Prostate Cancer Stem Cells and Their Potential Roles in Metastasis

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Most solid tumors have now been reported to contain stem cell-like cells called cancer stem cells (CSCs). These cells are endowed with high tumorigenic capacity and may be the cells that drive tumor formation, maintain tumor homeostasis, and mediate tumor metastasis. Since these self-renewing cancer cells may be the sole population to develop a primary tumor, it is predicted that CSCs may also represent the lethal seeds of metastasis, as supported by a flurry of recent studies on the relationship between CSCs and metastasis. Herein, we summarize current knowledge of stem/progenitor cells and CSCs, especially in the context of normal human prostate and prostate cancer. We further update the recently gained knowledge on the involvement of CSCs in metastasis. We also discuss the fundamental influence of tumor microenvironment on the manifestation of CSCs and metastasis. Finally, we discuss the clinical implication of CSC-based therapy.


KEY WORDS: prostate cancer; cancer stem cells; metastasis; tumor microenvironment

INTRODUCTION

In North America, prostate cancer (PCa) is the second leading malignancy in American men with an estimated 186,320 new cases and 28,660 deaths in 2008 [1]. If patients only develop localized tumor, the 5-year survival rate is almost 100%. However, the 5-year survival rate for patients with metastatic PCa is only ~33%. Over 90% of PCa-related mortality results from systemic dissemination and metastasis [2]. Radical prostatectomy is the main treatment modality for localized, early-stage PCa patients and androgen deprivation therapy (ADT) represents the mainstay for advanced patients. Upon ADT, disease symptoms are alleviated in 70–80% of the patients for the first 2 years but most patients eventually fail ADT resulting in a more aggressive and lethal form of PCa referred to as androgen-independent PCa (AIPC), which progresses and metastasizes [3]. There is currently no effective therapy for PCa metastasis, largely due to our poor understanding of the complex multistage metastatic process, which encompasses invasion, survival, arrest in the bloodstream, and metastatic colonization [4]. The recently revived cancer stem cell (CSC) theory throws new light on our understanding of tumor initiation, progression, and metastasis. CSCs have now been reported in multiple solid tumors including cancers in the breast, brain, colon, and pancreas [5–8]. Since metastasis is the major cause for cancer-related death, the CSC hypothesis would be incomplete without linking to metastasis and, hence, a better understanding of CSCs in metastasis may lead to better treatment for cancer patients. A flurry of recent studies focuses on metastatic properties of CSCs [9–18]. Our lab has been studying prostate CSCs in long-term cultured cell lines, xenograft tumors, and primary patient samples and their involvement in PCa metastasis. We have demonstrated that the PCa cell population that expresses high levels of surface adhesion molecule CD44 (i.e., CD44+ cell) are enriched in not only tumorigenic but also metastatic potential [19–23]. In this study, we will summarize the new development of CSCs relevant to PCa.

NORMAL PROSTATE STEM/PROGENITOR CELLS

Human prostate is a tubular-alveolar exocrine gland comprised of three distinct cell types (basal, luminal, and neuroendocrine [NE] cells) embedded in a fibro-muscular stroma. Basal cells form the layer along the basement membrane and luminal cells sit above the basal-cell layer and secrete prostatic proteins into the lumen whereas NE cells are a minor population of neuron-like cells that produce biogenic amines and neupeptides that support epithelial growth [19,24,25]. Luminal cells express prostate-specific antigen (PSA), prostatic acid phosphatase (PAP), androgen receptor (AR), and cytokeratins (CK) 8 and 18. In contrast, basal cells express CK5 and CK14 but not PSA and PAP [24,25]. However, some basal cells also express low levels of AR and BCL-2 and more than 80% of the proliferative cells reside in the basal layer. Unlike the majority of secretory luminal cells, NE cells are quiescent and do not express AR or PSA but express NE-specific markers such as chromogranin A and synaptophysin [26].

