Send Orders for Reprints to reprints@benthamscience.net

Current Cancer Drug Targets, **2014, 14, 225-240**

Cancer Stem Cells in Prostate Cancer Chemoresistance

Jie Ni[1,2], Paul Cozzi[2,3,*], Jingli Hao[1,2], Wei Duan[4], Peter Graham[1,2], John Kearsley[1,2] and Yong Li[1,2,*]

[1]*Cancer Care Centre and Prostate Cancer Institute, St. George Hospital, Kogarah, NSW 2217, Australia;* [2]*St. George and Sutherland Clinical School, the University of New South Wales (UNSW), Kensington, NSW 2052, Australia;* [3]*Department of Surgery, St. George Hospital, Kogarah, NSW 2217, Australia;* [4]*School of Medicine, Deakin University, Waurn Ponds, VIC 3217, Australia*

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 intense 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 chemosensitivity. 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

*Address correspondence to these authors at the Cancer Care Centre, St George Hospital, Gray St, Kogarah, Sydney, NSW 2217, Australia. Tel: +61-2-9113 2514; Fax: +61-2-9113 4044; E-mail: y.li@unsw.edu.au or Paul Cozzi, Department of Surgery, pcozzi@unsw.edu.au

1568-0096/14 $58.00+.00

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 chemotherapy, 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 CHEMO-RESISTANCE

Cancer Stem Cell Hypothesis

Despite the debate on CSCs' existence, cancer is becoming more recognized as a heterogenenous 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 assaying 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 ($CD44^{+}\alpha2\beta1^{high}CD133^{+}$) 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 $CD44^{+}\alpha2\beta1^{+/high}$ cell population from the LAPC-9 CaP tumor xenografts reveal a hierarchy in tumorigenic potential [31]. It was also reported that one population of $CD133^{high}/CD44^{high}$ cells isolated from established aggressive prostate PC-3-MM2 cell line have CSC characteristics and are potentially useful to study stem

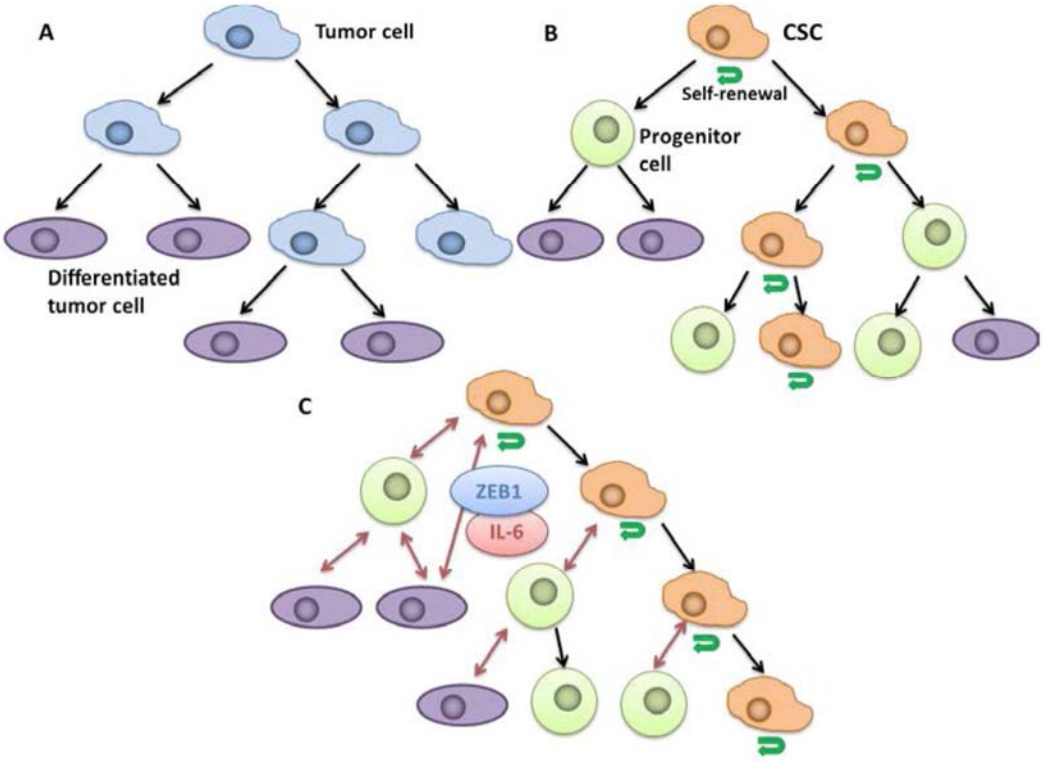

Fig. (1). Evolving models have been advocated to explain the heterogeneity of tumors and development of cancer stem cells (CSC). In the stochastic model of tumor growth, all tumor cells are biologically homogenous and equipotent, which stochastically self-renew or differentiate, leading to tumor heterogeneity (**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.

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 makers CD44, ATP-binding cassette sub-family G member 2 (ABCG2) and CD133. Qin *et al.* recently demonstrated that prostate specific antigen $(PSA)^{-/lo}$ CaP cells can initiate robust tumor development and resist androgen ablation in castrated hosts, and they harbor highly tumorigenic CPRC cells that can be prospectively enriched using aldehyde dehydrogenase $(ALDH)^+/CD44^+/\alpha2\beta1^+$ phenotype in CaP cell lines [35]. Moreover, CRPC cell lines contained infrequent DTX-resistant cytokeratin $(CK)^-$/HLA class I $(HLAI)^-$ CSCs, and $CK^-/HLAI^-$ cells were more frequent in patients treated with this antimitotic agent [36].

CD44 is a multifunctional protein involved in cell adhesion, migration, drug resistance, signal transmission, cell migration, and apoptosis [37, 38]. It is a primary receptor for hyaluronan, a major component of the extracellular matrix (ECM) and critical for cell signaling and cell-ECM interactions. In CaP, CD44 is an important marker used in conjunction with other CSC markers to enrich prostate CSCs. Our previous studies indicated that knocking downing CD44 expression by short hairpin RNA (shRNA) in PC-3M-luc CaP cells could increase chemosensitivity to DTX *in vitro* and *in vivo* [13]. The CD44 gene contains 20 exons alternatively spiced to give many CD44 variant isoforms (CD44v). Among numerous CD44v, CD44 variant 6 (CD44v6) was initially shown to be able to promote the metastatic potential of a rat pancreatic adenocarcinoma cell line in animal models [39]. Since then, it has been found that CD44v6 plays an important role in promoting tumor development and progression in various human cancers [40, 41]. Recently, CD44v6 has obtained substantive focus as being a potential CSC marker. Hebbard *et al.* reported that the expression of CD44v6 in mammary breast cancer reflects stem cell characteristics [42]. In a human bladder cancer study, CD44v6 was identified as a marker with CSC properties [43]. Our recent data showed that knocking down

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

Table 1. Putative prostate CSC markers identified in CaP cell lines, animal xenografts and human primary CaP tissues.

CSC Marker	Cell Line/Model/Tissue	Reference
$CD44^+\alpha2\beta1^{high}CD133^+$	Primary tumors	[28]
$CD44^+$	LAPC-4 and LAPC-9 models	[57]
$CD44^+/\alpha2\beta1^{+/high}$	LAPC-9 model	[31]
$CD133^{high}/CD44^{high}$	PC-3-MM2 cell line	[32]
$CD133^+/CD44^+$	PC-3 and DU145 cell lines	[33]
$CD44^+/CD24^-$	LNCaP and DU145 cell lines	[34]
$CD44^+ABCG2^+CD133^+$	PC-3, VCAP, LNCaP, 22RV1, and DU145, C4-2B cell lines	[58]
$PSA^{-/lo}$	LNCaP, LAPC-4 and LAPC-9 cell lines; primary CaP tumors	[35]
$CD133^+$	Primary tumors	[47]
$CD133^+$	LAPC-4, LNCaP and CWR22RV1 cell lines	[48]
$ALDH^{high}$	PC-3M-Pro4 and C4-2B cell lines; primary tumors	[54]
$ALDH1A1^+$	PC-3 and LNCaP cell lines	[59]
$TRA\text{-}1\text{-}60^+/CD151^+/CD166^+$	Primary tumors	[60]
$E\text{-}cadherin^+$	DU145 and PC-3 cell lines	[61]
$CD117^+/ABCG2^+$	22RV1 cell line	[62]
$OCT4^+$	Primary tumor cells	[23]
$ABCG2^+$	Tumorsphere cells derived from LNCaP, 22RV1, DU145 and PC-3 CaP cell lines	[63]

found to be positively correlated with Gleason score and pathologic stage, and inversely associated with overall survival and cancer-specific survival of the CaP patients, indicating ALDH1A1 could be a potential prostate CSC-related marker [24]. It was reported that $ALDH^{high}$ 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 heterogenecity 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 chemoresistannce 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 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 therapeutical 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-1alpha (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

