Cancer Stem Cells in Prostate Cancer Chemoresistance

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Abstract: There is currently no cure for metastatic castration-resistant prostate cancer (CRPC). Chemoresistance and metastatic disease remain the main causes of treatment failure and mortality in CaP patients. Although several advances have been made in the control of CRPC with some newly developed drugs, there is still an urgent need to investigate the mechanisms and pathways of prostate cancer (CaP) metastasis and chemoresistance, identify useful therapeutic targets, develop novel treatment approaches, improve current therapeutic modalities and increase patients’ survival. Cancer stem cells (CSCs), a minority population of cancer cells characterised by self-renewal and tumor initiation, have gained immense attention as they not only play a crucial role in cancer recurrence but also contribute substantially to chemoresistance. As such, a number of mechanisms in chemoresistance have been identified to be associated with CSCs. Therefore, a thorough and integral understanding of these mechanisms can identify novel biomarkers and develop innovative therapeutic strategies for CaP treatment. Our recent data have demonstrated CSCs are associated with CaP chemoresistance. In this review, we discuss the roles of putative CSC markers in CaP chemoresistance and elucidate several CSC-associated signaling pathways such as PI3K/Akt/mTOR, Wnt/β-catenin and Notch pathways in the regulation of CaP chemoresistance. Moreover, we will summarize emerging and innovative approaches for the treatment of CRPC and address the challenging CRPC that is driven by CSCs. Understanding the link between CSCs and metastatic CRPC will facilitate the development of novel therapeutic approaches to overcome chemoresistance and improve the clinical outcomes of CaP patients.

Keywords: Cancer stem cell, castration-resistant prostate cancer, chemoresistance, EMT, prostate cancer, treatment.

INTRODUCTION

Prostate cancer (CaP) remains a major medical burden in males in Western countries and accounts for an estimated 33,720 deaths in the USA in 2011 [1]. Localized CaP patients have an excellent long-term survival and high cure rates with standard approaches, such as surgical resection and radiotherapy. However, patients with advanced and metastatic disease are often associated with a poor prognosis, and up to 30% of treated CaP patients will suffer from a relapse and develop a prostate-specific antigen (PSA) recurrence within 18 months after surgical resection. Although some of the patients in the early stage initially respond to hormone therapy due to androgen dependence, almost all CaP patients invariably progress to recurrent castration-resistant CaP (CRPC) and eventually die from secondary disease (metastasis).

Chemotherapy remains the main treatment option in the setting of CRPC, only to provide very modest survival benefits. Docetaxel (DTX) is an anti-neoplastic agent and the most common choice for metastatic CaP treatment at the moment. The actions of DTX on cells involve the disruption of the cell cycle and the induction of apoptosis [2]. Two DTX-based clinical trials have unravelled the potential benefits of chemotherapy to prolong the survival time and improve the quality of life in CaP patients for the first time, at the cost of significant toxicity in elderly patient population [3, 4]. Mitoxantrone (MTX), a DNA intercalator, is less toxic but delivers only palliative benefits [5, 6]. Although newer chemotherapeutics such as satraplatin and cabazitaxel have demonstrated activity, survival benefits are still modest with median overall survival just more than one year [7, 8]. Inevitably, resistance to such therapies will develop and the disease then becomes difficult to control. Thus, it is important to investigate the mechanisms and pathways of CaP chemoresistance as well as identify useful therapeutic targets to improve current therapeutic modalities.

In some cases, intrinsic chemoresistance may result in the survival of a population of tumor cells that subsequently leads to relapse after treatment. This is particularly true for tumors that are composed of a heterogeneous population of cells such as CaP [9]. In the heterogeneous tumors, the tumor initiating potential and drug sensitivity of different tumor cells within the same tumor bulk have yielded two models of tumor initiation: the stochastic model and the hierarchical model [10]. In the hierarchical initiation, different subpopulations of cells within a tumor have various levels or
absence of tumor initiating potential. Those fractions of cells that have enhanced tumor initiating potential are referred to as cancer stem cells (CSCs).

While CSCs are not necessarily derived from normal stem cells, defining characteristics of CSCs includes the ability of self-renewal as well as differentiation into other tumor cell subtypes. They are considered to be responsible for tumor relapse and metastasis [11, 12]. Our recent data have demonstrated CSCs are associated with CaP chemosensitivity [13]. As such, a number of mechanisms in chemoresistance have been identified to be associated with CSCs, and several signaling pathways are involved in the self-renewal behavior of CSCs, including PI3K/Akt/mTOR, Wnt, Notch, Hedgehog and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathways, which mediate the resistance against chemotherapy [14] and insufficient elimination of CSCs is largely challenging the efficacy of current chemotheraphy, leading to tumor recurrence. Therefore, a thorough and integral understanding of these mechanisms can identify novel biomarkers (targets) and develop innovative therapeutic strategies to cure CaP disease.

In this review, we discuss the hypothesis of CSCs and relationship between CSCs and chemoresistance, investigate the roles of putative CSC markers (surface markers and transduction factors) in CaP chemoresistance and elucidate several CSC-associated signaling pathways such as PI3K/Akt/mTOR, Wnt/β-catenin and Notch pathways in the regulation of CaP chemoresistance. Moreover, we will address the challenging CRPC that is driven by CSCs and summarize emerging and innovative approaches for the treatment of CRPC. Understanding the link between CSCs and metastatic CRPC will facilitate the development of novel therapeutic approaches to overcome chemoresistance and improve the clinical outcomes of CaP patients.

CANCER STEM CELLS IN CAP CHEMORESISTANCE

Cancer Stem Cell Hypothesis

Despite the debate on CSCs’ existence, cancer is becoming more recognized as a heterogeneous disease with hierarchies of subpopulations that demonstrate a variety of phenotypes. Two models have been proposed to explain tumor heterogeneity: the stochastic and hierarchical models (Fig. 1). While the stochastic model proposed that all cells within a tumor are biologically homogenous and therefore have equal capacity to regenerate the tumor, the hierarchical model (also referred to as the CSC model) suggested that only a small subset of tumor cells possesses the capacity to regenerate the tumor [10, 15]. According to the hierarchical model, it should be possible to separate tumor cells into subpopulations that are tumor initiating and non-tumor initiating. The tumor-initiating cells, also referred to as CSCs, are defined by their capacity for self-renewal, potential to differentiate into any cells in a tumor, and proliferative capacity to drive expansion of the tumor [16]. They also embody certain refractory natures such as extremely aggressive metastatic ability, and increased resistance to conventional chemotherapy and radiotherapy.

