Cancer Letters 465 (2019) 94–104

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Cancer stem cells in prostate cancer radioresistance

Tsing Tsao[a,b], Julia Beretov[a,b,c], Jie Ni[a,b], Xupeng Bai[a,b], Joseph Bucci[a,b], Peter Graham[a,b], Yong Li[a,b,d,*]

[a] St George and Sutherland Clinical School, Faculty of Medicine, UNSW, Sydney, NSW, 2052, Australia
[b] Cancer Care Centre, St George Hospital, Kogarah, NSW, 2217, Australia
[c] Anatomical Pathology, NSW Health Pathology, St. George Hospital, Kogarah, NSW, 2217, Australia
[d] School of Basic Medical Sciences, Zhengzhou University, Henan, 450001, China

ARTICLE INFO

Keywords:
Cancer stem cells
Radioresistance
Prostate cancer
Radiosensitisation
Anticancer therapy

ABSTRACT

Cancer stem cells (CSCs) in prostate cancer (CaP) are regarded as major contributors to radioresistance due to complex mechanisms including enhanced DNA repair, increased intracellular reactive oxygen species scavenging, activation of anti-apoptotic pathways, microenvironment hypoxia, epithelial-to-mesenchymal transition (EMT) and autophagy. They are also believed to cause tumour recurrence and metastasis due to their unique capability to survive and replicate the heterogeneity of the original tumour. Finding markers of prostate CSCs (PCSC) for identification, prognostication and targeting is key in enhancing therapeutic and clinical outcomes. Markers such as aldehyde dehydrogenase, CD44, integrins and EMT markers have been proved to show great potential in being sensitive and specific to the presence of PCSCs. Novel therapies such as Hedgehog and Wnt pathway inhibitors, angiogenesis inhibitors and metformin show potential in eliminating PCSCs to improve therapeutic outcomes. Here, we review the current state of the literature regarding mechanisms of PCSC radioresistance, promising PCSC markers and novel PCSC-specific therapeutic approaches and their implications in CaP treatment and prognosis.

1. Introduction

Prostate cancer (CaP) is the fifth highest cause of cancer death and second most frequently diagnosed cancer among males worldwide, with an estimated 174,650 new cases and 31,620 deaths in the USA in 2019 [1,2]. CaP death rates have been decreasing due to earlier diagnosis and improved treatment modalities in many developed countries [2]. Currently, radical prostatectomy (RP), external-beam radiation therapy (EBRT) and brachytherapy (BT) are the main primary and adjuvant options for men diagnosed with localised CaP. However, radioresistance in localised CaP is a major therapeutic challenge, with 22–69% of CaP patients experiencing biochemical recurrence (BCR) following radiotherapy (RT) [3]. BCR is a rising prostate-specific antigen (PSA) level following primary curative therapy for CaP. Almost half of patients with BCR progress to clinical recurrence within 15 years, and 1/3 of CaP patients with BCR progress to metastatic castration-resistant prostate cancer (CRPC), eventually leading to cancer death [4].

So far, there is no consensus on the treatment of BCR following both primary RT or RP. Salvage RP following primary RT failure is highly dependent on surgical expertise, and has high potential for morbidity and poor functional outcomes compared to primary RP alone [3]. The current evidence for salvage RT following primary RP is considerably varied, with the estimated 5-year freedom from a second BCR ranging from 37% to 71% [5]. In either case, RT failure in both the primary and salvage setting is difficult to remedy, and positive outcomes apply to only a carefully selected subset of patients [3,5]. Other options include androgen deprivation therapy (ADT), which is usually only effective for the first 2–3 years, after which most patients inevitably progress to incurable metastatic CRPC. Chemotherapy offers a modest survival benefit at best of about 19 months after castration resistance [4]. Given the futility and morbidity of attempting to cure recurrent CaP, there is an urgent need to overcome RT failure in earlier stages when it is still localised, thus preventing disease progression.

This review aims to give an overview of the mechanisms of RT failure associated with the cancer stem cell (CSC) model, discuss the current knowledge of promising prostate cancer stem cell (PCSC) markers in CaP RT, and emphasise the importance of PCSCs as potential therapeutic targets.

* Corresponding author. Level 2, Research and Education Centre, St George Hospital, 4-10 South St, Kogarah, NSW, 2217, Australia.
E-mail address: y.li@unsw.edu.au (Y. Li).

https://doi.org/10.1016/j.canlet.2019.08.020
Received 1 August 2019; Received in revised form 29 August 2019; Accepted 30 August 2019
0304-3835/

Abbreviations

ADT	androgen deprivation therapy
AE	Adverse event
AKT	Protein Kinase B
ALDH1	Aldehyde dehydrogenase 1
BCR	Biochemical recurrence
BMI-1	B-lymphoma Mo-MLV insertion region 1 homolog
BPH	Benign prostatic hyperplasia
BT	Brachytherapy
CAF	Cancer-associated Fibroblast
CaP	Prostate cancer
CD44	Cluster of differentiation 44
CD44v	CD44 variant
CD133	Cluster of differentiation 133
CFLIP	cellular FLICE-like inhibitory protein
c-MYC	cellular-myelocytomatosis
CRPC	Castration-resistant prostate cancer
CSC	Cancer stem cell
CXCR4	C-X-C chemokine receptor type 4
CXCL12	C-X-C ligand type 12
DHH	Desert hedgehog
DTX	Docetaxel
EBRT	External-beam radiation therapy
EMT	epithelial-to-mesenchymal transition
HH	Hedgehog
HIF	Hypoxia inducible factor
HMGB1	high-mobility group box 1
HSP90	heat shock protein 90
IAP	inhibitors of apoptosis family of proteins
IHH	Indian hedgehog
IL6	Interleukin 6
MAB	monoclonal antibody
MCL-1	myeloid leukemia cell differentiation protein Mcl-1
MET	mesenchymal-to-epithelial transition
IMB	Intemumab
MiRNA	microRNA
MnSOD	manganese superoxide dismutase
MTOR	Mammalian target of rapamycin
NF-κB	nuclear factor kappa-light-chain-enhancer of actuated B cells
NOD	Non-obese diabetic
OCT3/4	Octamer binding transcription factor 3/4
OS	Overall survival
PAFR	platelet-activating factor receptor
PARP-1	poly(ADP-ribose) polymerase 1
PCSC	Prostate cancer stem cell
PFS	Progression free survival
PPFS	PSA progression free survival
PSA	Prostate specific antigen
PI3K	phosphatidylinositol 3-kinases
PIN	Progressive intrapepithelial neoplasia
PRD	Prednisone
PTCH	Patched
Re1B	redox-sensitive transcription factor 1B
RT	Radiotherapy
RP	Radical prostatectomy
ROS	Reactive oxygen species
SCID	Severe combined immunodeficiency
SHH	Sonic hedgehog
SMC1A	structural maintenance of chromosome 1A
SMO	Smoothened
SOX2	SRY-related HMG-box gene 2
STAT3	Signal transducer & activator of transcription 3
TGF-β	Transforming Growth Factor β
TMA	Tissue microarray
VEGFR	Vascular Endothelial Growth Factor Receptor