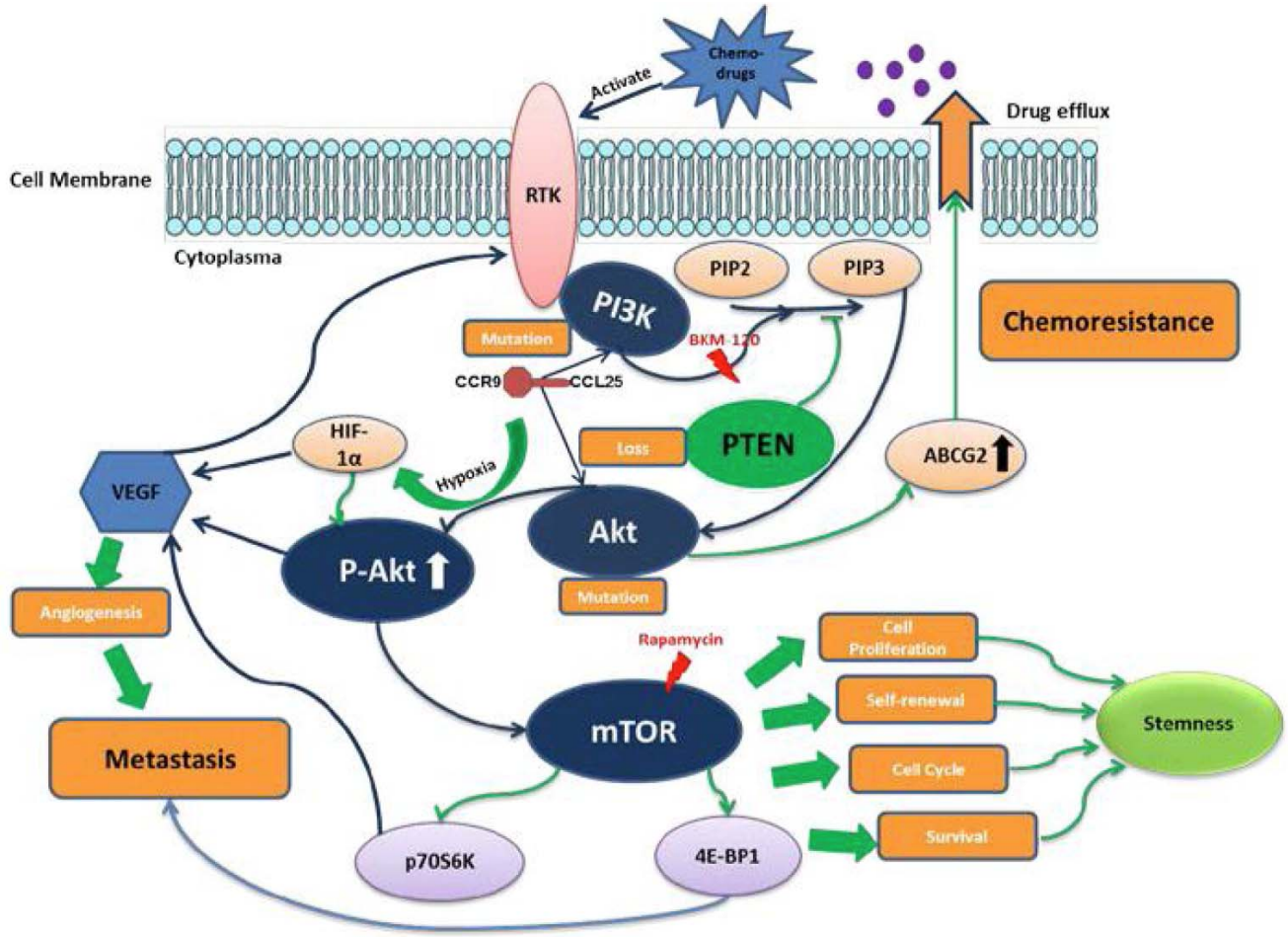

Fig. (2). Overview of PI3K/Akt/PTEN/mTOR signaling pathway in the regulation of cancer metastasis, chemoreistance as well as its association with CSCs in CaP cells. This pathway plays a crucial role in regulating a broad range of cellular functions including cell proliferation, self-renewal, cell cycle, survival, angiogenesis, adhesion and migration. PI3K converts PIP2 into PIP3 while PTEN antagonizes PI3K by converting PIP3 to PIP2, thus inhibiting downstream signaling. Akt, which is the downstream of PI3K, is activated and phosphorylated by PIP3 which subsequently causes alteration of cellular functions including the activation of mTOR and its substrates p70S6K and 4E-BP1. Under hypoxia, HIF-1α expression is significantly increased, which further affects the phosphorylation (activation) of Akt, followed by an overexpression of VEGF, which in turn activates this pathway and is crucial for metastasis and chemoresistance of CaP. Akt can also induce the expression of ABCG2, which is important in drug efflux response to chemotherapeutic agents. CCR9 and its ligand CCL25 can upregulate PI3K and Akt as well. Both BKM120 (a PI3K inhibitor) and Rapamycin (a mTOR inhibitor) can inhibit PI3K/Akt/PTEN/mTOR pathway effectively. ABCG2: ATP-binding cassette sub-family G member 2; CaP: prostate cancer; CCR9: chemokine receptor 9; CSCs: cancer stem cells; HIF-1α: hypoxia-inducible factor-1alpha; RTK: receptor tyrosine kinases; VEGF: vascular endothelial growth factor.

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 $OV6^+$ 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 upregualtion 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.

