CD133

Subjects: Cell Biology

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Prostate cancer stem cells (PCSCs), possessing self-renewal properties and resistance to anticancer treatment, are possibly the leading cause of distant metastasis and treatment failure in prostate cancer (PC). CD133 is one of the most well-known and valuable cell surface markers of cancer stem cells (CSCs) in many cancers, including PC. CSCs refer to a small subset of cancer cells, theoretically, this can be even a single cancer cell, which can differentiate into a heterogeneous and hierarchy of cancer cells. Sharing a number of characteristics with normal somatic stem cells, CSCs are capable of self-renewing, asymmetric division, generation of heterogeneous lineage, differentiation into various cancer cells which make up the tumor bulk, manifesting more aggressive phenotypes and exhibiting resistance to anticancer treatment. The existence of CSCs was first reported in acute myeloid leukemia in 1997 and later in a broad spectrum of common solid tumors, including PC.

Keywords: prostate cancer stem cells ; CD133

1. The Identification, Isolation and Enrichment of Prostate Cancer Stem Cells (PCSCs)

The availability of reliable PCSC markers is essential to isolate PCSCs. Just like many other cancer stem cells (CSCs), the PCSC is likely to share similar antigen expression with prostate stem cells (PSCs), its unmutated counterpart [1]. Accumulated evidence has shown that PCSCs express certain functional and non-functional (phenotypic) markers. With these markers labelled with antibodies, PCSCs can be identified by flow cytometry (FCM) and isolated by fluorescence-activated cell sorting (FACS) or magnetic cell sorting (MACS).

Identification of PCSCs was initially reported by three independent groups in 2005 [2][3][4]. All these initial PCSC research teams have isolated tumorigenic and self-renewing cells from prostate cancer tissues or cell lines with different PCSC markers. The most influential work was from Collins et al. group [2], who isolated PCSCs from human PC biopsies with $CD44^+/\alpha2\beta1^{high}/CD133^+$ phenotypes. The isolated PCSCs were capable of differentiation to $AR^+/PAP^+/CK18^+$ luminal cells. The fundamental evidence to prove the existence of PCSC is the reconstitution of a cancer bulk by inoculation of a small number of cancer cells in a xenograft model. After CD44+/α2β1high/CD133 cells were implanted subcutaneously in mice, formed acini-like structures were found to resemble prostate differentiation [2]. After that some researchers have utilized a variety of tentative PCSC markers to isolate PCSCs, or PC cells with stemness features, from patient derived tissue or PC cell lines. These markers include: CD133 [2][5], CD44 [2][6][7][8], ABCG-2 [3], CD24 [8], CD166 [9], ALDH1 [10], integrin α2β1 (CD49b) [2][6], Sca-1 [11] and so on .

Most published papers utilized CD133 based combined markers, only a few research teams applied a single marker such as CD133 alone. In addition, the above PCSC markers can be divided into extracellular or intracellular molecules. Extracellular markers technically do not require fixation and permeabilization for antibody binding, so they are more suitable for isolating living cells, which later will be collected for downstream in vitro or in vivo experiments to analyze PCSC properties. It is noteworthy that current PCSC markers do not exclusively express on PC, most of them express in CSCs of other cancer types.

Apart from the isolation from human PC tissue, significant research work also has shown that differentiated PC cell lines can be reprogrammed to PCSCs or stem-like PC cells by exposure to a variety of harsh experiment conditions, including chemotherapy [12][13], radiotherapy [14][15], serum-free medium [12][16], low attachment culture systems [16], sphere cultures [17], and androgen deprivation [18].(**Figure 1**) These experimental conditions can also induce tentative CSCs or stem-like cancer cells in other types of cancers, because the induced and sorted cells are capable of constituting tumors in a xenograft mouse model. The possible mechanism for these reprogrammed CSCs is inducing de-differentiation [15] and/or selecting resistant stem-like cells, while most of the non-CSC cancer cells die in unfavorable environments. Notably, genetic manipulation, such as PTEN deletion, can also lead to the expansion of the PCSC phenotype and tumor initiation [19][20].