However, although the CSC hypothesis states that there is a stem-like cell that maintains the tumor, it does not suggest that the CSCs are derived from normal stem cells. And, CSCs are not necessarily the origin of the initial primary tumor [17]. Interestingly, recent studies indicate that cancer cells can de-differentiate into CSCs under certain circumstances (tumor microenvironments), termed “plasticity” [18, 19] (Fig. 1).

Based on the refractory properties of CSCs, conventional chemotherapy may not be able to eliminate the CSCs that result in cancer recurrence after primary tumor treatment. Plasticity also makes it challenging to identify efficient biomarkers for development of novel therapeutic targets. Furthermore, because of a great overlap in features of regulators and markers between normal stem cells and CSCs (which will be discussed later in this review), it is intractable to develop unique drugs targeting only CSCs. Therefore, a lot of work remains to be done at both the levels of understanding the biological role of CSCs and their clinical relevance.

Putative Prostate CSC Surface Markers Involved in CaP Chemoresistance

Although some studies suggested that the cellular origins of CaP are terminally differentiated luminal cells [22], evidence still supports the existence of CSCs in CaP [23]. We have recently reviewed the literatures on CSCs origin, the identification and characterization in CaP as well as their clinical implications and therapeutic challenges [24]. There are also several reviews published by other authors elaborating the current status of research on CSCs in CaP, including characteristics of CSCs [25], methodologies of assessing CSCs [26] and the relationship of stem cells with therapy resistance [27]. In this section, we summarize putative CSC markers from human CaP cell lines, xenografts and primary tissues and only discuss prostate CSC markers associated with CaP chemoresistance.

Prostate CSCs express a number of same markers as prostate stem cells, such as CD44, CD133, integrins, breast cancer resistance protein (BCRP) and Sca-1, all of which have been utilized to identify prostate CSCs or prostate stem cells. The most frequently identified potential CSCs markers in CaP are summarized in Table 1. The cell surface CSC markers combined with cell sorting technology have been used to identify and isolate CSC subpopulations in CaP. Collin et al. reported the identification and characterization of a population (CD44+CD24−/−/CD133+/−) from human primary prostate tumors, which possesses a significant capacity for self-renewal and is also able to regenerate the phenotypically mixed populations of non-clonogenic cells such as androgen receptor (AR) and prostatic acid phosphatase (PAP) positive CaP cells [28]. They suggested that this population of CSCs can be used as a therapeutic target for CaP treatment [29, 30]. Later on, Patrawala et al. demonstrated that the CD44+CD24−/−/CD133+/− high cell population from the LAPC-9 CaP xenografts reveal a hierarchy in tumorigenic potential [31]. It was also reported that one population of CD133+/−/CD44+ high cells isolated from established aggressive prostate PC-3-MM2 cell line have CSC characteristics and are potentially useful to study stem...
cell behavior and their responses to CaP treatment [32]. Furthermore, Dubrovska et al. confirmed that the CD133+/CD44+ population of cells enriched in CaP progenitors from PC-3 and DU145 cell lines have tumor-initiating potential and that these progenitors can be expanded under non-adherent, serum-free, sphere-forming conditions [33].

Using flow cytometry, Hurt et al. isolated CD44+/CD24- LNCaP cells, which are able to form colonies in soft agar and form tumors in non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice when as few as 100 cells were injected [34], concluding that the CD44+/CD24- LNCaP CaP cells offer an attractive model system to explore the biology important to the maintenance and differentiation of prostate CSCs as well as to develop the therapeutics. Using CaP spheres model, Bisson and Prowse showed that prostate spheres from six metastatic CaP cell lines exhibit heterogeneous expression of proliferation, differentiation and stem cell-associated markers CD44, ATP-binding cassette sub-family G member 2 (ABCG2) and CD133. Qin et al. recently demonstrated that prostate specific antigen (PSA)+/CD44+ CaP cells offer an attractive model system to explore the biology important to the maintenance and differentiation of prostate CSCs as well as to develop the therapeutics.

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Fig. (1). Evolving models have been advocated to explain the heterogeneity of tumors and development of cancer stem cells (CSC). In the stochastic model of tumor growth, all tumor cells are biologically homogenous and equipotent, which stochastically self-renew or differentiate, leading to tumor heterogeneity (A). In the hierarchical model (CSC model) of tumor growth, only a subset of tumor cells have the ability of self-renewal and these cells (CSC) give rise to progenitor cells with limited proliferative potential that differentiate terminally (B). In the ‘de-differentiation’ model (also referred to as the fluid CSC model), both progenitor cells and differentiated cells are able to re-acquire the self-renewal potential under certain circumstances (tumor microenvironment), for example, at the presence of IL-6 [20] or ZEB-1 [21] (C). CSC: cancer stem cells.
CD44 variant 6 (CD44v6) by a small interfering RNA (siRNA) in different CaP cell lines could increase chemosensitivity to DTX, paclitaxel (PTX), doxorubicin (DOX) and MTX as well as reduce tumorsphere forming ability, respectively, suggesting that CD44v6 is a potential CSC marker and closely associated with chemoresistance of CaP (unpublished data). These results indicate both CD44 and CD44v6 are potential therapeutic targets and hold promise for improving CaP chemosensitivity.