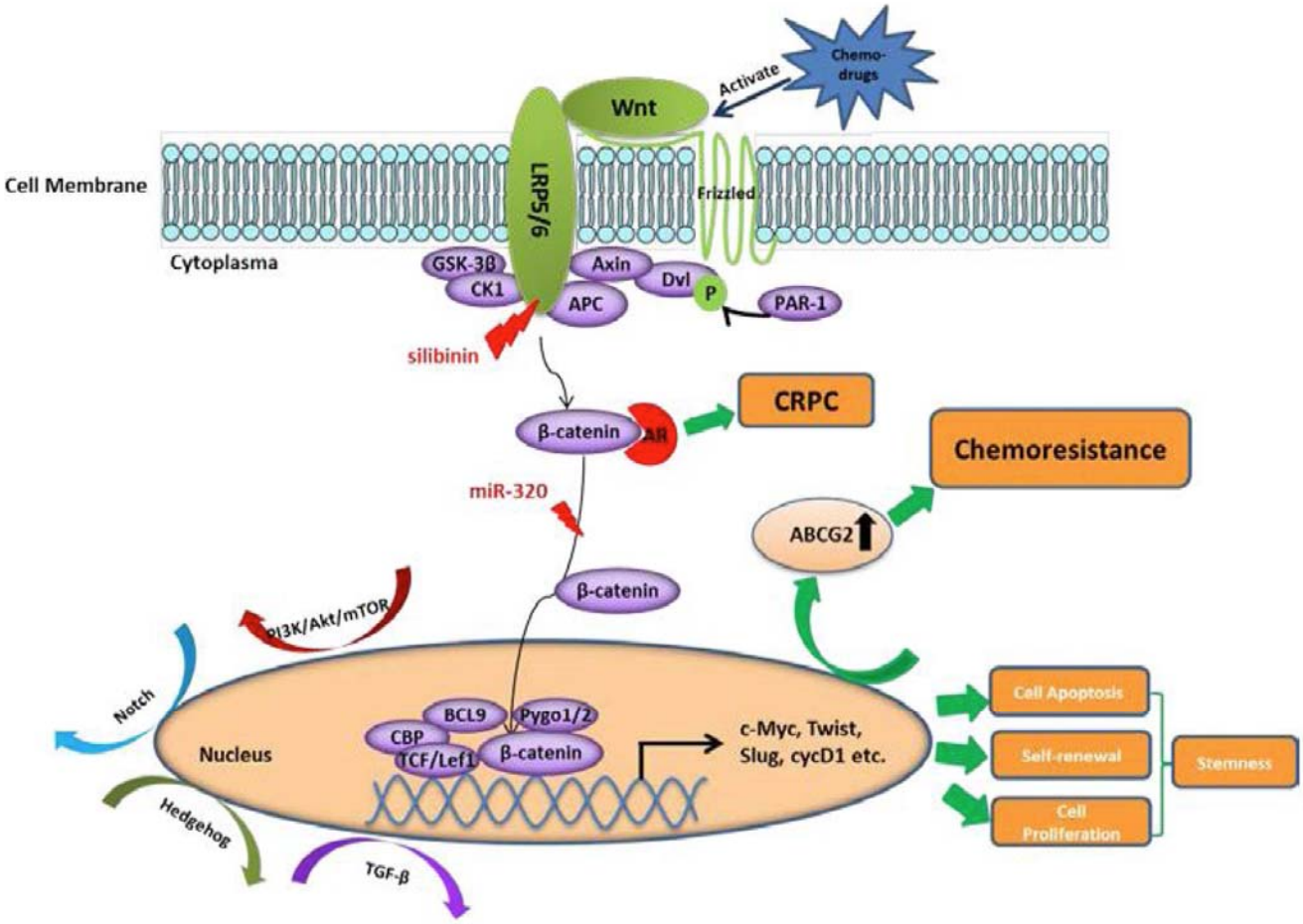

Fig. (3). Overview of on-state of canonical Wnt pathway, Wnt/β-catenin pathway. Wnt/β-catenin pathway is the canonical Wnt pathway that causes an accumulation of β-catenin in the cytoplasm and its eventual translocation into the nucleus to act as a transcriptional co-activator of TCF/LEF transcription factors, inducing the overexpression of c-Myc, Twist, Slug, cycD1, which plays crucial roles in cell apoptosis, self-renewal and cell proliferation. One potential mechanism of its regulation of chemoresistance in CaP is through the up-regualtion of ABCG2. Wnt/β-catenin signaling also cooparates with several other signaling pathways including PI3K/Akt/mTOR, Notch, Hedgehog, and TGF-β. It also reacts with AR, which plays a critical role in CRPC development. ABCG2: ATP-binding cassette sub-family G member 2; AR: androgen receptor; CaP: prostate cancer; CRPC: castration-resistant prostate cancer.

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 HLAI 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 CHEMO-RESISTANCE

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 $CD44^+\alpha_2\beta_1{}^{high}CD133^+$ 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 $CD166^{high}$ 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 $PSA^{(-/lo)}$ 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 $ALDH^+CD44^+\alpha2\beta1^+$ 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, Schatton 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 reconstitiute 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 transistion
EpCAM	=	Epithelial cell adhesion molecule
FACS	=	Fluorescence-activated cell sorting
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

[1] Beltran, H.; Beer, T.M.; Carducci, M.A.; de Bono, J.; Gleave, M.; Hussain, M.; Kelly, W.K.; Saad, F.; Sternberg, C.; Tagawa, S.T.; Tannock, I.F., New therapies for castration-resistant prostate cancer: efficacy and safety. *Eur Urol*, 2011, *60*, (2), 279-290.

[2] Logothetis, C.J., Docetaxel in the integrated management of prostate cancer. Current applications and future promise. *Oncology (Williston Park)*, 2002, *16*, (6 Suppl 6), 63-72.

[3] Petrylak, D.P.; Tangen, C.M.; Hussain, M.H.; Lara, P.N., Jr.; Jones, J.A.; Taplin, M.E.; Burch, P.A.; Berry, D.; Moinpour, C.; Kohli, M.; Benson, M.C.; Small, E.J.; Raghavan, D.; Crawford, E.D., Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. *N Engl J Med*, 2004, *351*, (15), 1513-1520.

[4] Berthold, D.R.; Pond, G.R.; Soban, F.; de Wit, R.; Eisenberger, M.; Tannock, I.F., Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer: updated survival in the TAX 327 study. *J Clin Oncol*, 2008, *26*, (2), 242-245.

[5] Tannock, I.F.; de Wit, R.; Berry, W.R.; Horti, J.; Pluzanska, A.; Chi, K.N.; Oudard, S.; Theodore, C.; James, N.D.; Turesson, I.; Rosenthal, M.A.; Eisenberger, M.A.; Investigators, T.A.X., Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med*, 2004, *351*, (15), 1502-1512.

[6] Kantoff, P.W.; Halabi, S.; Conaway, M.; Picus, J.; Kirshner, J.; Hars, V.; Trump, D.; Winer, E.P.; Vogelzang, N.J., Hydrocortisone with or without mitoxantrone in men with hormone-refractory prostate cancer: results of the cancer and leukemia group B 9182 study. *J Clin Oncol*, 1999, *17*, (8), 2506-2513.

[7] de Bono, J.S.; Oudard, S.; Ozguroglu, M.; Hansen, S.; Machiels, J.P.; Kocak, I.; Gravis, G.; Bodrogi, I.; Mackenzie, M.J.; Shen, L.; Roessner, M.; Gupta, S.; Sartor, A.O.; Investigators, T., Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. *Lancet*, 2010, *376*, (9747), 1147-1154.

[8] Sternberg, C.N.; Dumez, H.; Van Poppel, H.; Skoneczna, I.; Sella, A.; Daugaard, G.; Gil, T.; Graham, J.; Carpentier, P.; Calabro, F.; Collette, L.; Lacombe, D.; Group, E.G.T.C., Docetaxel plus oblimersen sodium (Bcl-2 antisense oligonucleotide): an EORTC multicenter, randomized phase II study in patients with castration-resistant prostate cancer. *Ann Oncol*, 2009, *20*, (7), 1264-1269.

[9] Ni, J.; Cozzi, P.J.; Duan, W.; Shigdar, S.; Graham, P.H.; Kearsley, J.H.; Li, Y., Role of the EpCAM (CD326) in prostate cancer metastasis and progression. *Cancer Metastasis Rev*, 2012, *31*, (3-4), 779-791.

[10] O'Brien, C.A.; Kreso, A.; Dick, J.E., Cancer stem cells in solid tumors: an overview. *Semin Radiat Oncol*, 2009, *19*, (2), 71-77.

[11] Clarke, M.F.; Fuller, M., Stem cells and cancer: two faces of eve. *Cell*, 2006, *124*, (6), 1111-1115.

[12] Gupta, P.B.; Chaffer, C.L.; Weinberg, R.A., Cancer stem cells: mirage or reality? *Nat Med*, 2009, *15*, (9), 1010-1012.

[13] Hao, J.; Madigan, M.C.; Khatri, A.; Power, C.A.; Hung, T.T.; Beretov, J.; Chang, L.; Xiao, W.; Cozzi, P.J.; Graham, P.H.; Kearsley, J.H.; Li, Y., *In vitro* and *in vivo* prostate cancer metastasis and chemoresistance can be modulated by expression of either CD44 or CD147. *PLoS One*, 2012, *7*, (8), e40716.

[14] McCubrey, J.A.; Abrams, S.L.; Stadelman, K.; Chappell, W.H.; Lahair, M.; Ferland, R.A.; Steelman, L.S., Targeting signal transduction pathways to eliminate chemotherapeutic drug resistance and cancer stem cells. *Adv Enzyme Regul*, 2010, *50*, (1), 285-307.

[15] Reya, T.; Morrison, S.J.; Clarke, M.F.; Weissman, I.L., Stem cells, cancer, and cancer stem cells. *Nature*, 2001, *414*, (6859), 105-111.

[16] Jordan, C.T.; Guzman, M.L.; Noble, M., Cancer stem cells. *N Engl J Med*, 2006, *355*, (12), 1253-1261.

[17] Visvader, J.E., Cells of origin in cancer. *Nature*, 2011, *469*, (7330), 314-322.

[18] Chaffer, C.L.; Brueckmann, I.; Scheel, C.; Kaestli, A.J.; Wiggins, P.A.; Rodrigues, L.O.; Brooks, M.; Reinhardt, F.; Su, Y.; Polyak, K.; Arendt, L.M.; Kuperwasser, C.; Bierie, B.; Weinberg, R.A., Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. *Proc Natl Acad Sci U S A*, 2011, *108*, (19), 7950-7955.

[19] Shigdar, S.; Li, Y.; Bhattacharya, S.; O'Connor, M.; Pu, C.; Lin, J.; Wang, T.; Xiang, D.; Kong, L.; Wei, M.Q.; Zhu, Y.; Zhou, S.; Duan, W., Inflammation and cancer stem cells. *Cancer Lett*, 2014, *345*, (2), 271-278.

[20] Iliopoulos, D.; Hirsch, H.A.; Wang, G.; Struhl, K., Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells *via* IL6 secretion. *Proc Natl Acad Sci U S A*, 2011, *108*, (4), 1397-1402.

[21] Chaffer, C.L.; Marjanovic, N.D.; Lee, T.; Bell, G.; Kleer, C.G.; Reinhardt, F.; D'Alessio, A.C.; Young, R.A.; Weinberg, R.A., Poised chromatin at the ZEB1 promoter enables breast cancer cell plasticity and enhances tumorigenicity. *Cell*, 2013, *154*, (1), 61-74.

