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Review

Prostate tumor-initiating cells: A new target for telomerase inhibition therapy?

Calin O. Marian *, Jerry W. Shay

University of Texas Southwestern Medical Center, Department of Cell Biology, 5323 Harry Hines Boulevard, Dallas, TX 75390-9039, USA

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ABSTRACT

Conventional therapies for prostate cancer, especially in its androgen-independent form, may result in the survival of small populations of resistant cells with tumor-initiating potential. These "cancer stem cells" are believed to be responsible for cancer relapse, and therapeutic strategies targeting these cells are of great importance. Telomerase is a ribonucleoprotein enzyme responsible for telomere elongation and is activated in the majority of malignancies, including prostate cancer, but is absent in most normal cells. Putative tumor-initiating cells have significant levels of telomerase, indicating that they are an excellent target for telomerase inhibition therapy. In this review, we present some evidence for the hypothesis that conventional therapies (standard chemotherapy and/or radiation therapy) in combination with telomerase inhibitors may result in effective and more durable responses.

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1. Introduction

Prostate cancer is the second most common malignancy found in men and is responsible for the highest rate of morbidity after lung cancer [1]. In most cases, localized prostate disease can be treated efficiently using surgery and androgen ablation therapy. However, the outcome for patients with metastatic disease remains poor [2]. Considering the advanced age for the majority of patients, the chemotherapy regimens have done little to improve median survival, and the lethality of the disease in patients with metastatic castrate-resistant disease remains high [3]. The Gleason classification of prostate tumors remains the best predictor for disease outcome, but more recently new molecular diagnostic techniques such as identification of TMPRSS2:ERG fusion transcripts [4,5], Glutathione-S-transferase P1 (GSTP1) gene promoter hypermethylation [6,7] and DD3 expression [8] can assist in early detection, prognosis, and monitoring of prostate cancer. In addition to diagnostics, current research in the prostate cancer field is focused on the establishment of new targeted therapies for the patients with metastatic disease. It is generally believed that cancer relapse in patients may be due to a small population of cells within the tumor mass which are resistant to conventional therapies.

2. The cancer stem cell hypothesis

The cancer stem cell hypothesis was described more than 150 years ago [9], but the modern revival of this concept arrived with the studies performed in leukemia, where it was shown that a single cell with the CD34+/CD38− phenotype had the capacity of inducing the disease in NOD-SCID mice [10]. More recently, cancer stem cells have been isolated from solid tumors, first in breast cancer, then in neurological malignancies [11,12]. The term "cancer stem cells" is still very controversial. Nevertheless, the general consensus is that these cells must have potent tumor initiation, self-renewal and differentiation capacity [13]. The tumor initiation aspect refers to the capacity of these cells to form tumors in immunocompromised mice using very small numbers of cells. Self-renewal capacity is tested by serial transplantation experiments, where re-isolated cancer stem cells can be transplanted in secondary and tertiary recipients. The differentiation ability of these cells does not refer to multilineage differentiation but rather to the capacity of the resulting tumors to be a phenocopy of the original tumor. An important characteristic of cancer stem cells is their ability to survive various therapies by activating anti-apoptotic pathways, increasing activity of membrane transporters and high DNA repair capacity [14,15]. It is important to point out that the definition of cancer stem cells does not imply the cell type from which these cells originated. This is the reason why for the purpose of this review we are going to use the term tumor-initiating cells. While the origin of tumor-initiating cells is highly debated, this review will focus on the intrinsic properties of these cells in prostate cancer, specifically on telomerase as both a biomarker and therapeutic target for this type of malignancy. The study of these populations of cells is very important, not only for the basic understanding of malignant transformation and pathogenesis, but also as a way to investigate and implement new therapies.

3. Isolation of prostate tumor-initiating cells

To some extent, the amount of knowledge about prostate tumor-initiating cells is still limited compared to that reported in other

* Corresponding author. Tel.: +1 214 648 2684; fax: +1 214 648 8694.
E-mail address: calin.marian@utsouthwestern.edu (C.O. Marian).

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cancers types. This is due, in part, to the small amounts of primary tumors samples available for investigation, and the difficulty in distinguishing between normal and malignant prostate cells based on surface markers alone. Because prostate tumor-initiating cells are present in very low numbers within a primary tumor (usually less than 1%), the use of cancer cell lines provides an efficient alternative to clinical samples. The caveat is that one needs to validate any scientific knowledge derived from prostate cell lines with studies in primary tumor counterparts. Cell lines are usually grown in culture medium supplemented with serum and high Ca^{2+}, conditions that generally permit growth but also encourage differentiation. Some researchers are strong advocates of xenograft propagation of human tumors, but the mouse environment is very different from the human prostate stromal niche, especially when using subcutaneous or renal capsule inoculations, and some amount of differentiation is unavoidable. An alternative is the prostate orthotopic xenograft, but these are difficult to establish, with high rates of mortality. The combinatorial use of primary samples, xenografts and cell lines will likely provide the tools for the most rigorous scientific investigations.