CD133 (human prominin-1) is a 5 transmembrane domain glycoprotein. The biological function of CD133 is not thoroughly understood yet. It was originally identified as a cell surface antigen present on CD34+ hematopoietic [44] and neural [45] stem cells. Besides its role in normal stem cells, CD133 has been well documented to be a putative CSC surface marker in a number of tumors including CaP [46]. Richardson et al. found that in primary CaP tissues CD133+ cells exhibit characteristics of stem cells including tumorsphere formation and the development of prostatic-like acini in SCID mice [47]. Within a series of AR+ human CaP cell lines including LAPC-4, LNCaP and CWR22Rv1 cells, CD133+ cells present at a low frequency, self-renew, express AR, generate phenotypically heterogeneous progeny negative for CD133, and possess an unlimited proliferative capacity [48]. However, other investigators found that CD133 was only expressed in DU145 cells but not in DuCaP, LAPC-4, CWR22Rv1, LNCaP and PC-3 CaP cells, and that CD133+ cells from the DU145 cell line were not more clonogenic than CD133- cells [49], considering CD133 selection does not enrich stem-like cells in CaP cell lines. Moreover, almost all CD133-related experiments performed to date can only detect the expression of AC133 and AC141 epitopes rather than the total CD133 protein. Recent evidence suggests that CD133 mRNA and protein seem to be constant upon differentiation in colon cancer whereas only AC133 epitope is lost after the differentiation [50]. Reasons for the discrepancies may be the application of different antibodies to CD133, different passages of tissue culture or experimental methodology. Yang et al. recently demonstrated that the expression of testicular nuclear receptor 4 (TR4) is significantly higher in CaP CD133+ stem/progenitor cells compared with CD133- non-stem/progenitor cells and knockdown of TR4 levels in the established CaP stem/progenitor cells and the CD133+ population of the C4-2B CaP cell line with lentiviral TR4 shRNA led to increased drug sensitivity to the two commonly used chemotherapeutic drugs, DTX and etoposide, suggesting that targeting TR4 may alter chemosensitivity of CaP stem/progenitor cells and overcome the chemoresistance problem in CaP therapeutics [51]. Wang et al. also demonstrated that CD133+/CD44+ cell population were only present in the DU145 cell line (0.1%) and had increased to 9.8% enriched by DTX chemotherapy [52]. These data support that CD133 as a CSC marker is closely associated with CaP chemosensitivity and can be used as a therapeutic target to overcome chemoresistance.

ALDH is an enzyme involved in intracellular retinoic acid production [53]. In prostate CSCs studies, the high expression of ALDH1A1, a member of ALDH family, was

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<th>Table 1</th>
<th>Putative prostate CSC markers identified in CaP cell lines, animal xenografts and human primary CaP tissues.</th>
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<tr>
<td><strong>CSC Marker</strong></td>
<td><strong>Cell Line/Model/Tissue</strong></td>
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<tr>
<td>CD44+v2β1h/LDH1h/CD133+</td>
<td>Primary tumors</td>
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<tr>
<td>CD44+</td>
<td>LAPC-4 and LAPC-9 models</td>
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<td>CD44+v2β1h/LDH1h/CD133+</td>
<td>LAPC-9 model</td>
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<td>CD133h/CD44v</td>
<td>PC-3-MM2 cell line</td>
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<td>CD133+/CD44+</td>
<td>PC-3 and DU145 cell lines</td>
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<td>CD44+/CD24+</td>
<td>LNCaP and DU145 cell lines</td>
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<td>CD44+/ABC2+/CD133+</td>
<td>PC-3, VCAP, LNCaP, 22RV1, and DU145, C4-2B cell lines</td>
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<td>PSAh</td>
<td>LNCaP, LAPC-4 and LAPC-9 cell lines; primary CaP tumors</td>
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<tr>
<td>CD133+</td>
<td>Primary tumors</td>
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<tr>
<td>CD133+</td>
<td>LAPC-4, LNCaP and CWR22RV1 cell lines</td>
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<tr>
<td>ALDH+/TR4+</td>
<td>PC-3M-Pro4 and C4-2B cell lines; primary tumors</td>
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<tr>
<td>ALDH1A1+</td>
<td>PC-3 and LNCaP cell lines</td>
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<tr>
<td>TRA-1-60+/CD151+/CD166+</td>
<td>Primary tumors</td>
</tr>
<tr>
<td>E-cadherin+</td>
<td>DU145 and PC-3 cell lines</td>
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<tr>
<td>CD117+/ABC2+</td>
<td>22RV1 cell line</td>
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<tr>
<td>OCT4+</td>
<td>Primary tumor cells</td>
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<tr>
<td>ABCG2+</td>
<td>Tumorsphere cells derived from LNCaP, 22RV1, DU145 and PC-3 CaP cell lines</td>
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with this knowledge, OCT4, SOX2 and NANOG have been revealed to be potential prostate CSC-related markers [24]. It was reported that ALDH1A1+ CaP cells from CaP cell lines (PC-3M-Pro4 and C4-2B) and primary CaP tissues not only display strongly elevated clonogenicity and migratory behavior in vitro, but also show enhanced tumorigenicity and metastatic ability in vivo [54]. By ALDEFLUOR assay and fluorescence-activated cell sorting (FACS), Li et al. isolated ALDH1A1+ cells from PC-3 and LNcap CaP cell lines and the isolated ALDH1A1+ CaP cells demonstrated high clonogenic and tumorigenic capacities in vitro, and serially reinitiated transplantable tumors that resembled histopathologic characteristics and heterogeneity of the parental CaP cells in vivo [24]. Therefore, ALDH and ALDH1A1 are promising prostate CSC-related markers for future therapy. ALDH plays an important role in chemoresistance in gastric cancer [55] and breast cancer [56]. However, the role of ALDH in CaP chemoresistance needs to be elucidated in the future studies.