2. The CSC model

The importance of CSCs is widely accepted in the field of cancer research and will be the basis of the ensuing discussion. CSCs are unique cancer cells which display the hallmark characteristics of self-renewal, quiescence and differentiation. They form a small subpopulation amongst the remaining tumour cell bulk and are responsible for tumour initiation, distinguishing CSCs from other end tumour cells [6]. This maintenance of both cell types is possible through symmetric cell division of CSCs, forming two new CSCs, or asymmetric cell division, creating a new CSC and a daughter cell which is more differentiated [7].

To date, there are several hypotheses regarding the origins of CSCs, including mutation of normal stem cells and cancer cells acquiring stem-cell properties through de-differentiation, fusion with progenitor cells and horizontal gene transfer although the evidence remains inconclusive [7].

PCSCs have been found to be innately radio-/chemo-resistant and highly metastatic [8]. RT can reduce the large non-PCSC tumour bulk but not PCSCs as shown in Fig. 1. PCSC survival results in BCR, clinical recurrence, progression to metastatic disease and cancer death [9]. The exact mechanisms underpinning these PCSC attributes are complex and overlapping. Considerable research is still being conducted in this area to uncover potential PCSC-specific therapies.

3. Mechanisms underlying PCSC radioresistance

There are multiple complex mechanisms underlying PCSC radioresistance. It is well-established that RT kills cells directly through DNA single-strand or double-strand breaks, resulting in activation of caspase-dependent and caspase-independent pro-apoptotic molecules or indirectly through excessive reactive oxygen species (ROS) production [10]. PCSC-associated radioresistance is explained by increased DNA repair capabilities including autophagy, decreased intracellular ROS levels through increased scavenging and over-activation of anti-apoptotic signalling pathways, all influenced by the hypoxic CSC niche and epithelial-to-mesenchymal transition (EMT) [11]. In addition, the quiescent status of PCSCs confers a natural resistance to RT [8]. These mechanisms are summarised in Fig. 2.

3.1. Tumour hypoxic microenvironment

It has been reported that CSCs exist in niches within the tumour, supported and maintained through factors secreted by the surrounding stroma. These CSC niches consist of multiple components, including non-CSCs, inflammatory cells, immune cells, vascular endothelial cells and fibroblasts along with extracellular matrix. These various components collaborate through cytokines, chemokine and growth factors to manufacture a hypoxic, inflammatory, immunosuppressive microenvironment facilitating tumour growth, progression and metastasis [6]. A hypoxic PCSC niche promotes radioresistance of PCSCs through impaired ROS production and activation of hypoxia-inducible factor (HIF) signalling [12]. Subunits HIF-1α and HIF-2α regulate genes implicated in cell quiescence, hypoxia tolerance and maintenance of an undifferentiated phenotype, thus promoting a radioresistant CSC population in solid tumours [13]. The influence of the hypoxic microenvironment on PCSCs is currently a growing area of research.

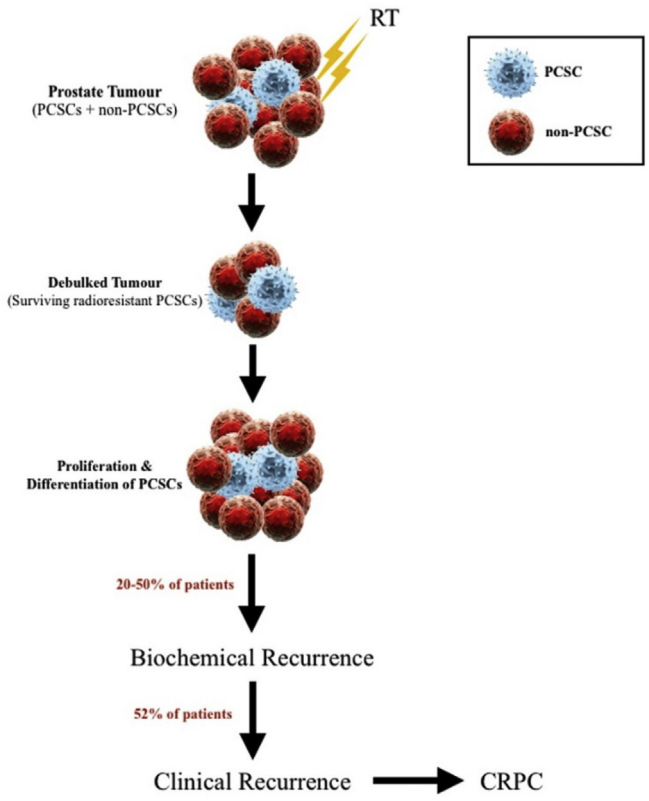

Fig. 1. Hypothetical model of PCSCs and tumour recurrence following RT. Notably, RT can debulk tumours due to the large proportion of non-PCSCs to PCSCs. However, the ability of PCSCs to survive, differentiate and initiate tumours inevitably results in tumour recurrence and CRPC. Abbreviation: CRPC, castration-resistant prostate cancer; PCSC, prostate cancer stem cell; RT, radiotherapy.

