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Identification, characterization, and biological relevance of prostate cancer stem cells from clinical specimens

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Abstract

Cancer stem cells (CSCs) are a reservoir of tumor cells which exhibit the properties of self-renewal and the ability to re-establish the heterogeneous cell population of a tumor. They appear therapy resistant and may be the underlying cause of recurrent disease. Using prostate as a model, this review presents the CSC hypothesis and discusses the role of the androgen receptor in CSCs, the methods used for isolating CSCs, and the therapeutic challenges CSCs have for cancer therapy.

Introduction

The concept that adult tissues contained dormant embryonic cells which when activated could develop into cancer was initially proposed in 1875 [1,2]. It required another 122 years to demonstrate that a subpopulation of cells expressing the cell surface markers $CD34^{+}CD38^{-}$ was capable of initiating human acute myeloid leukemia in non-obese diabetic mice with severe combined immunodeficiency disease [3]. These experiments suggested that normal primitive cells could undergo leukemic transformation. In mixed lineage leukemia (MLL), most chromosomal translocations occur in the 8.3 kb breakpoint cluster region between exons 8 and 13 [4]. The resulting in-frame fusion proteins have lost the SET (Su(var)3–9, enhancer of zeste, trithorax) domain required to mediate histone H3 lysine 4 (H3K4) methyltransferase activity, thereby efficiently transforming hematopoietic cells into leukemia stem cells [4]. Thus, epigenetic changes [reviewed in [2,5,6]] may provide important links between normal and cancer stem cells. Hemopoietic stem cell studies have provided the paradigms for identifying and isolating CSCs in solid tumors such as prostate [7,8,9], breast [10], colon [11], brain [12], ovarian [13] and pancreatic [10] cancers. Similar to hematopoietic malignancies, key properties exhibited by solid tumor CSCs include self-renewal and the ability to differentiate into the heterogeneous cancer cell lineages comprising a tumor [14].

Androgen receptor and prostate CSCs

The androgen receptor (AR) appears to play a central role in normal prostate function and in the emergence of therapy resistant disease. Regression/regeneration studies in the rat model have shown that even after 30 rounds of androgen deprivation and replacement, androgen treatment could completely restore the normal architecture of the ventral prostate [15]. During this process, basal cells preferentially survived whereas 90% of luminal epithelial cells were lost through apoptosis [16]. The surviving epithelial cells appeared androgen independent but

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responsive to exogenous androgen treatment [17]. Normal Prostate Stem Cells (PSCs) are thought to be AR^- yet able to undergo transit amplification to generate the epithelial cell lineages of the prostate [18,19]. The signaling pathways which promote AR expression in progenitor and secretory luminal epithelium are not known. Similarly, prostate CSCs do not express AR; however they reconstitute AR^+ tumor cell populations *in vivo* [19]. Thus, it is conceivable that CSCs could clonally expand in an androgen-independent environment, resulting in the development of therapy resistance and recurrent disease [20,21,22].

HPET CSC models

Clonal selection of human prostate cancer epithelial cells can be utilized to study CSCs. Thus, primary Human Prostate Epithelial (HPE) cells obtained from patient samples upon radical retropubic prostatectomy were immortalized utilizing the human telomerase reverse transcriptase (hTERT) gene to generate cell lines [19]. The parental cell line termed HPET (where T indicated hTERT) and 4 clonal cell lines did not express AR or AR-regulated secretory proteins such as Prostate Specific Antigen (PSA) and Prostatic Acid Phosphatase (PAP) [19]. Further characterization determined that both parental and clonal lines expressed embryonic stem and early progenitor markers, including Oct-4, Nanog, Sox2, and nestin, as well as CD44, CD133 and c-kit [19]. Utilizing the *in vivo* tissue recombination assay, these lines reconstituted the histopathology of the original tumor they were derived from, differentiating into the three epithelial cell lineages of the prostate. Indeed, the resulting tumors could be scored using the Gleason system, a method exclusively reserved for the evaluation of human clinical Prostate Cancer (PCa) specimens [19]. Interestingly, AR was re-expressed in the appropriate microenvironment, indicating that the stem cell niche was crucial in regulating CSC phenotype and function [19,23].