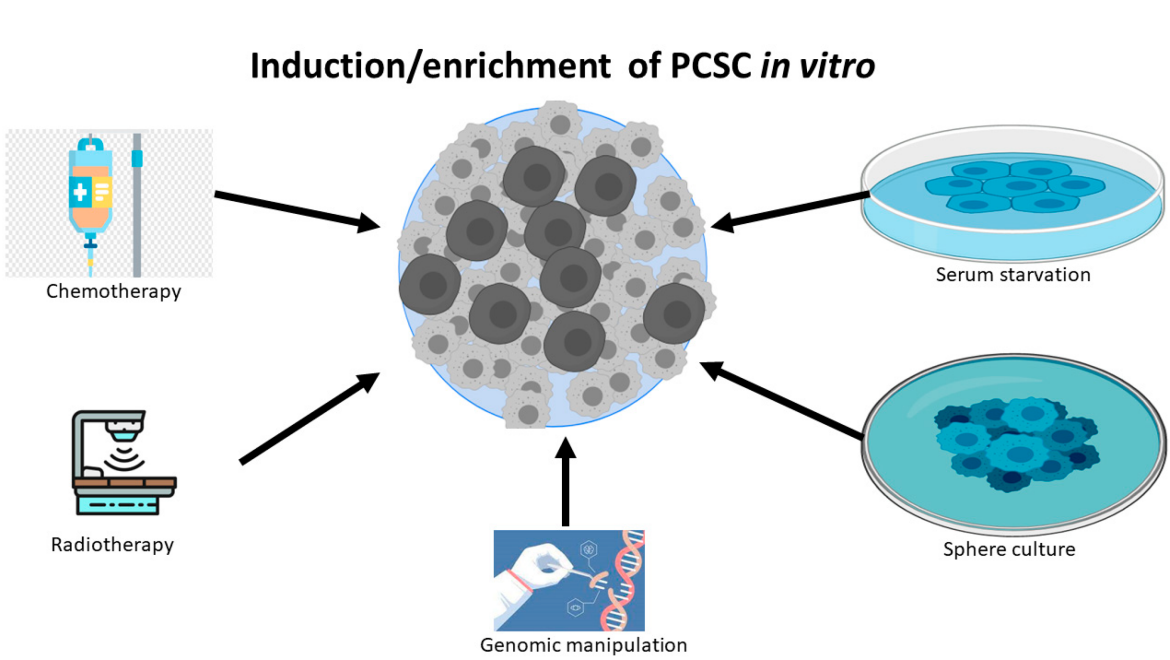

Figure 1. PC cell lines can be de-differentiated to PCSCs or stem-like PC cells by chemotherapy, radiotherapy, serum starvation, sphere culture, and genomic manipulation.

As mentioned earlier, the most convincing evidence of PCSC is the ability to reconstitute a tumor by the limited dilution assay, in which only a tiny amount of the tentative CSCs can demonstrate a significant ability to reconstitute the cancer bulk in severe combined immunodeficiency (SCID) mice. The Collin group showed that a few CD133+ cells, as few as 10, were able to develop a tumor [21]. In glioma cells, 100 CD133+ stem cells were sufficient to reconstitute a brain tumor [22].

2. CD133 Is a Robust Biomarker to Identify PCSC

In 1997, it was reported that CD133 was expressed in a subset of human CD34(+) hematopoietic stem cells (HSC) derived from human bone marrow and cord blood, so CD133 was first regarded as a marker of HSC [23][24]. In 2003, brain tumor stem cells were exclusively isolated using neural extracellular stem cell marker CD133. These CD133+ cells differentiated into tumor cells bearing some resemblance to patient-derived tumors [22]. Since then, in a variety of solid tumors, CD133 has become the most frequently used extracellular marker to detect CSCs [2][22][25][26][27][28][29][30][31][32]. Such universal expression tends to confirm CD133 as an essential maker of CSCs, despite contradicting data regarding the ambiguous role of CD133 expression in certain CSCs [25][33].