3.2. Enhanced DNA repair

An integral part of the cell DNA damage response is the activation of cell cycle checkpoints, which temporarily induce cell cycle arrest, allowing correction of DNA defects [14]. Cojoc et al. found that radioresistant PCSCs had increased baseline phosphorylation of Chk2 which enhances arrest of cell proliferation via ATM-Chk2 pathways, and increased phosphorylation of AKT which enhances DNA repair through non-homologous end joining (NHEJ) via activation of PI3K signalling pathway [15]. Inhibition of the PI3K/AKT/mTOR pathway using BEZ235, a dual PI3K and mTOR inhibitor, could radiosensitise PCSCs through inhibition of NHEJ and homologous recombination (HR) repair pathways [16]. Yadav et al. also found that SMC1A, the cohesin protein involved in structural maintenance of chromosome-1, is a phosphorylation target of ATM kinase in response to ionising radiation, resulting in S-phase delay for DNA reparation. Consequently, suppression of SMC1A resulted in radiosensitisation of CaP cells through alteration of the stem-like phenotype among CaP cells and attenuating the NHEJ DNA repair pathway [10]. These findings highlight a crucial role, and therapeutic target potential, of DNA repair-associated pathways in PCSCs in CaP radioresistance.

3.3. Activation of anti-apoptotic pathways

Another cellular response to significant DNA damage is to activate apoptotic pathways, the evasion of which also confers radioresistance [17]. The Wnt/β-catenin pathway which regulates livin, a preventer of cell apoptosis, has been reported to be overactivated in PCSCs. This results in evasion of apoptosis and allows PCSCs to turn on DNA repair mechanisms and survive [18]. Inhibition of cellular FLICE-like inhibitory protein (cFLIP) and MCL-1 has been found to sensitise PCSCs to anoikis, a form of apoptosis induced through detachment from epithelium, implicated in EMT [19]. Jia et al. also found that SRY-related HMG-box gene 2 (SOX2) over-expression in PCSCs increased resistance against apoptosis by delaying the cleavage of Caspase-3 and reducing store-operated $Ca2^{+}$ entry. Subsequent SOX2 knockdown increased the sensitivity of PCSCs to apoptosis, suggesting that SOX2 overexpression confers radioresistance [20]. The inhibitors of apoptosis family of proteins (IAP) also have a role to play, whereby inhibition of IAP by SH-130 resulted in significantly enhanced radiation-induced caspase activation and apoptosis in CaP cells [17]. The data shows that radioresistance of PCSCs is highly associated with activation of anti-apoptotic pathways.

3.4. Increased intracellular ROS scavenging

Irradiation causes excessive ROS production which leads to cell death by damaging intracellular components. Radioresistant PCSCs were found to have much lower baseline levels of intracellular ROS, suggesting increased ROS scavenging as a mechanism of radioresistance [15]. This is supported by studies showing CSCs having enhanced expression of ROS scavengers [15]. SMC1A suppression was also found to increase intracellular levels of ROS and lower glutathione levels in CaP cells, indicating the role of SMC1A in enhancing intracellular ROS scavenging [10].

3.5. Autophagy

Autophagy is a naturally occurring process allowing cells to survive stress through recycling of damaged cellular components. Conversely, prolonged induction of autophagy can result in cell death due to the excessive degradation of key intracellular components [21]. Thus, autophagy seems to perform a dual, paradoxical role by suppressing tumour growth in early stages through removal of damaged proteins, but promoting tumour growth and survival in later stages under nutrient deprivation and hypoxia [22]. The role of autophagy in being either pro-survival or pro-death for CSCs seems to differ according to cancer type and is unclear in CaP [23]. Paglin et al. and Chang et al. found that inhibition of autophagy radiosensitised PCSCs [24,25], while Yao et al. found that there was increased CaP cell viability and decreased cell death with inhibition of autophagy [26]. Therefore, more focused research is required to ascertain the role and mechanism of autophagy in PCSC radioresistance.

3.6. EMT

EMT involves an exchange of epithelial characteristics, such as non-motility, for mesenchymal traits, such as motility. During EMT, there is down-regulation of cell-cell adhesion and loss of polarity and interaction with the extracellular matrix [18]. Thus, EMT is critical in tumour invasion, metastasis, recurrence, and is linked to radioresistance and chemoresistance in CSC populations [25]. EMT is closely associated with CRPC, and CaP cells with more mesenchymal markers such as Snail, Vimentin, SOX2 and N-cadherin, exhibit a greater invasive phenotype *in vitro*, more aggressive behaviour and radioresistance [16,25]. Although the association between EMT and radioresistance has been shown, the exact underlying mechanism remains poorly understood. Theys et al. found that reduced expression of E-cadherin, a result of EMT, conferred radioresistance in breast cancer cells. Conversely an increased expression of E-cadherin resulted in radiosensitisation [27]. A new perspective on EMT is that it confers tumour radioresistance through promoting acquisition of the CSC phenotype in non-CSCs, involving TGF-β, Wnt, Hedgehog (HH), β-catenin, Notch, Nanog and STAT3 pathways [11]. Aberrant activation of the HH signalling pathway, involved in tissue patterning during embryogenesis and tissue homeostasis in adults, has been implicated specifically in CaP EMT