[22] Nagle, R.B.; Ahmann, F.R.; McDaniel, K.M.; Paquin, M.L.; Clark, V.A.; Celniker, A., Cytokeratin characterization of human prostatic carcinoma and its derived cell lines. *Cancer Res*, 1987, *47*, (1), 281-286.

[23] Gu, G.; Yuan, J.; Wills, M.; Kasper, S., Prostate cancer cells with stem cell characteristics reconstitute the original human tumor *in vivo*. *Cancer Res*, 2007, *67*, (10), 4807-4815.

[24] Li, Y.; Cozzi, P.J.; Russell, P.J., Promising tumor-associated antigens for future prostate cancer therapy. *Med Res Rev*, 2010, *30*, (1), 67-101.

[25] Li, H.; Chen, X.; Calhoun-Davis, T.; Claypool, K.; Tang, D.G., PC3 human prostate carcinoma cell holoclones contain self-renewing tumor-initiating cells. *Cancer Res*, 2008, *68*, (6), 1820-1825.

[26] Li, H.; Jiang, M.; Honorio, S.; Patrawala, L.; Jeter, C.R.; Calhoun-Davis, T.; Hayward, S.W.; Tang, D.G., Methodologies in assaying prostate cancer stem cells. *Methods Mol Biol*, 2009, *568*, 85-138.

[27] Lang, S.H.; Frame, F.M.; Collins, A.T., Prostate cancer stem cells. *J Pathol*, 2009, *217*, (2), 299-306.

[28] Collins, A.T.; Berry, P.A.; Hyde, C.; Stower, M.J.; Maitland, N.J., Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res*, 2005, *65*, (23), 10946-10951.

[29] Collins, A.T.; Maitland, N.J., Prostate cancer stem cells. *Eur J Cancer*, 2006, *42*, (9), 1213-1218.

[30] Maitland, N.J.; Collins, A.T., Prostate cancer stem cells: a new target for therapy. *J Clin Oncol*, 2008, *26*, (17), 2862-2870.

[31] Patrawala, L.; Calhoun-Davis, T.; Schneider-Broussard, R.; Tang, D.G., Hierarchical organization of prostate cancer cells in xenograft tumors: the CD44+alpha2beta1+ cell population is enriched in tumor-initiating cells. *Cancer Res*, 2007, *67*, (14), 6796-6805.

[32] Rowehl, R.A.; Crawford, H.; Dufour, A.; Ju, J.; Botchkina, G.I., Genomic analysis of prostate cancer stem cells isolated from a highly metastatic cell line. *Cancer Genomics Proteomics*, 2008, *5*, (6), 301-310.

[33] Dubrovska, A.; Kim, S.; Salamone, R.J.; Walker, J.R.; Maira, S.M.; Garcia-Echeverria, C.; Schultz, P.G.; Reddy, V.A., The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations. *Proc Natl Acad Sci U S A*, 2009, *106*, (1), 268-273.

[34] Hurt, E.M.; Kawasaki, B.T.; Klarmann, G.J.; Thomas, S.B.; Farrar, W.L., CD44+ CD24(-) prostate cells are early cancer progenitor/stem cells that provide a model for patients with poor prognosis. *Br J Cancer*, 2008, *98*, (4), 756-765.

[35] Qin, J.; Liu, X.; Laffin, B.; Chen, X.; Choy, G.; Jeter, C.R.; Calhoun-Davis, T.; Li, H.; Palapattu, G.S.; Pang, S.; Lin, K.; Huang, J.; Ivanov, I.; Li, W.; Suraneni, M.V.; Tang, D.G., The PSA(-/lo) prostate cancer cell population harbors self-renewing long-term tumor-propagating cells that resist castration. *Cell Stem Cell*, 2012, *10*, (5), 556-569.

[36] Domingo-Domenech, J.; Vidal, S.J.; Rodriguez-Bravo, V.; Castillo-Martin, M.; Quinn, S.A.; Rodriguez-Barrueco, R.; Bonal, D.M.; Charytonowicz, E.; Gladoun, N.; de la Iglesia-Vicente, J.; Petrylak, D.P.; Benson, M.C.; Silva, J.M.; Cordon-Cardo, C., Suppression of acquired docetaxel resistance in prostate cancer through depletion of notch- and hedgehog-dependent tumor-initiating cells. *Cancer Cell*, 2012, *22*, (3), 373-388.

[37] Ponta, H.; Sherman, L.; Herrlich, P.A., CD44: from adhesion molecules to signalling regulators. *Nat Rev Mol Cell Biol*, 2003, *4*, (1), 33-45.

[38] Naor, D.; Wallach-Dayan, S.B.; Zahalka, M.A.; Sionov, R.V., Involvement of CD44, a molecule with a thousand faces, in cancer dissemination. *Semin Cancer Biol*, 2008, *18*, (4), 260-267.

[39] Gunthert, U.; Hofmann, M.; Rudy, W.; Reber, S.; Zoller, M.; Haussmann, I.; Matzku, S.; Wenzel, A.; Ponta, H.; Herrlich, P., A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. *Cell*, 1991, *65*, (1), 13-24.

[40] Charpin, C.; Secq, V.; Giusiano, S.; Carpentier, S.; Andrac, L.; Lavaut, M.N.; Allasia, C.; Bonnier, P.; Garcia, S., A signature predictive of disease outcome in breast carcinomas, identified by quantitative immunocytochemical assays. *Int J Cancer*, 2009, *124*, (9), 2124-2134.

[41] Ween, M.P.; Oehler, M.K.; Ricciardelli, C., Role of Versican, Hyaluronan and CD44 in Ovarian Cancer Metastasis. *Int J Mol Sci*, 2011, *12*, (2), 1009-1029.

[42] Hebbard, L.; Steffen, A.; Zawadzki, V.; Fieber, C.; Howells, N.; Moll, J.; Ponta, H.; Hofmann, M.; Sleeman, J., CD44 expression and regulation during mammary gland development and function. *J Cell Sci*, 2000, *113 (Pt 14)*, 2619-2630.

[43] Yang, Y.M.; Chang, J.W., Bladder cancer initiating cells (BCICs) are among EMA-CD44v6+ subset: novel methods for isolating undetermined cancer stem (initiating) cells. *Cancer Invest*, 2008, *26*, (7), 725-733.

[44] Bidlingmaier, S.; Zhu, X.; Liu, B., The utility and limitations of glycosylated human CD133 epitopes in defining cancer stem cells. *J Mol Med (Berl)*, 2008, *86*, (9), 1025-1032.

[45] Coskun, V.; Wu, H.; Blanchi, B.; Tsao, S.; Kim, K.; Zhao, J.; Biancotti, J.C.; Hutnick, L.; Krueger, R.C., Jr.; Fan, G.; de Vellis, J.; Sun, Y.E., CD133+ neural stem cells in the ependyma of mammalian postnatal forebrain. *Proc Natl Acad Sci U S A*, 2008, *105*, (3), 1026-1031.

[46] Pellacani, D.; Oldridge, E.E.; Collins, A.T.; Maitland, N.J., Prominin-1 (CD133) Expression in the Prostate and Prostate Cancer: A Marker for Quiescent Stem Cells. *Adv Exp Med Biol*, 2013, *777*, 167-184.

[47] Richardson, G.D.; Robson, C.N.; Lang, S.H.; Neal, D.E.; Maitland, N.J.; Collins, A.T., CD133, a novel marker for human prostatic epithelial stem cells. *J Cell Sci*, 2004, *117*, (Pt 16), 3539-3545.

[48] Vander Griend, D.J.; Karthaus, W.L.; Dalrymple, S.; Meeker, A.; DeMarzo, A.M.; Isaacs, J.T., The role of CD133 in normal human prostate stem cells and malignant cancer-initiating cells. *Cancer Res*, 2008, *68*, (23), 9703-9711.

[49] Pfeiffer, M.J.; Schalken, J.A., Stem cell characteristics in prostate cancer cell lines. *Eur Urol*, 2010, *57*, (2), 246-254.

[50] Kemper, K.; Sprick, M.R.; de Bree, M.; Scopelliti, A.; Vermeulen, L.; Hoek, M.; Zeilstra, J.; Pals, S.T.; Mehmet, H.; Stassi, G.; Medema, J.P., The AC133 epitope, but not the CD133 protein, is lost upon cancer stem cell differentiation. *Cancer Res*, 2010, *70*, (2), 719-729.

