the prostate following castration and androgen replacement [65], in the same time regulating the activity of $CK8^{+}$ $CK14^{+}$ transit-amplifying cells. The best Notch inhibitors are the gamma secretase molecules, which prevent the release of the intracellular Notch domain by inhibiting its cleavage. Such substances, like DAPT or MK0752 are currently under phase I clinical trial investigation for $CD34^{+}CD38^{-}$ CSC in acute lymphoblastic leukemia and $CD133^{+}$ CSC in central nervous system malignancies [66].

Plerixafor, also known as AMD3100, is a bicyclam molecule, which binds reversibly to CXCR4. Even though initially developed as a potential therapeutic agent against HIV, preclinical data have shown that AMD3100 blocks CXCL12 binding of CXCR4 and as a

result it inhibits SDF-1α-induced calcium flux and chemotaxis. Clinical trials have demonstrated that Plerixafor is effective for the mobilization of peripheral blood stem cells for use in autologous hematopoietic stem cell transplantation, but blocking the same CXCR4–CXCL12 axis can also play an important role in the control of CSC dissemination from the primary prostate lesion and metastasis. New drugs such as CTCE-9908 are currently undergoing preclinical confirmation in this setting [67].

The current view of prostate cancer is that of a complex disease caused by genomic and epigenetic aberrations that affect a defined set of cellular properties. Yet a primary cause of several cancers, accounting for about one-fifth of all cancer causes in the world, is a defined virus, bacterium or some other unknown element that may alter the normal physiology of a cell to such extent that it changes beyond control. Although the prostate cancer stem cell model is only at its early stage of development, this hypothesis is crucial for the understanding of prostate carcinogenesis as well as for assessing successes and failures of future early treatment using targeted molecular agents.

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