There are several strategies to isolate prostate tumor-initiating cells. The most popular strategy employs the use of surface markers that share the same immunological profile with normal prostate stem cells. One of these surface markers is CD44, an adhesion molecule with multiple functions that appears to be important in tumor dissemination and metastasis [16–18]. One research group reported an in-depth study using $CD44^{high}$ cells isolated from various prostate cell lines [19]. These putative tumor-initiating cells were more proliferative, clonogenic, tumorigenic, and metastatic than the $CD44^{low}$ cells. The CD44 cells also show properties of progenitor cells, such as BrdU label retention and expression of several "stemness" factors, such as Oct-3/4, BMI, β-catenin, and SMO. Moreover, while these cells were AR−, they had the capacity to differentiate into AR+ cells. The authors recognized that the $CD44^{high}$ population of cells was still very heterogeneous and tried to further purify the tumor-initiating component using additional surface markers. In a subsequent study, it was shown that $CD44^{high}/\alpha_2\beta_1$ $integrin^{high}$ populations were more tumorigenic than $CD44^{low}/\alpha_2\beta_1$ $integrin^{low}$ populations when injected in immunocompromised mice and the authors proposed a tumorigenic hierarchy of prostate cancer cells based on the expression of these two markers [20].

Based on the similarities between mouse prostate and breast stem-like cells, another study sought to determine if a population of CD44+/CD24− cells identified tumor-initiating cells in the LNCaP prostate cancer cell line [21]. These cells were present at a very low level in the population (0.04%), and show increased clonogenic and differentiation capacity. Importantly, very low numbers of CD44+/CD24− cells were capable of forming tumors in NOD/SCID mice. These cells were also able to grow as spheroids in attachment-independent conditions and possessed an invasive gene signature.

Another important stem cell marker is prominin-1 (CD133), a pentaspan membrane protein with unclear function [22]. Collins et al. used a $CD44/\alpha_2\beta_1$ $integrin^{high}$/CD133+ phenotype to isolate tumor-initiating cells from primary prostate biopsies [23]. The cells isolated with these markers have a high clonogenic and proliferative capacity, are highly invasive through matrigel and capable of differentiation.

The percent age of CD133+ cells is low after the initial purification from primary tumor samples. Using the prostate cancer cell line DU145 we also find low numbers of CD133+ cells (Table 1). CD133+ cells isolated from primary tumors or DU145 cells can be placed back in culture, using both serum-supplemented media and adherent conditions or chemically defined media and attachment-independent conditions (spheroids). Regardless of the media and culture conditions used, the percentage of cells expressing CD133 remains very low (less than 1%), without any apparent enrichment over time in culture. This indicates that the culture conditions commonly employed in vitro for the propagation of prostate tumor stem cells do not allow the enrichment of this rare population of cells. This is in stark contrast with brain tumor stem cells, where some degree of positive enrichment is possible when isolated CD133+ cells are placed in culture [12]. Importantly, the experiments performed in DU145 cells indicate that the biology of CD133+ cells in primary tumor samples and cancer cell lines might be similar, and if true, the prostate cancer cell lines can be used as a valuable source of research material.

Table 1
The percentage of DU145 CD133+ cells is maintained at low levels in culture after initial FACS isolation in both monolayer and spheroid cultures

Percentage of DU145 CD133+ cells			
Days in culture	14	30	33
Monolayer[a]	0.19	0.19	0.18
Spheroids[b]	0.21	0.19	0.21

[a] The sorted CD 133+ cells were cultured in DMEM/199 media with 10% FBS.
[b] The sorted CD133+ cells were cultured as spheroids in DMEM/F12 media.

A recent study confirmed the significance of CD133 as a marker for both normal and tumor-initiating prostate cells [24]. Within several androgen receptor positive (AR+) human prostate cancer cell lines, CD133+ cells were found at low frequency and were able to self-renew, generate heterogeneous progenies and were capable of an unlimited proliferation capacity. The authors of this study also speculated that CD133 may function differently between normal and cancer prostate cells and that malignant CD133+ cells are originating from a malignantly transformed intermediate cell. Finally, it was confirmed that in addition to CD133+, the $CD44/\alpha_2\beta_1$ $integrin^{high}$/CD133+ population from the DU145 prostate cancer cell line [25] had high capacity of self-renewal and differentiation as well as strong proliferative and tumorigenic potential.

Another popular method to identify tumor-initiating cells is the isolation of the "side population" (SP). The SP cells are isolated based on the ability of cells to retain Hoechst dye, and in the LAPC-9 prostate cancer cell line the SP cells were shown to be more tumorigenic than the corresponding main population [26]. The LAPC-9 SP cells possessed other stem cell properties such as capacity of differentiation in vivo, as well as the ability to sustain subsequent transplantation. Additional information about stem cell surface makers (e.g. CD133, CD44, and $\alpha_2\beta_1$ integrin) was not provided by this study.

A different strategy adopted to identify tumor-initiating cells is based on their capacity to form holoclones — tightly packed clones with specific morphology that contain self-renewing cells and have been hypothesized to contain tumor-initiating cells [27]. The other two types of clones formed by epithelial cells (meroclones and paraclones) do not have the sustained proliferation capacity required for tumor initiation. Holoclones derived from the PC3 prostate cancer cell line were shown to contain stem-like cells that could initiate serially transplantable tumors [28]. In contrast, meroclones and paraclones did not proliferate and failed to initiate tumor development. Perhaps not surprising, the holoclones had high levels of CD44, $\alpha_2\beta_1$ integrin and β-catenin expression, whereas meroclones and paraclones show reduced expression of these stem cell markers. However, CD133 expression was not reported in this study.

In our experiments, we examined by immunofluorescence imaging the signature of DU145 prostate cancer cells grown at clonal density in attachment-independent conditions (spheroids). The attachment-independent conditions exert even more strain on the cells, and because spheroid formation was used extensively to enrich for stem cells, the clonogenic spheroid formation assay probably identifies the population of cells that have the highest tumorigenic potential. We specifically focused on common tumor-initiating cells markers such as CD44 and CD133. CD44 is present at high levels in the majority of DU145 cells, regardless of culture conditions (monolayer or spheroids). The CD133+ cells were also clearly identified in the spheroids,

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