[51] Yang, D.R.; Ding, X.F.; Luo, J.; Shan, Y.X.; Wang, R.; Lin, S.J.; Li, G.; Huang, C.K.; Zhu, J.; Chen, Y.; Lee, S.O.; Chang, C., Increased chemosensitivity *via* targeting testicular nuclear receptor 4 (TR4)-Oct4-interleukin 1 receptor antagonist (IL1Ra) axis in prostate cancer CD133+ stem/progenitor cells to battle prostate cancer. *J Biol Chem*, 2013, *288*, (23), 16476-16483.

[52] Wang, D.; Zhu, H.; Zhu, Y.; Liu, Y.; Shen, H.; Yin, R.; Zhang, Z.; Su, Z., CD133(+)/CD44(+)/Oct4(+)/Nestin(+) stem-like cells isolated from Panc-1 cell line may contribute to multi-resistance and metastasis of pancreatic cancer. *Acta Histochem*, 2013, *115*, (4), 349-356.

[53] Yoshida, A.; Hsu, L.C.; Dave, V., Retinal oxidation activity and biological role of human cytosolic aldehyde dehydrogenase. *Enzyme*, 1992, *46*, (4-5), 239-244.

[54] van den Hoogen, C.; van der Horst, G.; Cheung, H.; Buijs, J.T.; Lippitt, J.M.; Guzman-Ramirez, N.; Hamdy, F.C.; Eaton, C.L.; Thalmann, G.N.; Cecchini, M.G.; Pelger, R.C.; van der Pluijm, G., High aldehyde dehydrogenase activity identifies tumor-initiating and metastasis-initiating cells in human prostate cancer. *Cancer Res*, 2010, *70*, (12), 5163-5173.

[55] Nishikawa, S.; Konno, M.; Hamabe, A.; Hasegawa, S.; Kano, Y.; Ohta, K.; Fukusumi, T.; Sakai, D.; Kudo, T.; Haraguchi, N.; Satoh, T.; Takiguchi, S.; Mori, M.; Doki, Y.; Ishii, H., Aldehyde dehydrogenase high gastric cancer stem cells are resistant to chemotherapy. *Int J Oncol*, 2013, *42*, (4), 1437-1442.

[56] Liu, P.; Kumar, I.S.; Brown, S.; Kannappan, V.; Tawari, P.E.; Tang, J.Z.; Jiang, W.; Armesilla, A.L.; Darling, J.L.; Wang, W., Disulfiram targets cancer stem-like cells and reverses resistance and cross-resistance in acquired paclitaxel-resistant triple-negative breast cancer cells. *Br J Cancer*, 2013, *109*, (7), 1876-1885.

[57] Patrawala, L.; Calhoun, T.; Schneider-Broussard, R.; Li, H.; Bhatia, B.; Tang, S.; Reilly, J.G.; Chandra, D.; Zhou, J.; Claypool, K.; Coghlan, L.; Tang, D.G., Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene*, 2006, *25*, (12), 1696-1708.

[58] Bisson, I.; Prowse, D.M., WNT signaling regulates self-renewal and differentiation of prostate cancer cells with stem cell characteristics. *Cell Res*, 2009, *19*, (6), 683-697.

[59] Li, T.; Su, Y.; Mei, Y.; Leng, Q.; Leng, B.; Liu, Z.; Stass, S.A.; Jiang, F., ALDH1A1 is a marker for malignant prostate stem cells and predictor of prostate cancer patients' outcome. *Lab Invest*, 2010, *90*, (2), 234-244.

[60] Rajasekhar, V.K.; Studer, L.; Gerald, W.; Socci, N.D.; Scher, H.I., Tumour-initiating stem-like cells in human prostate cancer exhibit increased NF-kappaB signalling. *Nat Commun*, 2011, *2*, 162.

[61] Bae, K.M.; Parker, N.N.; Dai, Y.; Vieweg, J.; Siemann, D.W., E-cadherin plasticity in prostate cancer stem cell invasion. *Am J Cancer Res*, 2011, *1*, (1), 71-84.

[62] Liu, T.; Xu, F.; Du, X.; Lai, D.; Liu, T.; Zhao, Y.; Huang, Q.; Jiang, L.; Huang, W.; Cheng, W.; Liu, Z., Establishment and characterization of multi-drug resistant, prostate carcinoma-initiating stem-like cells from human prostate cancer cell lines 22RV1. *Mol Cell Biochem*, 2010, *340*, (1-2), 265-273.

[63] Zhang, L.; Jiao, M.; Li, L.; Wu, D.; Wu, K.; Li, X.; Zhu, G.; Dang, Q.; Wang, X.; Hsieh, J.T.; He, D., Tumorspheres derived from prostate cancer cells possess chemoresistant and cancer stem cell properties. *J Cancer Res Clin Oncol*, 2012, *138*, (4), 675-686.

[64] Visvader, J.E.; Lindeman, G.J., Cancer stem cells: current status and evolving complexities. *Cell Stem Cell*, 2012, *10*, (6), 717-728.

[65] Daley, G.Q., Common themes of dedifferentiation in somatic cell reprogramming and cancer. *Cold Spring Harb Symp Quant Biol*, 2008, *73*, 171-174.

[66] Park, I.H.; Zhao, R.; West, J.A.; Yabuuchi, A.; Huo, H.; Ince, T.A.; Lerou, P.H.; Lensch, M.W.; Daley, G.Q., Reprogramming of human somatic cells to pluripotency with defined factors. *Nature*, 2008, *451*, (7175), 141-146.

[67] Moad, M.; Pal, D.; Hepburn, A.C.; Williamson, S.C.; Wilson, L.; Lako, M.; Armstrong, L.; Hayward, S.W.; Franco, O.E.; Cates, J.M.; Fordham, S.E.; Przyborski, S.; Carr-Wilkinson, J.; Robson, C.N.; Heer, R., A Novel Model of Urinary Tract Differentiation, Tissue Regeneration, and Disease: Reprogramming Human Prostate and Bladder Cells into Induced Pluripotent Stem Cells. *European Urology*, 2013, *64*, (5), 753-761.

[68] Yu, J.; Vodyanik, M.A.; Smuga-Otto, K.; Antosiewicz-Bourget, J.; Frane, J.L.; Tian, S.; Nie, J.; Jonsdottir, G.A.; Ruotti, V.; Stewart, R.; Slukvin, II; Thomson, J.A., Induced pluripotent stem cell lines derived from human somatic cells. *Science*, 2007, *318*, (5858), 1917-1920.

[69] Loh, Y.H.; Wu, Q.; Chew, J.L.; Vega, V.B.; Zhang, W.; Chen, X.; Bourque, G.; George, J.; Leong, B.; Liu, J.; Wong, K.Y.; Sung, K.W.; Lee, C.W.; Zhao, X.D.; Chiu, K.P.; Lipovich, L.; Kuznetsov, V.A.; Robson, P.; Stanton, L.W.; Wei, C.L.; Ruan, Y.; Lim, B.; Ng, H.H., The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nat Genet*, 2006, *38*, (4), 431-440.

[70] Boyer, L.A.; Lee, T.I.; Cole, M.F.; Johnstone, S.E.; Levine, S.S.; Zucker, J.P.; Guenther, M.G.; Kumar, R.M.; Murray, H.L.; Jenner, R.G.; Gifford, D.K.; Melton, D.A.; Jaenisch, R.; Young, R.A., Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell*, 2005, *122*, (6), 947-956.

[71] Lin, Y.; Yang, Y.; Li, W.; Chen, Q.; Li, J.; Pan, X.; Zhou, L.; Liu, C.; Chen, C.; He, J.; Cao, H.; Yao, H.; Zheng, L.; Xu, X.; Xia, Z.; Ren, J.; Xiao, L.; Li, L.; Shen, B.; Zhou, H.; Wang, Y.J., Reciprocal regulation of Akt and Oct4 promotes the self-renewal and survival of embryonal carcinoma cells. *Mol Cell*, 2012, *48*, (4), 627-640.

[72] Rodriguez-Pinilla, S.M.; Sarrio, D.; Moreno-Bueno, G.; Rodriguez-Gil, Y.; Martinez, M.A.; Hernandez, L.; Hardisson, D.; Reis-Filho, J.S.; Palacios, J., Sox2: a possible driver of the basal-like phenotype in sporadic breast cancer. *Mod Pathol*, 2007, *20*, (4), 474-481.

[73] Noh, K.H.; Kim, B.W.; Song, K.H.; Cho, H.; Lee, Y.H.; Kim, J.H.; Chung, J.Y.; Kim, J.H.; Hewitt, S.M.; Seong, S.Y.; Mao, C.P.; Wu, T.C.; Kim, T.W., Nanog signaling in cancer promotes stem-like phenotype and immune evasion. *J Clin Invest*, 2012, *122*, (11), 4077-4093.