In the past two decades, CD133 alone [5][17], or in the combination with other markers [2][34][35][36][37][38], is one of the most well-characterized biomarkers used to identify PCSCs. The CD133 based PCSC marker combination includes: CD133+/CD44 [35], CD133+/CD44+/integrin α2β1 [2][34], CD133+/CXCR4 [36], CD133+/Trop-2+/integrin α2β1 [37], CD133+/CD44+/ABCG2+/CD24- [38]. It is unclear whether combined markers are more valuable than CD133 alone to identify PCSCs, but in colorectal cancer, it has been suggested that the CSC marker pool is more precise than CD133 alone [39][40]. In the first and key publication of PC, the marker combination $CD44^{+}/\alpha2\beta1^{high}/CD133^{+}$ was used to isolate PCSCs from 40 patient biopsies [2].

It is not surprising that CD133 expressed at low levels in prostate cancer tissues and four patient -derived PC cell lines, including PC-3, CWR22Rv1, DU-145 and LNCaP [17]. However, in the combined PCSC markers such as $CD44^{+}/\alpha2\beta1^{high}/CD133^{+}$, not all markers are weakly expressed in all PC tissue or cell lines. For example, PC-3 and DU-145 expressed > 93% CD44+ cells, while CWR22Rv1 and LNCaP cells expressed < 4% CD44+ cells [41]. The unpublished data confirmed the broad and strong expression of CD44 in PC-3 or DU-145 cells by FCM and Western blot. According to the definition of a CSC, cancer stem cells are less than 1% of all cancer cells, and the expression of a CSC marker in histological slides is supposed to be weak or not even expressed in non-CSC cancer cells. However, this general rule may not apply to all cancer cell lines because a cancer cell line was initially established from one or only a few cells, and the broad expression of CD44 or other markers cannot disqualify them as PCSC markers simplistically. As a well-recognized CSC marker in PC and other cancers, CD44+ PC cells displayed significantly enhanced tumorigenicity and metastasis compared with CD44- cells [7], and small numbers of CD44+/CD24- initiated tumors in a xenograft model [8]. The CSC marker combination CD133+/CD44+, with [2][34] or without [35] integrin α2β1, may help PCSC isolation from human PC tissue compared with CD133+ alone.

2. Gene Regulation and Functional Analysis of CD133 and CSC Stemness

The human CD133 (FROM1, prominin-1, AC133) gene locates on chromosome 4p15 and has 37 distinctive exons, resulting in the 12 alternatively spliced isoforms [42] of CD133 mRNA in a tissue-dependent manner. The CD133 gene transcription is regulated by five alternative promoters, three of which locate in the CpG island where DNA methylation occur. Methylation of these 133 promoters in vitro completely inhibits their activity, suggesting that methylation plays a vital role in gene regulation [43][44][45]. On the contrary, an abnormal DNA hypomethylation status of the CpG island in the promoter is positively correlated with elevated CD133 expression in some types of CSCs [43][46].

CD133 mRNA is detected in most adult tissues and in many cell lines, but CD133 protein expression is restricted and mainly expressed on normal stem cells [47][48][49][50], including prostate stem cells [49]. Only a few normal prostate cells express CD133, and most basal and luminal cells are negative, indicating CD133 expression is strictly defined during the development of epithelial hierarchy in prostate tissue. Due to heavy hypermethylation of the CpG island, DNA methylation inhibited CD133 expression in a number of prostate epithelial cell lines [51]. On the contrary, histone deacetylase inhibitors restored CD133 expression in prostate cell lines. However, in malignant prostate primary tissues, regulation of CD133 is under the dynamic control of chromatin condensation but not dependent on DNA methylation [51].

Three transcriptional factors are identified to regulate the transcription of CD133, which include the ALL1-fused gene from chromosome 4 protein (AF4), Sex determining region Y-box17(Sox17), and E26 transformation-specific (ETS). AF4 was identified to be a regulator of CD133 in Caco-2 cells (a colorectal carcinoma cell line) by shRNA screening [52]. Sox17 was identified as a critical regulator of self-renewal of fetal and adult HSC [53]. Forced expression of Sox17 induced expression of CD133 in CD133—cells, and reduction of SOX17 by siRNA induced a reduction in the level of CD133 in CD133 + cells [54]. The RAS/ERK/ETS conduct pathway was regulated at ETS binding site within the CD133 promoter [55], while suppression of the ERK pathway downregulated the expression of the CD133 protein.