Transduction Factors as CSC Markers Involved in CaP Chemoresistance

As CSCs do not necessarily originate from the transformation of a normal stem cell, they may arise from mutations attained by restricted progenitors and differentiated cells that have subsequently acquired self-renewal capacity [64]. Recent studies have suggested that non-tumorigenic cells can acquire de novo stem-like properties, and that cancer cells can reversibly transition stochastically between tumorigenic and non-tumorigenic states [18]. Insights into the mechanisms of cellular plasticity have arisen following studies that have achieved reprogramming of somatic cells towards a pluripotent stem-like state [65]. This reprogramming method, a process that produces induced pluripotent stem cells (iPSCs), utilizes the transcription factors of OCT4, SOX2, KLF4 and c-MYC to reset the epigenetic state of differentiated cells to a pluripotent state [66]. In one study, prostate stroma were transduced with OCT4, SOX2, KLF4, and c-MYC to reset the epigenetic state of differentiated cells to a pluripotent state [66]. In another study, prostate stroma were transduced with OCT4, SOX2, KLF4, and c-MYC genes to generate induced pluripotent stem cells of CaP, and successful reprogramming of prostate tissue into Pro-iPSCs was demonstrated by embryo stem cell morphology, marker expression, and functional pluripotency in generating germ-layer lineages [67]. The functions of c-MYC and KLF4 can be substituted by NANOG and LIN28 [68]. In embryonic stem cells, pluripotency is maintained by the core transcription factors OCT4, SOX2 and NANOG, which co-occupy the promoters of various target genes [69, 70]. Collectively, accumulating evidence reveals an essential role for OCT4, SOX2 and NANOG in the maintenance and acquisition of stem-like features. In line with this knowledge, OCT4, SOX2 and NANOG have been implicated in tumorigenesis, suggesting that these proteins may be critical for the generation of CSCs [71-73].

OCT4, also known as POU5F1, is a well-established transcription factor critical for maintaining pluripotency in embryonic stem cells. It remains unclear what roles if any OCT4 serves in somatic cells or during carcinogenesis. A novel function of OCT4 in tumorigenesis was proposed when its ectopic expression induced dysplastic growth of epithelial tissue [74]. Linn et al. observed that OCT4 was up-regulated in two drug-resistant CaP cell lines which also demonstrated significant tumorigenicity in vivo, suggesting that OCT4 re-expression in cancer cells may play an important role in tumor initiation and provide one possible mechanism by which cancer cells acquire/maintain a drug-resistant phenotype [75]. Over the years, accumulating evidence suggests that OCT4 is involved in promoting tumorigenicity, malignancy and chemoresistance in human cancers [76]. OCT4 transcripts are consistently detected in human tumors and OCT4 is also expressed in CSCs, including those of CaP [23], further implicating its participation in tumorigenesis and the development of an aggressive phenotype. Yang et al. found that knockdown of TR4 levels in the established prostate CSC and the CD133+ population of the C4-2 CaP cell line led to increased drug sensitivity to DTX and etoposide, along with down-regulation of OCT4 expression [51]. On the contrary, one of OCT4 isoforms, OCT4B has been reported as a strong marker of good prognosis for CaP patients [77]. Thus, the elusive role of OCT4 requires further investigations into it.

ABCG2 is a member of the ATP binding cassette (ABC) transporters, which can pump a wide range of endogenous and exogenous compounds out of cells. Accumulating evidence shows that ABCG2 is one of the most important multidrug-resistance transporters and its substrates include many commonly used chemodrugs in CaP chemotherapy including MTX [78]. In addition, recent studies suggest that ABCG2 may be involved in CSCs [79]. In CaP studies, Zhang et al. discovered that tumorsphere cells derived from LNCap, 22Rv1, DU145 and PC-3 CaP cell lines displayed enhanced self-renewal, chemoresistance and tumor-initiating capacity when compared with the adherent cells, along with high-level expression of “stemness” gene ABCG2 [63]. Ma et al. found that ABCG2 was significantly induced by either stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF), and these cytokines showed a synergistic effect on the induction of this protein expression in the CaP cell lines. The up-regulation of ABCG2 by these cytokines may partly explain why bone marrow metastatic CaP cells are chemoresistant [80]. In another study, a subpopulation of CD117+/ABCG2+ 22Rv1 CaP cells overexpressed stem cells markers such as NANOG, OCT4, SOX2, Nestin, and CD133, which are highly prolific and are also resistant to treatment with a variety of chemotherapeutics such as cisplatin, PTX, adriamycin, and methotrexate, indicating that ABCG2 is correlated with prostate CSCs and chemoresistance. Moreover, expression of ABCG2 is regulated by many CSC-associated signaling pathways, such as Hedgehog, Notch and PI3K/Akt/mTOR signaling pathways [81-83], which will be discussed in detail in the next section. Collectively, these data suggest that ABCG2 may serve as a promising biomarker for the identification of CSCs in tumors including CaP. New strategies targeting ABCG2 may effectively eliminate CSCs and overcome chemotherapeutic limitations in future CaP treatment.

CSC-ASSOCIATED SIGNALING PATHWAYS IN CAP CHEMoresistance

The mounting evidence that CSCs contribute to chemoresistance across a broad range of malignancies has...
stimulated great interests in investigating the underlying mechanisms. In this section, we summarize several CSC-associated signaling pathways that might contribute to CaP chemoresistance and constitute therapeutic vulnerabilities that can be exploited for the development of novel therapeutic strategies.

**PI3K/Akt/mTOR Signaling Pathway**

Among several independent signaling pathways, the PI3K/Akt/mTOR signaling pathway has a diverse array of functions, including the regulation of cellular survival, differentiation and stem cell-like properties, growth, proliferation, metabolism, migration, and angiogenesis, and it is a key pathway that has been linked to both tumorigenesis and resistance to therapy in CaP and other solid tumors [84]. The possible regulation of CaP chemoresistance by the PI3K/Akt/mTOR pathway is shown in Fig. 2. Activation of the PI3K/Akt/mTOR pathway has been strongly implicated in CaP progression [85, 86]. Alterations of components of the PI3K/Akt/mTOR pathway, including mutation, altered expression, and copy number alterations, have been reported in 42% of primary prostate tumors and 100% of metastatic tumors [86]. Several lines of evidence indicate that this signaling system plays a key role in CSC biology [87]. Preclinical studies suggest that the PI3K/Akt/mTOR pathway is important in maintaining a prostate CSC population [33] and is involved in epithelial-mesenchymal transistion (EMT) in CaP cells [88, 89]. Moreover, under hypoxia, the PI3K/Akt/mTOR pathway significantly increases the expression of hypoxia-inducible factor-1 alpha (HIF-1α), which further affects the phosphorylation of Akt. The positive interaction between Akt and HIF-1α results in an over-expression of vascular endothelial growth factor (VEGF), which is crucial for homeostasis and chemoresistance of CSCs [90]. Akt could also induce the expression of ABCG2, which is important in drug efflux response to chemotherapeutic agents [91]. Ma et al. reported that activation of PI3K/Akt/mTOR pathway is associated with the high level of CSC marker CD133 in hepatocellular carcinoma [92]. In one study, Sharma et al. found that the interaction between chemokine receptor-9 (CCR9) and its natural ligand CCL25 upregulates antiapoptotic proteins, including PI3K and Akt as well as downregulates activation of Caspase-3 in CaP cells. Furthermore, the cytotoxic effect of etoposide was significantly inhibited in the presence of CCL25 via PI3K/Akt/mTOR pathway [93]. Kumar et al. recently found that Rottlerin (an active molecule isolated from Mallotus philippinensis, a medicinal plant used in Ayurvedic Medicine for anti-allergic and anti-helminthic treatments) induces autophagy and apoptosis in CaP stem cells via PI3K/Akt/mTOR signaling pathway in human CaP samples [94]. These findings demonstrate the close link between the PI3K/Akt/mTOR pathway and prostate CSCs.