Two fundamental traits of stem cells (SCs), i.e., self-renewal and multi-potency, were first established in studies of hemopoiesis [18,27]. The adult SCs are primitive cells with the self-renewal ability to differentiate into all different lineages of progeny and even to reconstitute an organ or regenerate the damage tissues, the best example of which are hematopoietic stem cells (HSCs) that can reconstitute the whole blood and rescue a lethally irradiated mouse [19,27]. Identification of various SCs has generated great hopes for cell therapies of neurodegenerative diseases, burn, diabetes, and heart diseases [9]. On the other hand, although the HSCs can be defined as long-term (LT) HSCs, short-term (ST) HSCs, and multi-potent progenitor (MPP) cells, we still lack in-depth knowledge on the lineage development in most other solid tissue SCs [19] including the prostate.

Evidence that strongly supports the existence of SCs in the prostate is from John Isaac’s classic androgen cycling experiments [28]. The
observation that the adult rodent prostate can undergo multiple rounds of castration-induced regression and testosterone-induced regeneration indicates that only a small population of SCs possesses the ability to both self-renew and differentiate while the bulk, androgen-dependent, terminally differentiated cells lack such an ability [24,27,28]. Several candidate populations of prostate stem/progenitor cells have been reported including those expressing high levels of CD44, integrin α2β1, or CD133 [19]. CD44 is expressed on most basal cells and has been implicated in tumor metastasis [20,21]. The CD133+ cells are enriched in CD44+/α2β1hi basal cell population and possess high proliferative potential in vitro and the ability to produce prostate glandular structures [29]. Interestingly, two recent independent studies in the mouse prostate have identified two different populations of SCs. One, marked by CD117 (c-Kit), is localized in the basal layer [30] and the other, called castration-resistant Nkx3.1-expressing cells, in the luminal layer [31].

Identification and characterization of normal prostate SCs clearly will have relevance to understanding the cell of origin for human PCAs. The experimental evidence that most of the cells that survive castration appear to be basal rather than luminal cells has led to the traditional hypothesis that the basal-cell layer harbors self-renewing SCs [24,28]. There are several pieces of evidence that support this hypothesis. First, some key molecules that normally regulate SC self-renewal and survival, e.g., p63, hTERT, and Bcl-2, are preferentially localized in the basal layer [19,32]. Second, the proximal region of mouse prostatic ducts that highly expresses basal cell marker CK14 but not luminal marker CK8 is found to be the prostatic SC niche. Furthermore, basal cells that express SC markers such as CD44, CD49f, CD117, CD133, Bcl2, Tert, and p63 are all located in proximal region but not distal and intermediate regions [33]. Third, when the basal-cell marker p63 is knocked out, mice are born without prostate or mammary gland [34]. Fourth, basal cells have been shown to differentiate into luminal cells [24,32]. Fifth, all primary normal human prostate (NHP) epithelial cells that can expand in vitro express basal-cell markers such as CD44, α2β1, CK5, hTERT, and p63 but not luminal markers [35], suggesting that these primary cells may contain a small number of prostate SCs. Finally, a single, basally localized Lin-Sca-1+CD133+CD44+CD117+ cell can regenerate prostatic glands when recombined with urogenital sinus mesenchyme and transplanted under the kidney capsule [30], strongly arguing for the presence of SCs in the basal layer. This defined SC population also possesses long-term self-renewal and can produce secretory prostatic product [30]. Moreover, the SC marker CD117 is specifically expressed in the proximal region and enriched upon castration [30].

In human PCAs, however, the majority of tumor cells express luminal markers such as PSA and AR and most prostate tumors have a notable lack of cells expressing basal-cell markers (in fact, loss of basal cell markers has been used in assisting the clinical diagnosis of PCAs) [25,31]. In the mouse prostate, the long-term BrdU label-retaining cells (or LRCs), which have been shown to possess functional SC properties in many other tissues, are localized in not only basal but also the luminal layer in the proximal region [33], suggesting that the luminal cell layer may also harbor SCs. The luminal origin of prostatic SCs is further supported by recent demonstration of a rare population of luminal cells that expresses the regulator of prostate epithelial differentiation, the homeobox gene Nkx3.1, and manifest SC properties upon castration [31]. These luminally localized SCs, termed CARNs for castration-resistant Nkx3.1-expressing cells, can self-renew and regenerate prostatic outgrowth with single-cell transplantation [31]. Intriguingly, Leong et al. [30] demonstrated that the sympathetic-expressing NE cells are enriched in Lin-Sca-1+CD133+CD44+CD117+ mouse prostate SCs. Huang et al. studied the relationship between the SC marker CD44 and NE cells and found that most NE cells expressed CD44 [26]. These latter observations raise the possibility that even NE cells might have some SC properties.