[74] Hochedlinger, K.; Yamada, Y.; Beard, C.; Jaenisch, R., Ectopic expression of Oct-4 blocks progenitor-cell differentiation and causes dysplasia in epithelial tissues. *Cell*, 2005, *121*, (3), 465-477.

[75] Linn, D.E.; Yang, X.; Sun, F.; Xie, Y.; Chen, H.; Jiang, R.; Chen, H.; Chumsri, S.; Burger, A.M.; Qiu, Y., A Role for OCT4 in Tumor Initiation of Drug-Resistant Prostate Cancer Cells. *Genes Cancer*, 2010, *1*, (9), 908-916.

[76] Tai, M.H.; Chang, C.C.; Kiupel, M.; Webster, J.D.; Olson, L.K.; Trosko, J.E., Oct4 expression in adult human stem cells: evidence in support of the stem cell theory of carcinogenesis. *Carcinogenesis*, 2005, *26*, (2), 495-502.

[77] de Resende, M.F.; Chinen, L.T.; Vieira, S.; Jampietro, J.; da Fonseca, F.P.; Vassallo, J.; Campos, L.C.; Guimaraes, G.C.; Soares, F.A.; Rocha, R.M., Prognostication of OCT4 isoform expression in prostate cancer. *Tumour Biol*, 2013, *34*, (5), 2665-2673.

[78] Robey, R.W.; Polgar, O.; Deeken, J.; To, K.W.; Bates, S.E., ABCG2: determining its relevance in clinical drug resistance. *Cancer Metastasis Rev*, 2007, *26*, (1), 39-57.

[79] Dean, M.; Fojo, T.; Bates, S., Tumour stem cells and drug resistance. *Nat Rev Cancer*, 2005, *5*, (4), 275-284.

[80] Ma, Y.; Liang, D.; Liu, J.; Axcrona, K.; Kvalheim, G.; Giercksky, K.E.; Nesland, J.M.; Suo, Z., Synergistic effect of SCF and G-CSF on stem-like properties in prostate cancer cell lines. *Tumour Biol*, 2012, *33*, (4), 967-978.

[81] Sims-Mourtada, J.; Izzo, J.G.; Ajani, J.; Chao, K.S., Sonic Hedgehog promotes multiple drug resistance by regulation of drug transport. *Oncogene*, 2007, *26*, (38), 5674-5679.

[82] Bhattacharya, S.; Das, A.; Mallya, K.; Ahmad, I., Maintenance of retinal stem cells by Abcg2 is regulated by notch signaling. *J Cell Sci*, 2007, *120*, (Pt 15), 2652-2662.

[83] Bleau, A.M.; Hambardzumyan, D.; Ozawa, T.; Fomchenko, E.I.; Huse, J.T.; Brennan, C.W.; Holland, E.C., PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. *Cell Stem Cell*, 2009, *4*, (3), 226-235.

[84] Bitting, R.L.; Armstrong, A.J., Targeting the PI3K/Akt/mTOR pathway in castration-resistant prostate cancer. *Endocr Relat Cancer*, 2013, *20*, (3), R83-99.

[85] Pourmand, G.; Ziaee, A.A.; Abedi, A.R.; Mehrsai, A.; Alavi, H.A.; Ahmadi, A.; Saadati, H.R., Role of PTEN gene in progression of prostate cancer. *Urol J*, 2007, *4*, (2), 95-100.

[86] Taylor, B.S.; Schultz, N.; Hieronymus, H.; Gopalan, A.; Xiao, Y.; Carver, B.S.; Arora, V.K.; Kaushik, P.; Cerami, E.; Reva, B.; Antipin, Y.; Mitsiades, N.; Landers, T.; Dolgalev, I.; Major, J.E.; Wilson, M.; Socci, N.D.; Lash, A.E.; Heguy, A.; Eastham, J.A.; Scher, H.I.; Reuter, V.E.; Scardino, P.T.; Sander, C.; Sawyers, C.L.; Gerald, W.L., Integrative genomic profiling of human prostate cancer. *Cancer Cell*, 2010, *18*, (1), 11-22.

[87] Martelli, A.M.; Evangelisti, C.; Chappell, W.; Abrams, S.L.; Basecke, J.; Stivala, F.; Donia, M.; Fagone, P.; Nicoletti, F.; Libra, M.; Ruvolo, V.; Ruvolo, P.; Kempf, C.R.; Steelman, L.S.; McCubrey, J.A., Targeting the translational apparatus to improve leukemia therapy: roles of the PI3K/PTEN/Akt/mTOR pathway. *Leukemia*, 2011, *25*, (7), 1064-1079.

[88] Lim, M.; Chuong, C.M.; Roy-Burman, P., PI3K, Erk signaling in BMP7-induced epithelial-mesenchymal transition (EMT) of PC-3 prostate cancer cells in 2- and 3-dimensional cultures. *Horm Cancer*, 2011, *2*, (5), 298-309.

[89] Mulholland, D.J.; Kobayashi, N.; Ruscetti, M.; Zhi, A.; Tran, L.M.; Huang, J.; Gleave, M.; Wu, H., Pten loss and RAS/MAPK activation cooperate to promote EMT and metastasis initiated from prostate cancer stem/progenitor cells. *Cancer Res*, 2012, *72*, (7), 1878-1889.

[90] Lau, C.K.; Yang, Z.F.; Ho, D.W.; Ng, M.N.; Yeoh, G.C.; Poon, R.T.; Fan, S.T., An Akt/hypoxia-inducible factor-1alpha/platelet-derived growth factor-BB autocrine loop mediates hypoxia-induced chemoresistance in liver cancer cells and tumorigenic hepatic progenitor cells. *Clin Cancer Res*, 2009, *15*, (10), 3462-3471.

[91] Lee, T.K.; Castilho, A.; Cheung, V.C.; Tang, K.H.; Ma, S.; Ng, I.O., Lupeol targets liver tumor-initiating cells through phosphatase and tensin homolog modulation. *Hepatology*, 2011, *53*, (1), 160-170.

[92] Ma, S.; Lee, T.K.; Zheng, B.J.; Chan, K.W.; Guan, X.Y., CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. *Oncogene*, 2008, *27*, (12), 1749-1758.

[93] Sharma, P.K.; Singh, R.; Novakovic, K.R.; Eaton, J.W.; Grizzle, W.E.; Singh, S., CCR9 mediates PI3K/AKT-dependent antiapoptotic signals in prostate cancer cells and inhibition of CCR9-CCL25 interaction enhances the cytotoxic effects of etoposide. *Int J Cancer*, 2010, *127*, (9), 2020-2030.

[94] Kumar, D.; Shankar, S.; Srivastava, R.K., Rottlerin induces autophagy and apoptosis in prostate cancer stem cells *via* PI3K/Akt/mTOR signaling pathway. *Cancer Lett*, 2014, *343*, (2), 179-189.

[95] Di Cristofano, A.; Pandolfi, P.P., The multiple roles of PTEN in tumor suppression. *Cell*, 2000, *100*, (4), 387-390.

[96] Yilmaz, O.H.; Valdez, R.; Theisen, B.K.; Guo, W.; Ferguson, D.O.; Wu, H.; Morrison, S.J., Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. *Nature*, 2006, *441*, (7092), 475-482.

[97] Lee, J.T., Jr.; Steelman, L.S.; McCubrey, J.A., Phosphatidylinositol 3'-kinase activation leads to multidrug resistance protein-1 expression and subsequent chemoresistance in advanced prostate cancer cells. *Cancer Res*, 2004, *64*, (22), 8397-8404.

[98] Priulla, M.; Calastretti, A.; Bruno, P.; Azzariti, A.; Paradiso, A.; Canti, G.; Nicolin, A., Preferential chemosensitization of PTEN-mutated prostate cells by silencing the Akt kinase. *Prostate*, 2007, *67*, (7), 782-789.

[99] Huang, H.; Cheville, J.C.; Pan, Y.; Roche, P.C.; Schmidt, L.J.; Tindall, D.J., PTEN induces chemosensitivity in PTEN-mutated prostate cancer cells by suppression of Bcl-2 expression. *J Biol Chem*, 2001, *276*, (42), 38830-38836.

[100] Morikawa, Y.; Koike, H.; Sekine, Y.; Matsui, H.; Shibata, Y.; Ito, K.; Suzuki, K., Rapamycin enhances docetaxel-induced cytotoxicity in a androgen-independent prostate cancer xenograft model by survivin downregulation. *Biochem Biophys Res Commun*, 2012, *419*, (3), 584-589.