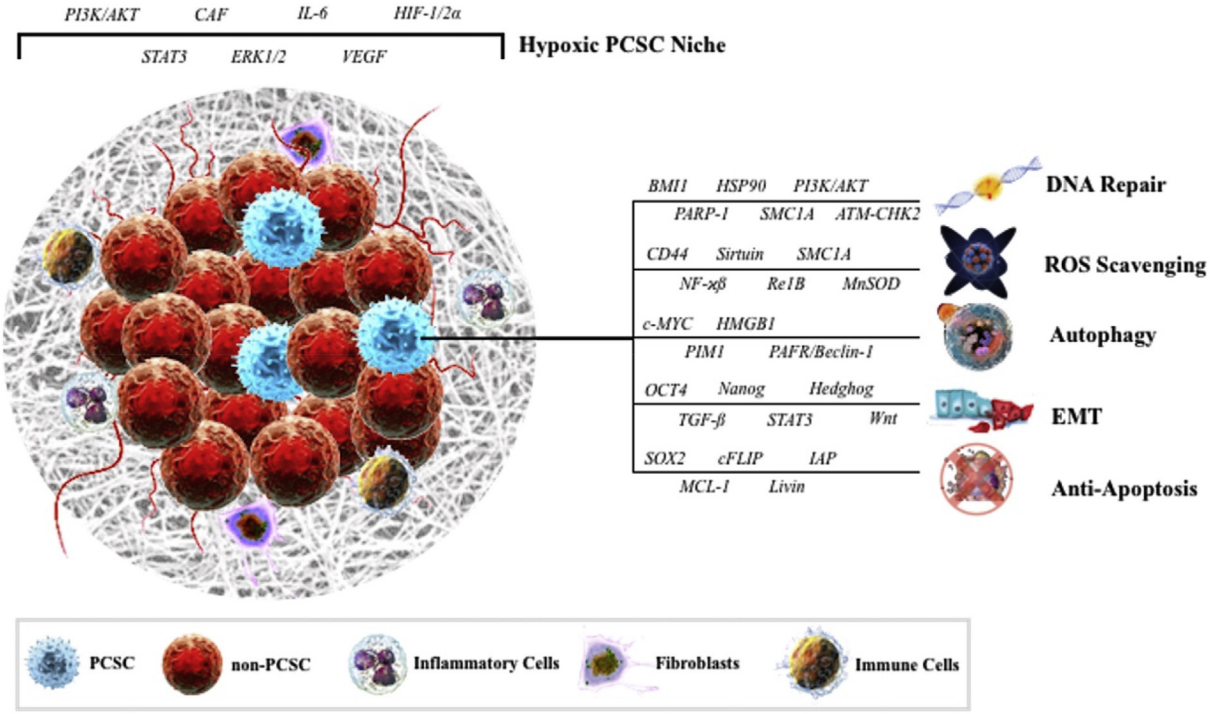

Fig. 2. Overview of the mechanisms of radioresistance in PCSCs. Under the influence of the hypoxic tumour microenvironment, CSC phenotypes can be induced, resulting in increased expression of CSC markers, enhanced DNA repair, increased intracellular ROS scavenging, activation of anti-apoptotic pathways, autophagy and EMT. A complex interplay of these mechanisms confers radioresistance and results in tumour recurrence after RT. Abbreviation: PCSC, prostate cancer stem cell; HIF, hypoxia inducible factor; CAF, cancer-associated fibroblast; IL6, interleukin 6; miRNA, microRNA; VEGFR, vascular endothelial growth factor receptor; PI3K, phosphatidylinositol 3-kinases; AKT, protein kinase B; BMI-1, B-lymphoma Mo-MLV insertion region 1 homolog; cFLIP, cellular FLICE-like inhibitory protein; SOX2, SRY-related HMG-box gene 2; MCL-1. myeloid leukemia cell differentiation protein Mcl-1; IAP, inhibitors of apoptosis family of proteins; TGF-β, transforming growth factor β; STAT3, signal transducer & activator of transcription 3; c-MYC, cellular-myelocytomatosis; HMGB1, high-mobility group box 1; PAFR, platelet-activating factor receptor; SMC1A, structural maintenance of chromosome 1A; HSP90, heat shock protein 90; Re1B, redox-sensitive transcription factor 1B; MnSOD, manganese superoxide dismutase; NF-κB, nuclear factor kappa-light-chain-enhancer of actuated B cells; PARP-1: poly(ADP-ribose) polymerase 1.

[28]. More detailed research on the exact mechanisms linking EMT to PCSCs and CaP radioresistance needs to be conducted for future therapeutic benefit.

4. Promising PCSC markers

The CSC model mandates that specific targeting of PCSCs will lead to greater therapeutic benefit than just conventional tumour bulk reduction [9]. This would require PCSC-specific inhibition in addition to conventional therapies.

As briefly illustrated above, the mechanisms and pathways conferring radioresistance to PCSCs are a complex interplay of overlapping chemical signals that research has only begun to unravel. There is a need to find central, upstream PCSC markers that aid in the identification of PCSCs for pre-clinical studies and prognostication, and that confer radioresistance for therapeutic targeting and radiosensitisation. This section focuses on the most prominent PCSC markers in current research and the state of the literature in both clinical prognostication and therapeutic intervention via PCSC targeting and/or radiosensitisation. The studies of these PCSC markers are summarised in Table 1.

4.1. Aldehyde dehydrogenase (ALDH)

There are 19 isoforms of ALDH in the human genome that play important roles in detoxification by oxidising intracellular aldehydes to carboxylic acids. ALDH1A1 catalyses the oxidation of retinal to retinoic acid, a signalling molecule in developmental cellular differentiation and stem cell self-preservation [29]. Li et al. found that ALDH1A1$^+$ CaP cells showed significantly increased clonogenicity and heterogenous tumour formation *in vitro* and *in vivo* compared to ALDH1A1$^-$ CaP cells, suggesting a correlation between PCSCs and ALDH1A1 activity [29]. Le Magnen et al. also found that ALDH1A1 was the sole isoform whose expression correlated with ALDH activity in clinical CaP specimens [30]. This finding strongly suggests that PCSCs are associated with ALDH1A1$^+$ cells. Cojoc et al. demonstrated that high ALDH activity is associated with radioresistance, and that expression of ALDH1A1 is directly regulated by the Wnt/β-catenin signalling pathway [15]. Consequently, knockdown of β-catenin expression led to inhibition of ALDH activity and radiosensitisation of CaP cells [15,18]. Cojoc et al. also found ALDH$^+$ cells to have increased intracellular ROS scavenging, more efficient DNA repair mechanisms and high levels of Wnt/TGF-β signalling which are potent inducers of EMT - all established mechanisms of radioresistance [15].

Kalantari et al. found that overexpression of ALDH1A1 in CaP cells from clinical specimens was significantly correlated with higher Gleason score, higher PSA, advanced tumour stage and regional lymph node metastases, consistent with the findings of Li et al. and Huwait et al. [29,31,32]. This data suggests that ALDH1A1 has the potential to not only corroborate existing risk factors in CaP, but also add value in predicting a patient's response to RT, something sorely missing in the current CaP risk assessment system [15].

Although ALDHs seem to be effective for identifying PCSCs and prognostication, its utility as a therapeutic target remains doubtful. Many pre-clinical studies do not identify the isoform and greater specificity is needed to home in on the prime PCSC target [33]. At the same time, there is evidence of functional overlap between ALDH isoforms, indicating that targeting a specific isoform may have a minimal therapeutic effect [34]. Kimble-Hill et al. have developed small molecule inhibitors from Indole-2,3-diones with selectivity toward ALDH1A1,

Table 1
Summary of available studies on PCSC and EMT markers in CaP radioresistance.