PTEN is a potent tumor suppressor gene and functions as a negative regulator of the PI3K/Akt/mTOR pathway, which is the most frequently mutated gene in human cancers [95]. PTEN deletion leads to increased activation of Akt and mTOR, and CSC emergence and expansion [96]. Lee et al. found that PTEN-negative PC-3 CaP cells were observed to have increased resistance to both DOX and PTX when compared with PTEN-positive DU145 cells [97]. In another study, Priulla et al. found that potentiation by siRNA of taxol cytotoxicity was significantly greater in mutated-PTEN cells (PC-3 and LNCaP) than in prostate cells expressing wild-type PTEN (DU145) [98], suggesting that PTEN is important in the regulation of CaP chemoresistance. It was reported that PTEN induces chemosensitivity by staurosporine, DOX and vincristine in PTEN-mutated LNCaP CaP cells by suppression of Bcl-2 expression [99]. Furthermore, modulation of PI3K activity with the use of constitutively active and dominant-negative inhibitors was found to affect the ability to CaP cells responding to chemotoxic treatments. Additionally, inhibition of PI3K with an inhibitor (LY294002) was able to potentiate the antineoplastic activity of both DOX and PTX in CaP cells [97]. Morikawa et al. reported that the combination of rapamycin (mTOR inhibitor) with DTX resulted in a greater inhibition of proliferation than treatment with rapamycin or DTX alone in PC-3 cells in vitro and PC-3 animal model in vivo by downregulation of survivin [100]. The results from our previous study support that PI3K/Akt pathway is associated with CaP chemoresistance [13]. Our recent studies also demonstrated that the PI3K/Akt/mTOR pathway is associated with CD44v6 expression and CaP chemosensitivity, and inhibition of this pathway leads to reduced tumorsphere forming ability and increased chemosensitivity in CaP cell lines (unpublished data). These findings suggest that activated PI3K/Akt/mTOR pathway greatly contributes to the maintenance of CSCs and the development of chemoresistance, making itself an effective target for eliminating CSCs and overcoming chemoresistance in CaP. Small molecular inhibitors which specifically target PI3K/Akt/mTOR proteins are very promising in improving CaP chemosensitivity. Targeting PI3K/Akt/mTOR signaling pathway in the treatment of CRPC has been reviewed [84]. It was recently reported that a dual PI3K/mTOR inhibitor NVP-BEZ235 (targeting PI3K and mTOR) can sensitize DTX in CRPC cells in vitro and in animal model in vivo [101].

**Wnt/β-Catenin Signaling Pathway**

In addition to the roles of the PI3K/Akt/mTOR signaling pathway, there are a number of other signaling pathways that have been documented to contribute to CSC biology, including chemoresistance. One such pathway is the Wnt/β-catenin signaling pathway (see Fig. 3), which is required for normal stem cell and CSC self-renewal in a number of cell types including prostate [58]. Bisson et al. have found that Wnt/β-catenin pathway inhibition causes a significant decrease in tumorsphere size and relative sphere formation independently of apoptosis in CaP [58]. Yu et al. demonstrated that Wnt/β-catenin activation promotes prostate tumor progression in a mouse model [102]. Treatment with the ligand Wnt3a, which is an activator of canonical Wnt/β-catenin signaling, caused a significant increase in tumorsphere size and self-renewal, suggesting that Wnt/β-catenin treatment promotes the self-renewal of CaP cells with stem cell characteristics [58]. Hsieh et al. reported that MicroRNA (miR)-320 suppresses the stem cell-like characteristics of CaP cells (CD44, CD133 and OCT4) using CaP cell lines, animal model and human CaP tissues
by downregulating the Wnt/β-catenin signaling pathway, indicating that targeting the miR-320/β-catenin interaction or perturbing miR-320 expression may prove to be a new therapeutic strategy in the treatment of CaP patients [103].

In other cancer types, activation of the Wnt/β-catenin pathway enhanced renewal of OVC6 hepatic CSCs, which also exhibited enhanced chemoresistance to cisplatin that could be reversed by knockdown of β-catenin [104]. Similar studies demonstrate that Wnt/β-catenin pathway can also confer chemoresistance to 5-FU and DOX in neuroblastoma and hepatocellular carcinoma [105, 106]. Our study also shows the putative CSC marker CD44v6 regulates CaP stem cell properties and chemosensitivity via Wnt/β-catenin signaling pathway (unpublished data). However, the mechanisms via which the Wnt/β-catenin pathway regulates chemoresistance and tumor initiation are still unclear and likely to vary among cell lines and cancer types. One potential mechanism is through the upregulation of ABCG2 [107]. Another noteworthy point is that Wnt/β-catenin signaling is not a single unit but cooperating with several other signaling pathways, for example, PI3K/Akt/mTOR [108], Notch [109], Hedgehog [109], TGF-β [110] and also with many nuclear receptors, such as AR, which plays a critical role in CaP development, progression and chemoresistance. Lu et al. observed that silibinin, a novel small molecule Wnt/β-catenin signaling inhibitor, displayed anti-cancer activity in CaP by suppressing Wnt co-receptor LRP6 expression at the transcription level [111], suggesting targeting this pathway is promising in future CaP treatment.
Notch Signaling Pathway