[101] Yasumizu, Y.; Miyajima, A.; Kosaka, T.; Miyazaki, Y.; Kikuchi, E.; Oya, M., Dual PI3K/mTOR Inhibitor NVP-BEZ235 Sensitizes Docetaxel in Castration Resistant Prostate Cancer. *J Urol*, 2014, *191*, (1), 227-234.

[102] Yu, X.; Wang, Y.; DeGraff, D.J.; Wills, M.L.; Matusik, R.J., Wnt/beta-catenin activation promotes prostate tumor progression in a mouse model. *Oncogene*, 2011, *30*, (16), 1868-1879.

[103] Hsieh, I.S.; Chang, K.C.; Tsai, Y.T.; Ke, J.Y.; Lu, P.J.; Lee, K.H.; Yeh, S.D.; Hong, T.M.; Chen, Y.L., MicroRNA-320 suppresses the stem cell-like characteristics of prostate cancer cells by downregulating the Wnt/beta-catenin signaling pathway. *Carcinogenesis*, 2013, *34*, (3), 530-538.

[104] Yang, W.; Yan, H.X.; Chen, L.; Liu, Q.; He, Y.Q.; Yu, L.X.; Zhang, S.H.; Huang, D.D.; Tang, L.; Kong, X.N.; Chen, C.; Liu, S.Q.; Wu, M.C.; Wang, H.Y., Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. *Cancer Res*, 2008, *68*, (11), 4287-4295.

[105] Noda, T.; Nagano, H.; Takemasa, I.; Yoshioka, S.; Murakami, M.; Wada, H.; Kobayashi, S.; Marubashi, S.; Takeda, Y.; Dono, K.; Umeshita, K.; Matsuura, N.; Matsubara, K.; Doki, Y.; Mori, M.; Monden, M., Activation of Wnt/beta-catenin signalling pathway induces chemoresistance to interferon-alpha/5-fluorouracil combination therapy for hepatocellular carcinoma. *Br J Cancer*, 2009, *100*, (10), 1647-1658.

[106] Flahaut, M.; Meier, R.; Coulon, A.; Nardou, K.A.; Niggli, F.K.; Martinet, D.; Beckmann, J.S.; Joseph, J.M.; Muhlethaler-Mottet, A.; Gross, N., The Wnt receptor FZD1 mediates chemoresistance in neuroblastoma through activation of the Wnt/beta-catenin pathway. *Oncogene*, 2009, *28*, (23), 2245-2256.

[107] Chau, W.K.; Ip, C.K.; Mak, A.S.; Lai, H.C.; Wong, A.S., c-Kit mediates chemoresistance and tumor-initiating capacity of ovarian cancer cells through activation of Wnt/beta-catenin-ATP-binding cassette G2 signaling. *Oncogene*, 2013, *32*, (22), 2767-2781.

[108] Almeida, M.; Han, L.; Bellido, T.; Manolagas, S.C.; Kousteni, S., Wnt proteins prevent apoptosis of both uncommitted osteoblast progenitors and differentiated osteoblasts by beta-catenin-dependent and -independent signaling cascades involving Src/ERK and phosphatidylinositol 3-kinase/AKT. *J Biol Chem*, 2005, *280*, (50), 41342-41351.

[109] Liu, S.; Dontu, G.; Wicha, M.S., Mammary stem cells, self-renewal pathways, and carcinogenesis. *Breast Cancer Res*, 2005, *7*, (3), 86-95.

[110] James, D.; Levine, A.J.; Besser, D.; Hemmati-Brivanlou, A., TGFbeta/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells. *Development*, 2005, *132*, (6), 1273-1282.

[111] Lu, W.; Lin, C.; King, T.D.; Chen, H.; Reynolds, R.C.; Li, Y., Silibinin inhibits Wnt/beta-catenin signaling by suppressing Wnt co-receptor LRP6 expression in human prostate and breast cancer cells. *Cell Signal*, 2012, *24*, (12), 2291-2296.

[112] Espinoza, I.; Pochampally, R.; Xing, F.; Watabe, K.; Miele, L., Notch signaling: targeting cancer stem cells and epithelial-to-mesenchymal transition. *Onco Targets Ther*, 2013, *6*, 1249-1259.

[113] Ranganathan, P.; Weaver, K.L.; Capobianco, A.J., Notch signalling in solid tumours: a little bit of everything but not all the time. *Nat Rev Cancer*, 2011, *11*, (5), 338-351.

[114] Zhu, H.; Zhou, X.; Redfield, S.; Lewin, J.; Miele, L., Elevated Jagged-1 and Notch-1 expression in high grade and metastatic prostate cancers. *Am J Transl Res*, 2013, *5*, (3), 368-378.

[115] Wang, Z.; Li, Y.; Ahmad, A.; Banerjee, S.; Azmi, A.S.; Kong, D.; Wojewoda, C.; Miele, L.; Sarkar, F.H., Down-regulation of Notch-1 is associated with Akt and FoxM1 in inducing cell growth inhibition and apoptosis in prostate cancer cells. *J Cell Biochem*, 2011, *112*, (1), 78-88.

[116] Ye, Q.F.; Zhang, Y.C.; Peng, X.Q.; Long, Z.; Ming, Y.Z.; He, L.Y., siRNA-mediated silencing of Notch-1 enhances docetaxel induced mitotic arrest and apoptosis in prostate cancer cells. *Asian Pac J Cancer Prev*, 2012, *13*, (6), 2485-2489.

[117] Sarkar, F.H.; Li, Y.; Wang, Z.; Kong, D., Novel targets for prostate cancer chemoprevention. *Endocr Relat Cancer*, 2010, *17*, (3), R195-212.

[118] Janikova, M.; Skarda, J., Differentiation pathways in carcinogenesis and in chemo- and radioresistance. *Neoplasma*, 2012, *59*, (1), 6-17.

[119] Hutchin, M.E.; Kariapper, M.S.; Grachtchouk, M.; Wang, A.; Wei, L.; Cummings, D.; Liu, J.; Michael, L.E.; Glick, A.; Dlugosz, A.A., Sustained Hedgehog signaling is required for basal cell carcinoma proliferation and survival: conditional skin tumorigenesis recapitulates the hair growth cycle. *Genes Dev*, 2005, *19*, (2), 214-223.

[120] Hatsell, S.; Frost, A.R., Hedgehog signaling in mammary gland development and breast cancer. *J Mammary Gland Biol Neoplasia*, 2007, *12*, (2-3), 163-173.

[121] Mfopou, J.K.; Bouwens, L., Hedgehog signals in pancreatic differentiation from embryonic stem cells: revisiting the neglected. *Differentiation*, 2008, *76*, (2), 107-117.

[122] Shaw, G.; Prowse, D.M., Inhibition of androgen-independent prostate cancer cell growth is enhanced by combination therapy targeting Hedgehog and ErbB signalling. *Cancer Cell Int*, 2008, *8*, 3.

[123] Singh, S.; Chitkara, D.; Mehrazin, R.; Behrman, S.W.; Wake, R.W.; Mahato, R.I., Chemoresistance in prostate cancer cells is regulated by miRNAs and Hedgehog pathway. *PLoS One*, 2012, *7*, (6), e40021.

[124] Chang, H.H.; Chen, B.Y.; Wu, C.Y.; Tsao, Z.J.; Chen, Y.Y.; Chang, C.P.; Yang, C.R.; Lin, D.P., Hedgehog overexpression leads to the formation of prostate cancer stem cells with metastatic property irrespective of androgen receptor expression in the mouse model. *J Biomed Sci*, 2011, *18*, 6.

[125] Zhang, L.; Li, L.; Jiao, M.; Wu, D.; Wu, K.; Li, X.; Zhu, G.; Yang, L.; Wang, X.; Hsieh, J.T.; He, D., Genistein inhibits the stemness properties of prostate cancer cells through targeting Hedgehog-Gli1 pathway. *Cancer Lett*, 2012, *323*, (1), 48-57.

[126] Crawford, T.Q.; Roelink, H., The notch response inhibitor DAPT enhances neuronal differentiation in embryonic stem cell-derived embryoid bodies independently of sonic hedgehog signaling. *Dev Dyn*, 2007, *236*, (3), 886-892.

[127] Riobo, N.A.; Lu, K.; Ai, X.; Haines, G.M.; Emerson, C.P., Jr., Phosphoinositide 3-kinase and Akt are essential for Sonic Hedgehog signaling. *Proc Natl Acad Sci U S A*, 2006, *103*, (12), 4505-4510.

[128] Heiser, P.W.; Lau, J.; Taketo, M.M.; Herrera, P.L.; Hebrok, M., Stabilization of beta-catenin impacts pancreas growth. *Development*, 2006, *133*, (10), 2023-2032.