Marker	Investigation Model			Clinical Significance	Reference
	In Vitro	*In Vivo*	Clinical Sample/Trial		
ALDH	PC-3, LNCaP	NOD/SCID	CaP (64), normal (18)	Stemness	[29]
			CaP (198), PIN (38), BPH (54)	Prognostication	[30]
	PC-3, DU145, LNCaP	NMRI		Radioresistance, Stemness	[15]
			CaP (105), PIN (21), BPH (31)	Prognostication	[31]
			CaP (32), PIN (17), BPH (35)	Prognostication	[32]
CD44	PC-3, PC-3M, DU145, LNCaP-C4-2B, LNCaP, LNCaP-LN3, DuCaP, RWPE-1		CaP (10), BPH (10)	Radioresistance, Stemness	[18]
	PC-3, DU145, LNCaP, PPC-1	NOD/SCID		Radioresistance, Stemness	[36]
	LNCaP, DU145	NOD/SCID		Prognostication, Stemness	[37]
	PC-3, PC-3M-luc, LNCaP			Radioresistance	[38]
	DU145, PC-3M	BALB/c		Radioresistance	[39]
	PC-3M	NOD/SCID		Radioresistance	[40]
			CaP (52)	Prognostication	[41]
			CaP (94)	Prognostication	[42]
			CaP (92)	Prognostication	[43]
			CaP (43), BPH (39)	Prognostication	[44]
			CaP (73), PIN (19), BPH (20)	Prognostication	[45]
	DU145, LAPC4, PPC-1, HPCa (*Ex-vivo* culture)	NOD/SCID		Therapeutics (microRNA-34a)	[46]
Integrins	PC-3, LNCaP			Radioresistance	[50]
	Ex-vivo culture			Stemness	[70]
	PC-3M-Pro4/luc$^+$	BALB/c		Therapeutics (GLPG0187)	[51]
	PC-3GFP	SCID		Therapeutics (GLPG0187)	[52]
	PC-3	BALB/c		Therapeutics (S247)	[53]
			Phase 2, mCRPC IMB (66) Placebo (65)	Therapeutics (IMB/Placebo + DTX + PRD)	[54]
			Phase 1, mCRPC (10)	Therapeutics (IMB + DTX + PRD)	[55]
			Phase 2, non-mCRPC (13)	Therapeutics (Cilengitide)	[56]
			Phase 1, mCRPC (21)	Therapeutics (MK-0429)	[57]
EMT Markers	PC-3, LNCaP, DU145, TSU-PrL, IA8	Nu/nu		Stemness	[60]
			CaP (35)	Prognostication	[61]
	PC-3, LNCaP, DU145, RWPE-1			Radioresistance, Stemness, Therapeutics (BEZ235)	[16]
			CaP (197)	Prognostication	[62]
	PC-3, DU145, OPCT-1, OPCT-2			Stemness	[63]
	PC-3, LNCaP, DU145	NOD/SCID		Stemness	[64]
	HMLE			Stemness	[65]
	PC-3			Stemness	[66]
	PC-3, LNCaP, MDA-PCa-2b	SCID		Therapeutics (mAb)	[67]
	PC-3, DU145		CaP (97)	Radioresistance, Stemness	[10]

Notes. ***In vitro*:** Cell line studies; ***In vivo*:** Mouse models. Abbreviaton: **CaP:** Prostate cancer; **PIN**: Progressive intraepithelial neoplasia; **BPH**: Benign prostatic hyperplasia; **mCRPC**: metastatic castration resistant prostate cancer; **DTX**: Docetaxel; **PRD**: Prednisone; **IMB**: Intemumab; **NOD:** non-obese diabetic; **SCID:** Severe combined immunodeficiency; **EMT Markers:** closely-related to PCSCs; **mAB:** monoclonal antibody.

although this has not yet been tested on CaP cell lines and is far from clinical trials [35].

In summary, high ALDH expression, particularly ALDH1A1, has been identified as a functional PCSC marker highly correlated with radioresistance in CaP with great potential as a predictor of radioresistance in CaP. More research needs to be done to establish its utility as a therapeutic target for PCSCs.

4.2. Cluster of differentiation 44 (CD44)

CD44 is a transmembrane glycoprotein receptor functioning as a cellular adhesion molecule for hyaluronic acid, a key part of the extracellular matrix [18]. CD44$^+$ CaP cells were associated with greater clonogenicity, tumorigenicity, metastatic behaviour and radioresistance than CD44$^-$ CaP cells [36–39]. CD44 variants are also highly expressed in many carcinomas, and are closely related to tumour growth, metastasis and therapeutic resistance. CD44 variant 6 (CD44v6) in particular was found to be highly expressed in metastatic CaP cells compared to normal prostate cells and closely associated with CaP proliferation, metastasis, EMT induction and chemo/radioresistance via the PI3K/AKT/mTOR pathway, with subsequent knockdown increasing radiosensitivity both *in vitro* and *in vivo* [18,40]. Ma et al. found that CD44 exhibits its radioprotective effects in conjunction with ERBB2 by mediating phosphorylation of p38 which enhanced the HR DNA repair pathway [39]. The evidence for CD44 as a CaP prognostic indicator is limited, with human CaP specimen studies yielding mixed results. Some studies have found a negative correlation between CD44 expression and Gleason score [41–43]. Others have found no

correlation between CD44 and other CaP prognostic indicators [44,45]. At this stage, larger studies yielding consistent results are needed for CD44 to discover its true clinical prognostic value.

Pre-clinical studies using CD44 as a therapeutic target in CaP are sparse, with Liu et al. finding that microRNA-34a was able to inhibit $CD44^+$ PCSCs and CaP metastasis through repression of CD44 [46]. In other cancers, there has been more research in CD44 as a therapeutic target. Phase I trials of humanised antibody against CD44v6 coupled with a cytotoxic drug mertansine or radiolabelling failed due to toxic side effects and poor selectivity respectively [47]. Other pre-clinical studies targeting CD44 and its binding to hyaluronan are being tested in other cancers [47].

In summary, CD44 and CD44v6 are well-established as PCSC markers which are highly correlated with radioresistance and metastasis, with great potential for PCSC identification and prediction of CaP radioresistance. Their utility as a prognostic indicator in CaP and therapeutic target for PCSCs is yet to be established.

4.3. Integrins

The role of the large family of integrins involves cellular adhesion and the formation of complexes with ligands within the extracellular matrix. They are cell surface receptors extending from the cytoplasm into the extracellular matrix [48]. Given their role in cellular adhesion, it is not surprising that down-regulation of many integrins, including α2 and α6, correlates with CaP metastasis, invasiveness and prognosis [48]. The integrins and integrin-complexes mentioned below have been linked to CaP radioresistance and PCSCs as well.