**Prostate Cancer Stem Cells**

It was reported several decades ago that only a minor subset (0.01–1%) of cancer cells acutely isolated from tumors had the ability to regenerate a clonal growth or a tumor, suggesting that these rare cells may represent tumor stem cells [36]. Pioneering work by John Dick and Bonnet [37] on acute myeloid leukemia provided the first direct evidence for CSCs, which by now have been reported in many solid tumors [5–8]. Most of the reported CSCs have been identified using normal stem/progenitor cell markers. The gold standard for functionally defining CSCs is to show their enhanced tumor-initiating capacity and their “self-renewal” by serial transplantation assays. The regenerated tumors by CSCs should possess similar cellular heterogeneity of the parental tumor [19]. One limitation in most of the current CSC studies is the failure to demonstrate multi-potency and asymmetric division in CSCs. A recent study reveals that breast CSCs can undergo asymmetric cell division and asymmetrically segregate Numb [38]. Our own studies also demonstrate that prostate CSCs can undergo asymmetric cell division [39].

Work from Drs. Collins and Maitland suggests that putative human prostate epithelial CSCs bear the CD44+α2β1+ phenotype [29]. In 2005, Collins et al. reported that prostate tumor cells with the same surface phenotype represent potential prostate CSCs, although tumor experiments were not performed in this study [40]. Using several xenograft prostate tumors (Du145, LAPC4, and LAPC9), we have shown that the CD44+ cell population is enriched in prostate CSCs and that PCa cells are organized as a tumorigenic hierarchy [20]. First, putative CSCs that can initiate serially passagable spheres and serially transplantable tumors are marked by CD44 expression and constitute the minority. Importantly, most metastatic activity resides in the CD44+ cell population [20]. Second, the side population (SP) also contains tumorigenic cells and 97% of the SP is CD44+ [41]. Third, in contrast to the SP and CD44+ cells, the α2β1+ and ABCG2+ PCA cells identify fast proliferating tumor progenitors [21]. Fourth, essentially all ABCG2+ and >80% α2β1+ cells are encompassed in the CD44+ population. Therefore, the CD44+α2β1+ cell population is even more highly enriched in tumor-initiating cells, whereas the CD44+α2β1- cells virtually lack tumorigenicity. Fifth, most CD44+ PCA cells are AR- but can give rise to AR+ cells in the spheres and tumors, thus indicating their ability to self-renew and undergo asymmetric division. Recent work by others has confirmed the presence of stem-like PCA cells in cell lines and PCA tissue [42,15].

**Origin of CSCs**

CSCs may derive from their normal counterparts targeted by transforming events. The fact that most currently reported CSCs have been identified using the corresponding normal SC marker(s) supports this possibility. For instance, the CD44+ breast CSCs express many SC markers that are commonly expressed in CD44+ normal SCs [5,20]. Since normal SCs already possess the self-renewal properties, tumorigenic transformation would immediately confer CSCs the self-renewal capacity [18,24]. The longevity of SCs also allows greater opportunities to accumulate genetic changes. In contrast, the more differentiated cells that naturally have lost self-renewal ability may not have a long enough lifespan to accumulate mutations required for full malignant transformation [18,43]. Evidence from both human tumors and animal models also supports the SC origin of tumor development. For example, the Philadelphia chromosome, a hallmark for human chronic myelogenous leukemia, has also been detected in normal HSCs [44], suggesting that HSCs may be the targets of malignant transformation. In animal models, knocking down Pten in the basal-cell layer, which harbors normal prostate SCs, leads to PCAs [24].