[129] Yao, J.; An, Y.; Wie, J.S.; Ji, Z.L.; Lu, Z.P.; Wu, J.L.; Jiang, K.R.; Chen, P.; Xu, Z.K.; Miao, Y., Cyclopamine reverts acquired chemoresistance and down-regulates cancer stem cell markers in pancreatic cancer cell lines. *Swiss Med Wkly*, 2011, *141*, w13208.

[130] Steg, A.D.; Bevis, K.S.; Katre, A.A.; Ziebarth, A.; Dobbin, Z.C.; Alvarez, R.D.; Zhang, K.; Conner, M.; Landen, C.N., Stem cell pathways contribute to clinical chemoresistance in ovarian cancer. *Clin Cancer Res*, 2012, *18*, (3), 869-881.

[131] Perkins, N.D., The diverse and complex roles of NF-kappaB subunits in cancer. *Nat Rev Cancer*, 2012, *12*, (2), 121-132.

[132] Codony-Servat, J.; Marin-Aguilera, M.; Visa, L.; Garcia-Albeniz, X.; Pineda, E.; Fernandez, P.L.; Filella, X.; Gascon, P.; Mellado, B., Nuclear factor-kappa B and interleukin-6 related docetaxel resistance in castration-resistant prostate cancer. *Prostate*, 2013, *73*, (5), 512-521.

[133] Kwon, O.; Kim, K.A.; Kim, S.O.; Ha, R.; Oh, W.K.; Kim, M.S.; Kim, H.S.; Kim, G.D.; Kim, J.W.; Jung, M.; Kim, C.H.; Ahn, J.S.; Kim, B.Y., NF-kappaB inhibition increases chemosensitivity to trichostatin A-induced cell death of Ki-Ras-transformed human prostate epithelial cells. *Carcinogenesis*, 2006, *27*, (11), 2258-2268.

[134] O'Neill, A.J.; Prencipe, M.; Dowling, C.; Fan, Y.; Mulrane, L.; Gallagher, W.M.; O'Connor, D.; O'Connor, R.; Devery, A.; Corcoran, C.; Rani, S.; O'Driscoll, L.; Fitzpatrick, J.M.; Watson, R.W., Characterisation and manipulation of docetaxel resistant prostate cancer cell lines. *Mol Cancer*, 2011, *10*, 126.

[135] Syrovets, T.; Gschwend, J.E.; Buchele, B.; Laumonnier, Y.; Zugmaier, W.; Genze, F.; Simmet, T., Inhibition of IkappaB kinase activity by acetyl-boswellic acids promotes apoptosis in androgen-independent PC-3 prostate cancer cells *in vitro* and *in vivo*. *J Biol Chem*, 2005, *280*, (7), 6170-6180.

[136] Flynn, V., Jr.; Ramanitharan, A.; Moparty, K.; Davis, R.; Sikka, S.; Agrawal, K.C.; Abdel-Mageed, A.B., Adenovirus-mediated inhibition of NF-kappaB confers chemo-sensitization and apoptosis in prostate cancer cells. *Int J Oncol*, 2003, *23*, (2), 317-323.

[137] Lee, S.Y.; Cho, J.S.; Yuk, D.Y.; Moon, D.C.; Jung, J.K.; Yoo, H.S.; Lee, Y.M.; Han, S.B.; Oh, K.W.; Hong, J.T., Obovatol enhances docetaxel-induced prostate and colon cancer cell death through inactivation of nuclear transcription factor-kappaB. *J Pharmacol Sci*, 2009, *111*, (2), 124-136.

[138] Denmeade, S.R.; Isaacs, J.T., A history of prostate cancer treatment. *Nat Rev Cancer*, 2002, *2*, (5), 389-396.

[139] Edwards, J.; Krishna, N.S.; Grigor, K.M.; Bartlett, J.M., Androgen receptor gene amplification and protein expression in hormone refractory prostate cancer. *Br J Cancer*, 2003, *89*, (3), 552-556.

[140] Chen, C.D.; Welsbie, D.S.; Tran, C.; Baek, S.H.; Chen, R.; Vessella, R.; Rosenfeld, M.G.; Sawyers, C.L., Molecular determinants of resistance to antiandrogen therapy. *Nat Med*, 2004, *10*, (1), 33-39.

[141] Taplin, M.E.; Rajeshkumar, B.; Halabi, S.; Werner, C.P.; Woda, B.A.; Picus, J.; Stadler, W.; Hayes, D.F.; Kantoff, P.W.; Vogelzang, N.J.; Small, E.J.; Cancer; Leukemia Group, B.S., Androgen receptor mutations in androgen-independent prostate cancer: Cancer and Leukemia Group B Study 9663. *J Clin Oncol*, 2003, *21*, (14), 2673-2678.

[142] Debes, J.D.; Tindall, D.J., Mechanisms of androgen-refractory prostate cancer. *N Engl J Med*, 2004, *351*, (15), 1488-1490.

[143] Jiao, J.; Hindoyan, A.; Wang, S.; Tran, L.M.; Goldstein, A.S.; Lawson, D.; Chen, D.; Li, Y.; Guo, C.; Zhang, B.; Fazli, L.; Gleave, M.; Witte, O.N.; Garraway, I.P.; Wu, H., Identification of CD166 as a surface marker for enriching prostate stem/progenitor and cancer initiating cells. *PLoS One*, 2012, *7*, (8), e42564.

[144] Frank, N.Y.; Schatton, T.; Frank, M.H., The therapeutic promise of the cancer stem cell concept. *J Clin Invest*, 2010, *120*, (1), 41-50.

[145] Fabian, A.; Barok, M.; Vereb, G.; Szollosi, J., Die hard: are cancer stem cells the Bruce Willises of tumor biology? *Cytometry A*, 2009, *75*, (1), 67-74.

[146] Vidal, S.J.; Rodriguez-Bravo, V.; Galsky, M.; Cordon-Cardo, C.; Domingo-Domenech, J., Targeting cancer stem cells to suppress acquired chemotherapy resistance. *Oncogene*, 2013, 1-13.

[147] Zhou, Y.; Yang, J.; Kopecek, J., Selective inhibitory effect of HPMA copolymer-cyclopamine conjugate on prostate cancer stem cells. *Biomaterials*, 2012, *33*, (6), 1863-1872.

[148] Shigdar, S.; Lin, J.; Yu, Y.; Pastuovic, M.; Wei, M.; Duan, W., RNA aptamer against a cancer stem cell marker epithelial cell adhesion molecule. *Cancer Sci*, 2011, *102*, (5), 991-998.

[149] Baeuerle, P.A.; Gires, O., EpCAM (CD326) finding its role in cancer. *Br J Cancer*, 2007, *96*, (3), 417-423.

[150] Tang, D.G., Understanding cancer stem cell heterogeneity and plasticity. *Cell Res*, 2012, *22*, (3), 457-472.

[151] Kelly, P.N.; Dakic, A.; Adams, J.M.; Nutt, S.L.; Strasser, A., Tumor growth need not be driven by rare cancer stem cells. *Science*, 2007, *317*, (5836), 337.

[152] Schatton, T.; Murphy, G.F.; Frank, N.Y.; Yamaura, K.; Waaga-Gasser, A.M.; Gasser, M.; Zhan, Q.; Jordan, S.; Duncan, L.M.; Weishaupt, C.; Fuhlbrigge, R.C.; Kupper, T.S.; Sayegh, M.H.; Frank, M.H., Identification of cells initiating human melanomas. *Nature*, 2008, *451*, (7176), 345-349.

[153] Quintana, E.; Shackleton, M.; Sabel, M.S.; Fullen, D.R.; Johnson, T.M.; Morrison, S.J., Efficient tumour formation by single human melanoma cells. *Nature*, 2008, *456*, (7222), 593-598.

[154] Pienta, K.J.; Abate-Shen, C.; Agus, D.B.; Attar, R.M.; Chung, L.W.; Greenberg, N.M.; Hahn, W.C.; Isaacs, J.T.; Navone, N.M.; Peehl, D.M.; Simons, J.W.; Solit, D.B.; Soule, H.R.; VanDyke, T.A.; Weber, M.J.; Wu, L.; Vessella, R.L., The current state of preclinical prostate cancer animal models. *Prostate*, 2008, *68*, (6), 629-639.

[155] Liu, Z.; Turkoz, A.; Jackson, E.N.; Corbo, J.C.; Engelbach, J.A.; Garbow, J.R.; Piwnica-Worms, D.R.; Kopan, R., Notch1 loss of heterozygosity causes vascular tumors and lethal hemorrhage in mice. *J Clin Invest*, 2011, *121*, (2), 800-808.

Received: November 10, 2013 Revised: January 20, 2014 Accepted: March 03, 2014