The most well-studied integrin in CaP is αvß3 integrin due to its well-established role in bone metastases by allowing cell adhesion to and migration on vitronectin, fibronectin and osteopontin [49]. High αvβ3 integrin expression has also been linked to CaP tumour

Table 2
Advances in therapeutic targeting of PCSCs.

Drug	Study Type	Sample Source	Outcome	Reference
GANT61 (HH inhibitor)	Pre-clinical	*In vitro:* PC-3, DU145, 22Rv1 *In vivo:* NMRI Nu/Nu	↑ *Radiosensitivity*	[79]
	Pre-clinical	*In vivo:* BALB/c nu/nu	↓ tumour growth	[72]
Erismodegib (HH inhibitor)	Pre-clinical	*In vitro:* Ex-vivo culture *In vivo: NOD/SCID*	*In vitro*: ↓ EMT *In vivo:* ↓ tumour growth	[73]
	Phase I Clinical	High-risk local CaP (14)	Well-tolerated; > 60x HH inhibition	[74]
Vismodegib (HH inhibitor)	Phase I Clinical	Advanced/metastatic solid tumours; CaP (2), Others (66)	Well-tolerated; Pharmocdynamic GLI1 inhibition; No clinical effect	[75]
	Phase I Clinical	mCRPC (69)	Well-tolerated; Pharmocdynamic GLI1 inhibition; No clinical effect	[76]
Itraconazole (HH inhibitor)	Phase II Clinical	BCR patients (21)	Modest PSA ↓; Grade 1 AE risk	[77]
	Phase II Clinical	mCRPC (46)	High-dose (600 mg/day) has modest effect on PPFS and PFS; Grade 3 AEs	[78]
Foxy-5 (Wnt5a mimic)	Pre-clinical	*In vitro:* PC-3, LNCaP-C42B *In vivo:* NOD/SCID *TMA:* CaP (397), BPH (41)	*In vitro:* rWnt5a ↑apoptosis *In vivo:* ↑Wnt5a ~ ↓tumour growth *TMA:* ↑ Wnt5a ~ ↑ CaP survival	[84]
	Phase I Clinical	N/A	N/A	[82]
DKK1 (Wnt inhibitor)	Pre-clinical	*In vitro:* PC-3, LNCaP, DU145, 22RV1, VCAP	↓ sphere formation	[81]
sFRP2 (Wnt inhibitor)	Pre-clinical	*In vitro:* PC-3, LNCaP, DU145, 22RV1, VCAP	↓ sphere formation	[81]
Tasquinimod (Angiogenesis Inhibitor)	Phase III Clinical	mCRPC (1245)	↑ relative PFS, no ↑ in OS; 42.8% ≥ Grade 3 AE	[89]
	Phase II Clinical	mCRPC (201)	↑ 6 mth PFS/median PFS ↑ Grade 3-4 AE	[88]
	Phase I Clinical	CRPC (21)	Well-tolerated; Possible clinical effect	[87]
	Pre-clinical	*In vitro:* LNCaP, LAPC4, CWR22RV1 *In vivo:* CWR22R–H in nu/nu	*In vitro:* ↑ Radiosensitivity *In vivo:* ↑ Radiosensitivity	[92]
Sunitinib (Angiogenesis Inhibitor)	Phase I Clinical	Localised CaP (17) Combination with RT, ADT	Combination without ADT was well-tolerated; Grade 2-3 AEs	[91]
	Phase III Clinical	mCRPC (873) Combination with PRD vs placebo	↑ PFS, no ↑ in OS ↑Grade 3-4 AE	[90]
	Pre-clinical	*In vitro:* PC-3, LNCaP, DU145 *In vivo:* Nu/nu	*In vitro:* ↑ Radiosensitivity *In vivo:* ↓ tumour volume ± RT	[12]
	Pre-clinical	*In vitro:* PC-3, LNCaP, DU145 *In vivo:* Nu/nu	*In vitro:* ↑ Radiosensitivity *In vivo:* No tumour growth delay	[93]
Metformin	Phase II Clinical	non-diabetic mCRPC (95) Combination with DTX	No clinical benefit (full results yet to be published)	[100]
	Epidemiological	Meta-Analysis of Epidemiological Observation (21)	Association with ↓ CaP risk, BCR but not all-cause mortality	[97]
	Pre-clinical	*In vitro:* LNCaP *In vivo:* SCID/CD-1 *RC:* local CaP (504)	*In vitro:* No radiosensitisation *In vivo:* ↑ tumour growth delay/radiosensitivity due to hypoxia *RC:* ↓ BCR post-RT	[95]
	Pre-clinical	*In vitro:* PC-3, LNCaP, DU145, Vcap *TMA:* CaP (84)	*In vitro:* ↓ EMT; ↑miR30a + ↓SOX4 *TMA:* miR30a & SOX4 expression inversely related	[96]

Notes. ***In vitro:*** Cell line studies; ***In vivo:*** Mouse models. Abbreviation: **CaP:** prostate cancer; **BPH:** benign prostatic hyperplasia; **mCRPC:** metastatic castration-resistant prostate cancer; **HH:** Hedgehog; **AE**: Adverse Event; **PSA:** prostate-specific antigen**;** **PFS**: Progression Free Survival; **PPFS:** PSA Progression Free Survival; **OS**: Overall Survival; **DTX**: Docetaxel; **PRD**: Prednisone; **TMA:** tissue microarray; **NOD:** non-obese diabetic; **SCID:** severe combined immunodeficiency; **BCR:** biochemical recurrence; **RT:** radiotherapy; **ADT:** androgen-deprivation therapy; **EMT:** epithelial-to-mesenchymal transition; **miR30a:** microRNA-30a.

invasiveness and metastasis *in vitro* and radioresistance through inhibition of radiation-induced down-regulation of Survivin, an IAP [50]. Down-regulation of β3 integrin through cRGD, an αvβ3-selective cyclic peptide antagonist, resulted in significant radiosensitisation of CaP cells *in vitro* [50].