CD133 is a 97kDa transmembrane glycoprotein with five transmembrane domains, and due to heavy glycosylation, its apparent molecular weight is about 130 kDa. CD133 null mice usually grow normally except for a progressive degeneration of photoreceptors [56], which is consistent with the critical function of CD133 in photoreceptor cells [57]. CD133 selectively expresses in some types of stem cells during tissue development, and its expression is regulated in a development-dependent manner. The presentation of CD133 is rapidly lost upon differentiation.

In PC, as discussed earlier, subpopulations of CD133+ cells isolated from primary prostate cancer tissues or established cell lines exhibited stem cell-like characteristics. Ectopic over-expression of CD133 rendered LnCap cells significant CSC properties such as higher expression of Oct-4 and Nanog [58], promoted bone metastasis, and increased epithelial-to-mesenchymal transition (EMT) properties, which include increased vimentin and decreased E-cadherin.

3. Multiple Functional Roles of CD133 and CSC Stemness

The upstream or pertaining molecular events inducing transcriptional, translational or epigenetic regulation of CD133 are still largely unknown. It was reported that certain conduct pathways, extracellular oxygen levels, or mitochondria metabolism were mutually interconnected with CD133 gene expression and stemness of CSCs, as summarized below and illustrated in **Figure 2**:

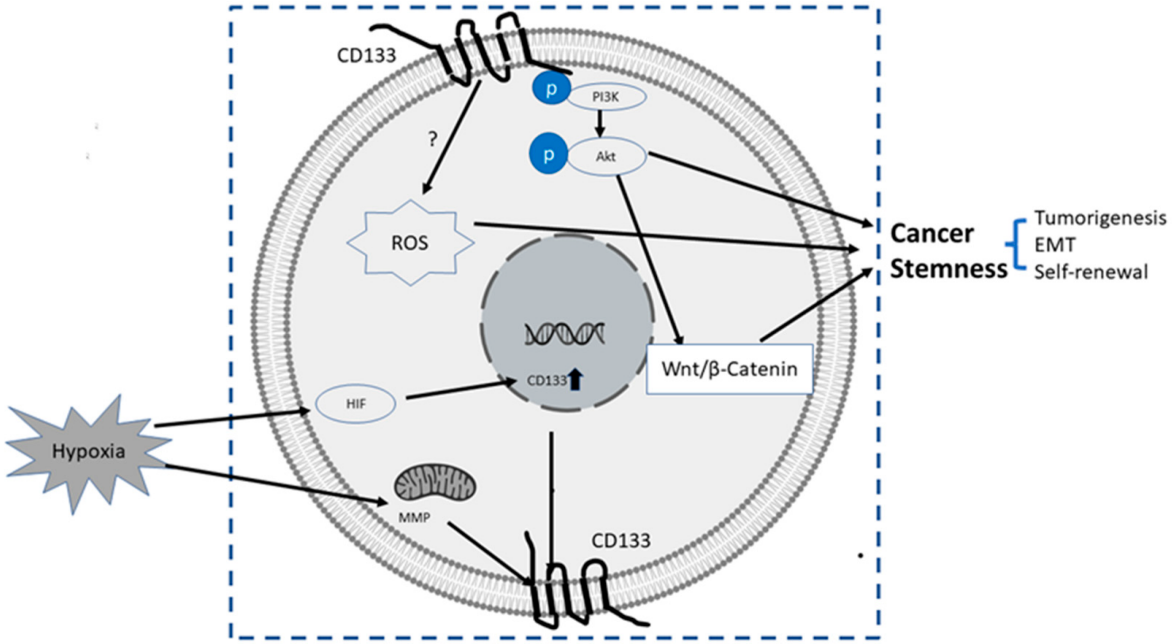

Figure 2. Summary of some known upstream and downstream molecular events related to CD133. The upstream events result in the upregulation of CD 133, and downstream events after CD133 induction are directly or indirectly related to increased stemness properties of PC cells, mainly via CD133/PI3K/AKT/Wnt/β-Catenin signaling axis.