Recent studies using mouse models suggest that cancer may also arise from the committed progenitors [19,25,31]. Over-expression of
mixed-lineage leukemia (MLL)-AF9 oncogenic fusion gene induces leukemia from the committed myeloid progenitors [45]. The fact that most tumor cells in early human PCas express luminal makers such as CK8/18, AR, and PSA but rarely basal cell makers suggests that luminal progenitor cells could be the origin of prostate CSCs, as supported by a recent study [31]. Since normal SCs are generally rare and quiescent, they might have fewer opportunities to sustain genetic mutations compared to the more abundant and faster dividing progenitor cells.

Another possible origin of CSCs might be through cell fusion between SCs and other types of cells including SCs, progenitors, differentiated cells, stromal cells, and inflammatory cells. Cell fusion may allow the combination of self-renewal of CSCs with the accumulated mutations in differentiated cells to attain fully neoplastic transformation [46]. One example is that patients who have received allogeneic HSC transplantsations have higher tendency to develop renal cell carcinomas and metastasis. Tumor cells derived from the metastatic lesions in such patients often demonstrate a hybrid between the host and donor cells [47]. Other studies have shown that bone marrow derived cells (BMDCs) can fuse with neoplastic epithelium to promote tumor develop-ment and metastasis, suggesting that fusion between BMDCs and tumor cells could potentially create CSCs [48]. Recent studies on transformation of induced pluripotent SCs (i.e., iPSCs) reveal that reprogramming of differentiated human cells could initiate cancer development [49].

CSCs AND METASTASIS

If CSCs are the only cells that have the capacity to generate a tumor, theoretically they must be responsible for metastasis and therefore CSC-targeted therapies should be able to eliminate metastasis, the main killer of cancer patients. In other words, the CSC hypothesis will be considered incomplete without making connection to metastasis [16,18,50]. In fact, many pieces of evidence support CSC involvement in metastasis. First, eight out of nine breast cancer specimens in which the first solid-tumor CSCs were reported were actually metastases [4,5], implying that metastases may be enriched in CSCs. The CD44+ breast CSC-enriched cells are also highly metastatic [20]. Second, gene expression profile studies on PCa identified an 11-gene signature that included “stemness” genes such as BMI-1 in highly metastatic PCAs and also predicted poor outcomes in PCa patients [51,52]. Also, the poorly differentiated aggressive human tumors are found to possess an ES cell-like gene expression profile [53]. In addition, early-disseminated breast cancer cells detected in the bone marrow are enriched in CD44+/CD24−/lo CSC phenotype [11]. Third, in principle, only CSCs that are endowed with the self-renewal ability could founder a colony in a distant site while differentiated cells that generally lack the self-renwal capacity would not proliferate well to establish a metastatic colony [9,54]. Forth, epithelial-mesenchymal transition (EMT) plays an important role in metastasis and a recent study demonstrates that breast cancer cells induced to undergo EMT also acquires CSCs traits [55]. Fifth, tumor dormancy has long been recognized as a cause of metastasis in the clinic, particularly in breast and prostate tumors and metastasis can occur many years after treatment [18,54]. CSCs may stay quiescent and switch from being dormant to proliferative due to environmental changes, giving rise to recurrence and metastasis. Finally, development of resistance to therapeutics frequently signals the presence of metastatic lesions. Recent studies indicate that CSCs appear to be generally more resistant to chemical and radiation therapies [56]. These discussions raise the possibility that CSCs could be the lethal seeds in Paget’s “seed and soil hypothesis” to spread metastasis [54,57].