Pre-clinical data targeting integrins has been promising. Van der Horst et al. and Reeves et al. showed that the αvß3 integrin inhibitor GLPG0187 inhibited CaP metastasis and EMT *in vivo,* and reduced the αv-integrin$^+$ PCSC population *in vitro* [51,52]. Abdollahi et al. showed that S247, another αvß3 integrin inhibitor, synergistically enhanced RT in CaP both *in vitro* and *in vivo* by inhibiting radiation-induced protective angiogenic effects [53]. However, clinical data has been underwhelming. Four Phase I and II clinical trials involving integrin inhibitors Intetumumab (monoclonal antibody against αv integrins) in combination with docetaxel, Cilengitide (αvß3 and αvß5 selective antagonist) monotherapy and MK-0249 (αvß3 inhibitor) have concluded good tolerability, but without any significant clinical activity in PSA response or efficacy worse than placebo in progression-free survival [54–57].

However, there still remains potential for the integrins as prognostic markers in CaP. Studies have shown that αvß3 integrin has utility as a binding target for radiolabelled peptides for non-invasive imaging assessment of tumour angiogenesis, presence of metastases, risk of future metastases and therapeutic response to angiogenesis inhibitors [58,59]. While the clinical trials for therapeutic integrin inhibition have yielded disappointing results, it remains viable to explore the targeting of integrins for radiosensitisation and prognostication in CaP.

4.4. EMT markers

The relationship between EMT and PCSCs is intricate and mysterious. There are many common molecular mechanisms in the induction of EMT and generation of CSCs [60]. This is further supported by established links between EMT and measures normally attributed to the presence of PCSCs, such as radioresistance, castration resistance, BCR, metastasis formation, poor prognosis and their rarity in solid tumours. Stark et al. found that a key EMT marker signature (decreased E-cadherin with increased N-cadherin and Vimentin) was significantly evident in post-RT specimens compared with pre-RT specimens in a cohort with diagnosed CaP, BCR and tissue-diagnosed local CaP recurrence [61]. Chang et al. found that radioresistant CaP cells showed down-regulation of E-cadherin (epithelial marker) and up-regulation of N-cadherin, Vimentin, OCT3/4, SOX2 and alpha smooth muscle actin (α-SMA) (mesenchymal markers) over control CaP cells [20]. Induction of EMT and the CSC phenotype were found to be associated with the PI3K/AKT/mTOR pathway activation and subsequent inhibition resulted in radiosensitisation [20]. The EMT-associated markers Twist and Vimentin were found by Behnsawy et al. to be independently predictive of BCR from immunohistochemical staining of 197 RP specimens [62].

While E-cadherin, N-cadherin, Vimentin and the associated transcriptional factors Nanog, OCT4 and SOX2 have been well-linked to EMT, their utility as markers for PCSCs remains limited until the exact nature of the relationship between EMT and PCSCs can be unambiguously answered. Multiple studies have found that inducing EMT in PCSCs produces stem-like characteristics such as self-renewal, clonogenicity, tumorigenic potential and sphere-forming capability [60,63–65]. However, Celià-Terrassa et al. found contrary evidence that inducing EMT inhibited stem-like attributes in PCSCs [66]. The combined understanding is that CaP cells undergoing EMT and PCSCs comprise two separate non-linear spectrums with a significant overlap, with factors such as the method of EMT induction, its transient nature and a concomitant mesenchymal-to-epithelial transition (MET) process influencing where cells end up on the spectrum [66]. Simply put, many but not all CaP cells undergoing EMT are PCSCs, and many but not all PCSCs undergo EMT.

Nonetheless, CaP cells undergoing EMT can still be targeted for therapeutic benefit - eliminating either more invasive tumour cells or PCSCs. Pre-clinical studies have shown that inhibiting EMT has therapeutic potential. Tanaka et al. used a monoclonal antibody to target N-cadherin, causing inhibition of CaP cell growth, metastasis and castration resistance *in vivo* [67]. Chang et al. inhibited expression of EMT and PCSC markers such as CD44, CD44v6 and ALDH1 through BEZ235, which resulted in radiosensitisation and induced apoptosis *in vitro* [16]. Yadav et al. found that suppression of SMC1A inhibited EMT and stem-like properties in CaP cells and resulted in radiosensitisation [10]. While it cannot yet be concluded whether EMT markers can serve as a proxy for PCSC markers, its role in CaP disease progression and therapeutic resistance is inarguable and it is a ripe area of research.

In summary, many markers with links to PCSCs have been discovered in research. However, the heterogeneity and plasticity of PCSCs both *in vitro* and *in vivo* indubitably casts doubt on the sensitivity and specificity of these markers. Although there are other markers such as CD133 and CXCR4/CXCL12 that hold much potential in the identification of PCSCs, to the best of our knowledge, their links to radioresistance have yet to be established and are thus outside the scope of this review [68,69].

5. Novel therapeutic approaches to PCSCs and CaP radioresistance

While challenges exist in utilising the abovementioned PCSC markers as therapeutic targets, their usefulness in identifying and evaluating PCSC populations has paved the way for evaluating the PCSC-targeting ability of other therapies. This section outlines current novel therapeutic approaches in various stages aiming to target PCSCs to overcome radioresistance and improve outcomes, summarised in Table 2.

5.1. HH pathway inhibitors

The HH signalling pathway mediates interactions between epithelial and mesenchymal cells during embryogenesis and maintains certain adult stem cell populations adults [33]. Simply, the canonical HH pathway starts with the peptide ligands Sonic Hedgehog (SHH), Indian Hedgehog (IHH) and Desert Hedgehog (DHH) which bind to the transmembrane receptor Patched (PTCH). This causes disinhibition of the protein Smoothened (SMO) which then results in alterations of post-translational processing of the Gli transcription factors which regulate cell proliferation, differentiation and angiogenesis [28,33].

This pathway has received much attention in the study of PCSCs due to its up-regulation in CaP and role in EMT, therapeutic resistance and metastasis [28,71]. Pre-clinical data regarding HH inhibitors and PCSCs is promising. Lauth et al. also found that the Gli small molecule inhibitor GANT61 suppressed CaP tumour growth *in vivo* [72]. Nanta et al. showed that Erismodegib, an SMO inhibitor, induced apoptosis, inhibited cell viability and EMT in PCSC spheroids *in vitro* and inhibited tumour growth *in vivo* through regulation of BMI-1 and microRNA-128 [73]. Erismodegib also passed a Phase I study showing good tolerability and bioavailability, albeit without obvious clinical effect [74]. The SMO inhibitor vismodegib has been approved by the Food and Drug Administration (FDA) for treatment of advanced basal cell carcinoma. However, LoRusso et al. and Maughan et al. found through Phase I studies that vismodegib did not have a significant clinical effect on PSA or the tumour, rendering Phase II studies unjustified [75,76]. High-dose Itraconazole, a common anti-fungal, has since received attention due to its serendipitous SMO inhibition and two Phase II trials have concluded modest effects in PSA response and PSA progression-free survival in patients with BCR and metastatic CRPC respectively [77,78].