On a different note, Notch signaling pathway has been identified to play an important role in a number of processes during tumor progression and metastasis including tumor initiation, angiogenesis, EMT-driven metastasis and self-renewal of CSCs [112, 113]. Recent evidence shows that Notch may also contribute to certain mechanisms of chemoresistance in CaP CSCs. Overexpression of Notch-1 was found in both human CRPC and high-grade CaP samples compared with those observed in low-grade CaP and benign prostatic hyperplasia (BPH) tissues [114]. Wang et al. found that down-regulation of Notch-1 by siRNA is associated with Akt and FoxM1 in inducing cell growth inhibition and apoptosis in CaP cells in vitro and in CaP animal models in vivo [115]. It has also been reported that silencing of Notch-1 by siRNA promoted DTX-induced cell growth inhibition, apoptosis and cell cycle arrest in PC-3 CaP cells. In addition, these effects were associated with decreased Akt expression in PC-3 cells [116]. Notch signaling pathway combined with other signaling pathways such as Wnt, Hedgehog and NF-κB plays a very important role in CaP chemoprevention [117].

Hedgehog Signaling Pathway

Hedgehog signaling pathway is involved in the development of many organs, which facilitates the differentiation of human embryotic stem cells [118]. In adults, the activity of Hedgehog signaling has to be exactly defined in time and space and there is unlimited capacity for self-renewal which means that Hedgehog pathway plays a crucial role in tumor initiation and metastasis [119]. In fact, the disturbances in Hedgehog pathway are commonly observed in a large number of tumors [119-121]. It was reported that increased levels of Hedgehog signaling proteins were found in androgen-independent CaP (AIPC) cells in culture and circulating CaP cells isolated from patients with AIPC, suggesting Hedgehog signaling contributes to the androgen-independent growth of CaP cells [122]. Using PTX resistant DU145-TXR and PC3-TXR cell lines and clinical CaP tissues, Singh et al. found that chemoresistance in CaP
is regulated by miR200c and 34a as well as Hedgehog pathway, and that a Hedgehog inhibitor CYA can reverse PTX chemoresistance and eliminate CSC side populations in androgen independent, metastatic CaP cells [123]. Using pCX-shh-IG mice that overexpress Hedgehog protein persistently in adult prostates, Chang et al. found that Hedgehog overexpression leads to the formation of prostate CSCs with metastatic property irrespective of AR expression in a mouse model, suggesting that Hedgehog signaling plays important roles in transforming normal prostate basal/stem cells into prostate CSCs and in the progression of prostate CSCs into metastatic tumor cells [124]. Zhang et al. demonstrated that genistein (a natural compound) targets prostate CSC-like cells (CD44+ cells) using 22RV1 and DU145 cell lines in vitro and animal models in vivo via targeting Hedgehog–Gli1 self-renewal pathway, showing its potential for CaP prevention and treatment in the clinical setting [125].

Similarly to the Notch signaling pathway, Hedgehog pathway depends on the cooperation with other signaling pathways, such as Notch [126], PI3K/Akt/mTOR [127], and Wnt/β-catenin [128] pathways for the regulation of CaP metastasis, progression and chemosensitivity. While the specific mechanisms remain elusive, inhibition of the Hedgehog pathway has been demonstrated to sensitize CSCs in a variety of tumor types including pancreatic cancer [129], ovarian cancer [130] and CaP [123]. Domingo-Domenech et al. recently identified a subpopulation of cells with potent tumor-initiating capacity from DU-145-DR and 22RV1-DR cells that survive DTX exposure, lack differentiation markers and HLA1 antigens, while overexpressing the Notch and Hedgehog signaling pathways. Notably, targeting Notch and Hedgehog signaling pathways depleted this population through inhibition of the survival molecules including Akt and Bcl-2, suggesting a therapeutic strategy for abrogating DTX-resistance in CRPC [36]. This study established the link between chemoresistance and Hedgehog signaling pathway, and is an excellent basis for further detailed research to clarify the involvement of this pathway in CaP chemoresistance and CSCs.

**NF-κB Signaling Pathway**

Regulators of inflammation, such as NF-κB pathway, can also contribute to chemoresistance and CSCs. As a key mediator of the inflammatory response, NF-κB has a diverse set of biological functions that can be either tumorigenic or tumor-suppressive [131]. Using human CaP tissues, Rajasekhar et al. recently identified a minor subset of stem-like human prostate tumor-initiating cells (TICs) with triple-marker-positive TRA-1-60, CD151 and CD166 that possess stem cell characteristics and multipotency as demonstrated by in vitro sphere-formation and in vivo tumor-initiation, respectively. These TICs recapitulate the original parent tumor heterogeneity in serial xeno-transplantations indicating a tumor cell hierarchy in human CaP development and exhibit increased NF-κB activity [60]. Activation of NF-κB in CD44+ ovarian CSCs was reported to be correlated with chemoresistance to PTX and carboplatin, while inhibition of NF-κB in vivo has been shown to enhance the growth of squamous cell carcinoma and promote the development of hepatocellular carcinoma [19]. Using human CaP samples and CaP cell lines, Codony-Servat et al. found that NF-κB and IL-6 are related with DTX resistance in CRPC [132]. Kwon et al. found that blocking the NF-κB pathway could improve the chemosensitivity to trichostatin A (a promising anticancer drug) in 267B1/K-ras human CaP cells [133]. O’Neill et al. demonstrated that inhibition of NF-κB with the BAY 11-7082 inhibitor reversed the PC-3 cell resistance to DTX, suggesting NF-κB plays an immensely important role in determining DTX-resistance [134]. It was also reported that inhibition of NF-κB pathway by acetyl-boswellic acids (natural compounds) could promote apoptosis in androgen-independent PC-3 CaP cells in vitro and PC-3 xenografts in vivo [135]. Moreover, using a dominant negative super-repressor IκB mutant adenoviral construct, Flynn et al. inhibited NF-κB pathway and observed the enhanced apoptotic potentials of PTX and rhTNF-α in chemoresistant DU145 CaP cells, which may provide therapeutic implications for CRPC [136]. Moreover, Lee et al. found that obovatol (an active compound isolated from Magnolia obovata) enhances DTX-induced CaP cell death through inactivation of NF-κB, further validating its close link to chemoresistance and CSCs in CaP [137].