On the other hand, as is always the case, the real picture may be more complicated. For instance, the CD44+/CD24− breast CSCs do not seem to possess higher metastatic potential than the CD44+/CD24+ cells when intra-cardiacaally injected into mice although the two populations show differences by in vitro invasion assays [16]. Also, the molecule CD24 is, in fact, highly expressed in breast cancer metastases [58]. CD133 is another widely used CSC marker; however, Hermann et al. reported that the CD133+ cell population alone could not produce metastasis in an orthotopic pancreatic cancer model but the combined CD133+/CXCR4+ subpopulation showed strong metastasis [12]. Also, the CD133− colon cancer cells have been shown to be even more aggressive and metastatic than their CD133+ counterparts although both populations could initiate tumor development [59]. These findings suggest that metastasis and tumor initiation might be processes medi-ated by distinct cancer cell populations [50] and there might exist metastatic CSCs [60,61]. The genetically-based clonal evolution hypothesis proposes that metastasis results from accumulation of genetic mutations and only the rare tumor cell clones that have attained the right and sufficient numbers of mutations will be “naturally selected” and able to disseminate [62,63]. This hypothesis is challenged by demon-strations that metastatic cells pre-exist in early-stage primary tumors [64,65]. As a matter of fact, CSCs might actually be the “naturally selected” clones in the clonal evolution model [66]. In summary, the true interrelationship between CSCs and metastasis awaits more in-depth studies and it is anticipated that novel therapeutics that specifically target CSCs may also root out metastasis-seeding cells.

TUMOR MICROENVIRONMENT AND METASTATIC PROSTATE CSCs

Most current human CSC studies employ xenotransplantation models by injecting human tumor cells into immune deficient mice [43]. The xenograft models are also widely used in metastasis studies [67,68], in which, human cancer cells are implanted in either orthotopic or ectopic sites in mice. It has been well established that the orthotopi-cally injected human tumor cells produces more widespread metastasis than ectopically (e.g., subcutaneous or s.c.) implanted isogenic tumor cells [68]. Dr. Fidler’s group first reported that human PCa cells injected into the nude mouse dorsal prostate (DP) generated more metastasis than s.c. injected cells [69]. However, the underlying molecular mech-anisms for the microenvironment-regulated PCa cell metastasis remain unclear. In our recent work, we also observed that multiple human PCa cells injected into mouse DP indeed exhibited much stronger metastasis to the lung as well as many other organs than the cells injected s.c. By comparing the gene expression profiles of the DP versus the s.c. tumors, we uncovered a very interesting CSC-enriched gene signature unique to the DP tumors. Specifically, the DP-implanted human PCa cells over-express many CSC genes including osteopontin (OPN), CXCR4, CD133, ABCG2, CD44, and CD24. We have already performed a systematic shRNA screening on these molecules and found that some of these molecules clearly have functional roles in PCa cell metastasis in the DP. In addition, when we put the purified OPN+/OPN- cells into the mouse DP, the OPN+ cells showed more aggressive and more metastatic phenotypes whereas the OPN- cells only initiated tumor regeneration without metastasis. Our observations suggest that the “orthotopic” microenvironment may facilitate cancer metastasis by promoting the manifestations of CSCs.

THERAPEUTIC IMPLICATIONS

Theoretically, the CSC-oriented tumor therapy strategies will focus on only a subpopulation of tumor cells rather than the bulk of the tumor. Conventional chemotherapy and radiation therapy seek to reduce the tumor burden, which is usually measured by immediate reduction in tumor size. CSC-targeted therapy, however, will only deplete the CSC subset and this may not necessarily be associated with a significant reduction in tumor volumes [50]. Changes in the CSC content, pro-gression-free survival time, and the time to first evidence of metastasis,
could be used as potential endpoints for evaluation of CSC therapy. In animal studies, labeled tumor cells (e.g., by GFP or luciferase) could be transplanted and used to track metastasis and the outcomes of metastasis and various interventions [70], which makes evaluation of metastasis therapy more practical and accurate. Using such strategies, recent evidence shows that targeting the ‘‘stemness’’ pathway by either neutralizing antibodies or chemical inhibitors may effectively deplete CSCs. For example, CXCR4 is one of the important molecules in both SCs and metastasis. Depletion of CD133+/CXCR4+ subpopulation using the anti-CXCR4 antibodies abrogated metastatic potential in a pancreatic cancer model [12]. In another example, inhibition of the PTEN/Akt/PI3K signaling has been shown to reduce the CSC content and dampen the tumorigenic ability of CD133+/CD44+ prostate CSCs [71]. These examples illustrate the clinical implications of CSC-targeted therapeutic strategies, which, when combined with conventional therapeutic regimens that target the bulk tumor cells, may deliver a long-lasting therapeutic efficacy and prevent tumor recurrence and distant metastasis.

REFERENCES