(1)PI3K/Akt pathway: In glioma CSCs, phosphorylation of tyrosine-828 in the CD133 C-terminal domain mediated interaction between CD133 and the phosphoinositide PI3K 85 kDa subunit (p85), which further activated the PI3K/Akt conduct pathway. On the contrary, CD133 knockdown significantly inhibited the activation of the PI3K/Akt pathway, accompanied by reduced properties of self-renewal and tumor-forming in glioma CSCs. Taken together, CD133 activated the PI3K/Akt pathway and regulated stemness in glioma CSCs [59][60].

(2)Wnt Signaling: In several patient-derived glioblastoma cell lines, compared with CD133 ^{low} cells, CD133 ^{high} cells showed higher levels of endogenous Wnt activity and self-renewal property, while inhibition of CD 133 by a novel anti CD133 antibody suppressed the function of CD133 as well as the activity of Wnt pathway. Interestingly, a pan-AKT inhibitor MK-2006 diminished overexpression of CD133 induced Wnt activation, indicating a CD133/AKT/Wnt signaling axis may play a role in regulating the stemness of glioblastoma [61].In PC, non-adherent prostaspheres cultures enriched stemness characteristics of prostate cell likes. Inhibition of Wnt signaling reduced the prostasphere size and the self-renewal properties of prostate cancer stem-like cells, while adding Wnt3α increased self-renewal and expression level of CD133 [62]. Therefore, Wnt-β-catenin signals promote the self-renewal of PCSC or progenitor cells [63], which may be independent of AR activity [62].

(3)CD133-transferrin-iron: The low oxygen niche is the microenvironment where the stem cell resides. In the tumor microenvironment, hypoxia upregulated the expression of hypoxia -inducible factor-1(HIF-1) and then indirectly induced CD133 expression [64][65] and other stem cell markers of PCSC [66]. In addition, hypoxia also disturbs mitochondrial membrane potential (MMP) to regulate CD133 post-transcriptionally [67].

(4)Reactive oxygen species (ROS): ROS are by-products of normal cellular metabolism but excess ROS leads to cell death. In CSCs, the Redox scavenger system is activated to keep ROS at a low level [68]. In PCSCs, CD133+ cells are more vulnerable to ROS-induced cell damage [69].

4. CD133 and Its Clinical Significance in PCSC

It is estimated that metastasis is responsible for about 90% of cancer deaths. [70]. An autopsy study showed that bone metastases were found in 90% of 1589 patients who died from metastatic prostate cancer, strongly suggesting this preponderance of bone metastasis in castrate resistance PC [71]. It is likely that bone metastasis is the ultimate result of PCSC disseminated from prostate cancer [72].

A cancer metastasis initiates from an invasion of cancer cells through the basement membrane, followed by multiple steps including angiogenesis, intravasation, extravasation, and colonization. In addition, cancer metastasis requires an epithelial status switch, including both an EMT [73] to leave the primary location and a Mesenchymal-to-Epithelial Transition (MET) to seed into the secondary site [74]. PCSCs are more apt to an epithelial status switch and metastasis because of the

capacity of cell plasticity. CD133 may be involved in cancer metastasis, especially bone metastasis. In a group of 131 cancer patients (26% prostate cancers), 111 metastatic patients had a significantly increased expression of CD133 mRNA ($p < 0.05$), especially patients with bone metastasis ($p < 0.001$) [75].

It is reasonable to postulate that CD133 might be a progress factor in some solid cancers, and several reports correlated the expression of CD133 with poor prognosis in a variety of solid cancers [76][77][78][79][80]. In a group of metastatic castration-resistant prostate cancer (mCRPC) patients, circulating tumor cells (CTCs) with CD133+ have an AR-independent, increased proliferative potential [81]. Very recently, a clinical trial was conducted to evaluate the clinical significance of CD133 in CTCs of newly diagnosed mCRPC patients. It was found that using CD133 in circulating tumor cells can independently predict progression-free survival (PFS) in mCRPC patients who received androgen deprivation therapy (ADT) therapy ($p < 0.05$) [82].

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