There are a number of other signaling pathways that are involved in the maintenance of CSCs and the development of chemoresistance in CaP which is beyond the scope of our current topic. More attention should be paid to the investigation of these signaling pathways to illuminate the underlying mechanisms and eventually, to cure CRPC.

**ASSOCIATION OF CRPC, CSC AND CHEMoresistance**

As early as 1940s, it was demonstrated that CaP, like prostate gland itself, is initially dependent on androgens. Consequently, androgen deprivation therapy (ADT) has been used to treat locally advanced CaP [138]. Though some of the patients are initially responsive to ADT, the majority of patients inevitably progress to CRPC, which develops metastasis rapidly and is incurable by current treatment strategies [138]. This predicament can be attributed to current therapies targeting differentiated cells without the AR. CSCs. ADT may promote disease progression by increasing the castration-resistant stem cell pool and/or activating quiescent stem cells to repopulate. Many possible mechanisms have been advocated to explain the etiology of CRPC, most of which centre on AR amplification [139], AR overexpression [140], AR mutations [141] and AR-independent and survival pathways upregulation [142].

Collins et al. found that CD44+αβ1highCD133+ cells are AR [28]. In addition, prostate CSCs isolated on the expression of CD44 were also found AR [57]. A cell surface marker CD166 was found to be highly expressed in human CRPC samples, and CD166high cells in the PTEN-null model demonstrated enhanced tumorsphere formation abilities [143], suggesting a potentially close association between CRPC and CSCs, making the CSC an ideal future therapeutic target for treating CRPC. Notably, a subpopulation of PSAα1+CaP cells can initiate robust tumor development and resist androgen ablation in castrated hosts, and it harbors highly tumorigenic CRPC cells that can be prospectively enriched using ALDH1+CD44+α2β1+ phenotype [35]. Interestingly,
unlike normal human prostate stem cells, in a recent study it was implied that CD133+ cells from CaP (putative prostate CSC) are AR+, suggesting that AR+ prostate CSCs are derived from a malignantly transformed intermediate cell that acquires stem-like activity [48]. The discrepancy may be explained by the fact that the study was performed in vitro, which did not thoroughly mimic the heterogeneity or tumor microenvironment in primary CaP. All the results indicate the close link between CRPC, CSCs and chemoresistance. Further investigation of roles and mechanisms of CSCs in CRPC holds promise to cure metastatic, chemoresistant and refractory CaP disease.

TARGETING CSCS IN CRPC TO OVERCOME CHEMORESISTANCE

The CSC hypothesis reveals that in order to cure CaP, elimination of the ‘root’ of CaP, prostate CSCs, is of paramount importance [15]. Given the quiescent, long-lived and therapeutic-resistant natures of CSCs, targeting them is not an easy task. More and more studies now conclude that when designing new therapies, CSCs must be taken into consideration, particular in tumors which are prone to relapse, for example, CaP [79, 144, 145]. Hence, novel therapeutic strategies to target CSCs to cure CRPC are urgently needed.

Current chemodrugs used in treating CRPC preferentially hinder cell proliferation or induce cell apoptosis, without significantly affecting the prostate CSCs, which are resistant to chemotherapy reagents due to expression of drug-resistant proteins and regulation by drug resistant signaling pathways as discussed above. Several recent studies found that some prostate CSC subpopulations express low level of AR and are resistant to castration [35, 36, 143], providing us with a hint that the expansion of this particular subpopulation may promote the development of CRPC. Based on these findings, these castration-resistant prostate CSCs manifest themselves as potential targets for novel drug development.

Shedding new light on the eradication of CSCs is the induction of differentiation from CSCs to their mature compartments, which should push the CSCs into the normal cell cycle and would make the cells more susceptible to conventional therapies such as ADT and chemotherapy. The bottleneck is how and when to induce the differentiation. Possible solutions include silencing the overexpressed gene in CSCs or switch on the genes that are not involved in CSCs. Thus, a better understanding of the interrelationship amongst genes, differentiation and maintenance of CSCs is very crucial.

Moreover, targeting the biomarkers that identify prostate CSC and signaling pathways that sustain the prostate CSCs and develop CaP chemoresistance could also lead to the development of new therapies using alone or in combination. A combination of GDC-0449 (a novel Hedgehog pathway inhibitor), Compound E (a novel Notch pathway inhibitor) and DTX has been used to target Hedgehog and Notch pathways to treat CaP and now in the Phase II clinical trial [146]. Zhou et al. recently designed an N-(2-hydroxypropyl) methacrylamide (HPMA)-based delivery system for delivery of the hedgehog-signaling inhibitor cyclopamine that is a selective therapeutic against CSCs. The newly-designed HPMA copolymer-cyclopamine conjugate binds to cells via the smoothened membrane receptor. The authors reported that the HPMA copolymer-cyclopamine conjugate shows a selective inhibitory effect on prostate CSCs in comparison with that on bulk cancer cells [147]. Our research team has recently isolated an RNA aptamer that interacts specifically with a number of live human cancer cells derived from breast, colorectal and gastric cancers that express putative CSC marker epithelial cell adhesion molecule (EpCAM) [148]. EpCAM, also known as CD326, is a transmembrane glycoprotein initially identified as a predominant antigen on human colon carcinoma [149]. Normal epithelia express EpCAM at a variable yet generally lower level than carcinoma cells. Recent data suggest a more pleiotropic role for EpCAM that is not only limited to the promotion or prevention of cell–cell adhesion but also involved in cell signaling, migration, proliferation and differentiation [9]. Importantly, this novel EpCAM RNA aptamer is efficiently internalized after binding to cell surface EpCAM. Furthermore, we also found that this EpCAM RNA aptamer can specifically bind human CaP tissues and lymph node metastases as well as PC-3 and DU145 CaP cell lines, PC-3M-luc xenografts followed by active internalization (unpublished data). It can be used as a novel drug delivery system carrying conventional drugs to kill CSCs with high affinity and specificity.