Methods for Identifying and/or Isolating CSC

Only a small number of cultured cells show proliferative potential in various *in vitro* and *in vivo* assays [24]. Utilizing stem cell surface markers to isolate these side populations of cells, prostate cell lines as well as primary prostate cells have been subjected to fluorescence activated cell sorting (FACS) (Table 1). Commonly used markers have included CD44 [25], Sca-1 [26], CD133/prominin-1 [27], the ATP-binding cassette transporter/breast cancer resistance protein (ABCG2/BCRP) [28] and integrins [29]. $CD44^+$-expressing prostate cancer cells, including NHP6-hTERT/T, NHP6-DNp53/T, DU-145 and PC-3 cells, displayed increased proliferation, tumorigenicity and metastatic potential compared to $CD44^-$ cells [30]. BrdU label-retention, one of the hallmarks of a stem cell, was observed in a small percentage of $CD44^+$ NHP6-hTERT/T cells [30]. Similarly, $CD44^+/CD24^-$ LNCaP cells formed prostaspheres *in vitro* [31]. $CD44^+/CD24^-$ xenografts consistently formed tumors in NOD/SCID with the sc injection of 1000 cells, whereas the depleted cell population did not generate xenografts [31].

Stem cell antigen-1 (Sca-1) has been extensively used to enrich for murine hematopoietic stem cells [32]. In the prostate, $Sca\text{-}1^+$ cells appeared to cluster around the proximal region of the prostatic ducts [33]. These cells demonstrated stem cell characteristics, including multi-lineage differentiation and regeneration of tubular structures containing basal and luminal epithelial cells [33]. $Sca\text{-}1^+$ cells exhibited increased proliferative capacity, with a subpopulation of $Sca\text{-}1^+$ cells also expressing Bcl-2 and integrin α6, markers observed in stem cells from other organs [26]. $Sca\text{-}1^+$ cells appear androgen-independent since castration results in a concomitant enrichment for $Sca\text{-}1^+$ cells [33].

CD133 (or prominin-1) is expressed on hematopoietic stem and progenitor cells [34]. In the prostate, $CD133^+/\alpha_2\beta_1^{hi}$ expression was detected in approximately 1 % of the basal cell

population [27]. $CD133^+$ cells formed prostaspheres in culture and prostatic-like acini in SCID mice [27]. Subpopulations of $CD133^+$ cells also co-expressed cytokeratin 14 or TERT, and generated more numerous and larger branching ducts consisting of luminal and basal cells compared to $CD133^+$ cells [35].

Flow cytometry based on stem cell behavior have also been utilized to isolate side populations. The ATP-binding cassette membrane transporter ABCG2 has been associated with the development of multi-drug resistance [36]. Huss *et al.* identified a subpopulation of $ABCG2^+/AR^-$ cells where ABCG2 appeared to isolate the CSC from its microenvironment through the constitutive efflux of androgens, suggesting that this process protected the CSC from androgen deprivation therapy, hypoxia, or adjuvant chemotherapy [28]. In further elucidating the function of ABCG2, Patrawala *et al.* determined that $ABCG2^-$ cells could generate $ABCG2^+$ cells as well as form more and larger clones, [36]. Their study suggested that $ABCG2^+$ cells were fast-cycling progenitors whereas the $ABCG2^-$ population contained primitive stem-like cancer cells [36]. Similar to that observed for hemopoietic stem cells [37], prostate cells which did not accumulate Hoechst 33342 dye exhibited stem cell properties such as developing into spheroids and branching structures in 3-dimentional Matrigel culture [38].

Therapeutic approaches to target CSCs

The role CSCs in metastatic and therapy resistant disease is an area of intense research (Table 2). A popular hypothesis is that recurrence is caused by the survival of a small subpopulation of CSCs which have retained or gained the property of self-renewal. If not eliminated by androgen-deprivation and/or chemotherapy, they remain dormant in bone marrow or other target organs until eventually triggered to regenerate the heterogenous cell populations of a tumor [7]. It still needs to be ascertained whether all solid tumors are maintained by CSCs and whether specific markers could differentiate between normal stem and CSCs. Current thinking is that cure rates of prostate and other cancers would be greatly improved by selectively eliminating CSCs while sparing normal stem cells.

Most, if not all, known CSC markers are similarly expressed by a diverse population of stem, progenitor, and differentiated cell types in numerous tissues and organs. Thus, they could be utilized in combination with negative selection against differentiation markers to isolate and characterize CSC populations from tissues or cell culture. Ideally, the identification of a prostate-specific CSC marker would facilitate the development of designer drugs to eliminate CSCs from the tumor cell population.

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Table I

Methods for Identifying and Isolating SC or CSC.

- BrdU labeling
- Primary cells immortalized with hTERT
- Fluorescence Activated Cell Sorting (FACS)
  - based on stem cell surface markers
    - CD133/prominin-1/α2β1hi
    - CD44
    - Sca-1
  - based on stem cell behavior
    - ABCG2/BCRP

Table II

Questions on CSC still to be answered.

- What markers identify normal stem and CSCs?
- How do CSCs differ from normal tissue stem cells?
- Are all tumors maintained by CSCs?
- Can CSCs be eliminated while sparing normal stem cells?
- Will eradicating CSCs prevent drug resistance and tumor recurrence?