CURRENT CHALLENGES FOR CSCS IN CAP RESEARCH

The evolving concept of CSC has attracted much attention. Much more work is needed to better understand where CSCs originate and how CSCs develop and sustain a tumor. As in other tumors, many divergent prostate CSC populations with different biomarkers have been reported, and it is essential to elucidate the interrelationship between phenotypically and functionally different prostate CSCs.

So far most prostate CSCs publications have been based on studies in cell lines or mouse xenograft models [150]. Little data have shown whether human primary CaP tissues also possess stem-like cancer cells, and whether distinct phenotypes of CSCs exist in individual CaP patients. Actually it is becoming clearer and clearer that the hierarchy of cells within mouse tumor models can be very different from those in human cancers [151]. This conundrum may be the result of inadequate models, inappropriate use of the models and irrational design of the experiment. And even in mouse tumor models, the frequency of CSC differs greatly according to recent work: in two studies on melanomas, Schatten found the frequency of melanoma tumor initiating cells was a NOD/SCID mouse model [152], while Quitana demonstrated that an average of 27% of unselected melanoma cells from four different patients formed tumors in single-cell transplants to NOD/SCID interleukin-2 receptor gamma chain null (Il2rg(-/-)) mice which are highly immunocompromised [153]. One possible explanation may involve the influence of the tumor environment where tumor grows. The discrepancy in frequency of the two studies may attribute to altered protocols including prolonging the observation period, injecting the tumor cells to an extract rich in laminin (an extracellular-matrix components) and using more highly immunocompromised strains of mice as hosts.
Since CaP is no longer considered as a disease of a single cell type but rather viewed as a complicated system composed of epithelial cells that exhibit immortalized growth within the framework of a microenvironment that supports the growth as well as the macroenvironment of the host with a unique genotype and immune system [154]. Thus it is difficult to reconstitute and fully mimic human CaP development in an immunodeficient host by using a single CaP cell line.

In the matter of therapeutic strategies based on targeting specific pathways that sustain the stemness, the danger of side-effects must be taken into account. A recent study raised concerns over targeting Notch pathway, in which silencing of Notch1 caused widespread vascular tumors and organism lethality secondary to massive haemorrhage especially in liver [155]. The safety of targeting drugs remains to be assessed.

Furthermore, it is worth mentioning that during carcinogenesis, characteristics of CSCs tend to be very changeable presenting different mobile targets, which only adds to the difficulties for treating CaP. Thus, combination of cytotoxic agents and targeted therapies will provide an opportunity to eradicate the cancer cells.

CONCLUSIONS

Despite the underlying mechanisms of CaP chemoresistance are being investigated somehow, a substantial number of patients progress to the metastatic disease and eventually die. Thus, CRPC will continue to be a major challenge to the medical community and a heavy burden to the older men. Currently, there is no cure available for CRPC. CSCs may shed new light on development of new therapeutic strategies. Although there is ample evidence for the existence of CSCs, many unresolved issues are apparent. Here, we summarize putative CSC markers and transduction factors for identification of CSCs and outline diverse CSC-associated signaling pathways in CaP chemoresistance. Furthermore, we delineate the association of CRPC, CSCs and chemoresistance, describe the current efforts we have made as well as challenges we are faced to cure CRPC. But suffice it to say that last decade has witness an exciting improvement including approval of novel drugs for the treatment of advanced CaP that hold much clinical promise and better understanding of the genetic and phenotypic properties of the CSC and its association with CaP chemoresistance. Nevertheless, further and thorough investigations on both research models and clinical samples are urgently needed, to facilitate the development of novel therapeutical strategies to tackle CRPC and improve the prognosis and survival of those patients who succumb to CRPC.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interested are disclosed.

ACKNOWLEDGEMENTS

This work was supported in part by a NH&MRC Career Development Fellowship (YL), Surgical & Urological Research Fund at Urology Sydney, Cancer Research Trust Fund at Cancer Care Centre, St George Hospital, and Prostate & Breast Cancer Foundation Ltd, Australia.

ABBREVIATIONS

ABC = ATP-binding cassette
ABCG2 = ATP-binding cassette sub-family G member 2
ADT = Androgen deprivation therapy
AIPC = Androgen-independent prostate cancer
ALDH = Aldehyde dehydrogenase
AR = Androgen receptor
BCRP = Breast cancer resistance protein
BPH = Benign prostatic hyperplasia
CaP = Prostate cancer
CCR9 = Chemokine receptor 9
CD44v = CD44 variant isoforms
CK = Cytokeratin
CRPC = Castration-resistant prostate cancer
CSCs = Cancer stem cells
DOX = Doxorubicin
DTX = Docetaxel
ECM = Extracellular matrix
EMT = Epithelial-mesenchymal transition
EpCAM = Epithelial cell adhesion molecule
FACS = Fluorescence-activated cell sorting
G-CSF = Granulocyte colony-stimulating factor
G-CSF = Granulocyte colony-stimulating factor
HIF-1α = Hypoxia-inducible factor-1alpha
HLAI = HLA class I
HPMA = N-(2-hydroxypropyl) methacrylamide
Il2rg(-/-) = Interlukin-2 receptor gamma chain null
iPSCs = Induced pluripotent stem cells
miR = MicroRNA
MTX = Mitoxantrone
NOD/SCID = Non-obese diabetic/severe combined immunodeficiency
PAP = Prostatic acid phosphatase
PSA = Prostate-specific antigen
PTX = Paclitaxel
SCF = Stem cell factor
shRNA = Short hairpin RNA
siRNA = Small interfering RNA
TICs = Tumor-initiating cells
TR4 = Testicular nuclear receptor 4
VEGF = Vascular endothelial growth factor

REFERENCES


Cancer Stem Cells in Prostate Cancer Chemoresistance