Interestingly, pre-clinical data also suggests repurposing HH inhibitors as chemo/radio-sensitising agents. Gonnissen et al. found that GANT61 sensitised CaP to RT via induction of apoptosis and cell cycle inhibition *in vitro* and *in vivo* [79]. More pre-clinical and clinical studies

need to be done in this area to fully explore the utility of HH inhibitors as chemo/radiosensitising agents.

In summary, inhibiting the HH pathway is promising for targeting PCSCs. Emerging pre-clinical data suggests utility of HH inhibitors as chemo/radiosensitising agents as well. So far, clinical data is most promising regarding Itraconazole for metastatic CRPC.

5.2. Wnt pathway inhibitors

The canonical ß-catenin-dependent pathway regulates expression of Wnt genes related to EMT, CSC maintenance and proliferation while the non-canonical β-catenin independent pathway activates transcription factors promoting cell growth, survival, angiogenesis and invasion [80]. These effects of the Wnt pathway are implicated in PCSCs self-renewal and maintenance, risk of metastasis, therapeutic resistance and disease progression [80,81].

Expression of prominent PCSC markers of radioresistance such as ALDH1A1 and CD44v6 mentioned earlier are also directly regulated by the canonical pathway, and inhibition of the Wnt/β-catenin pathway reduced ALDH1A1$^+$ and CD44v6$^+$ PCSC numbers and resulted in radiosensitisation [15,18]. Non-canonical Wnt signalling has also been linked to PI3K/AKT pathway activation in CSCs, which has also been implicated in PCSCs [16]. Thus, targeting the Wnt pathway is an area of great current research.

A Wnt5a mimicking peptide known as Foxy-5 is currently under Phase I trials in CaP with varying levels of Wnt5a expression [82]. Wnt5a is a non-canonical Wnt signalling pathway activator that has been associated with reduced metastasis and tumour suppression in CaP [83,84]. Pre-clinical studies with small-molecule Wnt inhibitors DKK1 and sFRP2 have also shown significant inhibition of PCSC sphere formation [81]. Targeting upstream of the Wnt pathway also seems to have therapeutic potential. Chen et al. reported blocking glucocorticoid receptor, which activates of the Wnt/β-catenin pathway via SGK1, prevented induction of the PCSC phenotype by radiation [85]. In other solid tumours and haematological malignancies, inhibition of the Wnt pathway is also being studied with Porcupine inhibitors, tankyrase inhibitors, Wnt antibodies and β-catenin inhibitors in Phase I studies [80].

In summary, more research on Wnt inhibitors in the context of CaP is needed. Due to the centrality of the Wnt pathway to key PCSC features such as EMT, radioresistance and self-renewal, there is much potential in watching this space.

5.3. Angiogenesis inhibitors

Hypoxic conditions in the CaP microenvironment result in a dysregulation of pro- and anti-angiogenic factors, resulting in 'leaky' tumour vasculature which delivers insufficient oxygen to the tumour, thus maintaining hypoxia and prolonging this angiogenic dysregulation [86]. Angiogenesis inhibitors delay tumour progression and induce vascular normalisation, reducing tumour hypoxia and resulting in radiosensitisation [86]. Tasquinimod is an oral quinoline-3-carboxamide derivative with anti-angiogenic and immunomodulatory effects that has garnered much attention in the treatment of CRPC on the back of promising Phase I and Phase II trials [87,88]. However, a Phase III trial failed to demonstrate overall survival benefit of tasquinimod monotherapy in metastatic CRPC [89]. Sunitinib, a receptor tyrosine kinase inhibitor with anti-angiogenic effect, follows a similar story with an unsuccessful Phase III trial failing to demonstrate overall survival benefit in metastatic CRPC [90,91].

Nonetheless, pre-clinical data suggests potential in repurposing tasquinimod and sunitinib as radiosensitising agents in CaP instead [12,92,93]. Dalrymple et al. found that tasquinimod prevents the radiation-induced angiogenic rebound, thus enhancing RT both *in vitro* and *in vivo* [92]. Brooks et al. also found that Sunitinib caused radiosensitisation on CaP cells *in vitro* without increasing tumour growth delay *in vivo* post-RT [93]. Supporting these findings, Diaz et al. found that Sunitinib specifically radiosensitised PCSCs *in vitro* and attenuated the HIF-1α pathway, implicated in PCSC maintenance and radioresistance [12].

While tasquinimod and sunitinib have hit a roadblock in the treatment of CRPC, they retain potential to be repurposed as radiosensitising agents which can aid in PCSC elimination via RT earlier in the disease. More pre-clinical and clinical research need to be conducted to further elucidate the potential of tasquinimod and sunitinib with RT.

5.4. Metformin

Metformin is a familiar, well-tolerated biguanide in the first-line treatment of type II diabetes. It has recently garnered attention due to its potential in targeting PCSCs [94]. Metformin inhibits mitochondrial oxidative phosphorylation which CSCs are more reliant on, compared to non-CSCs which primarily use glycolysis to support rapid proliferation [94,95]. This results in a preferential build-up of ROS in CSCs, ultimately leading to apoptosis [95]. Another effect of metformin is to decrease CaP cellular oxygen consumption, thereby reducing tumour hypoxia and causing radiosensitisation, demonstrated *in vitro* and *in vivo* [95]. Zhang et al. also found that metformin inhibits EMT in CaP through the action of microRNA-30a modulating SOX4 expression [96].

While the mechanism of metformin's effect on PCSCs is still being studied, its overall benefit is demonstrated in an association with a significant reduction in CaP risk and BCR [97]. So far, the main TAXOMET phase II trial comparing metformin in combination with docetaxel and prednisone against placebo in patients with metastatic CRPC has yielded no clinical benefit of the addition of metformin. Though disappointing, metformin's potential in targeting and radiosensitising PCSCs earlier in the disease has yet to be studied clinically, although pre-clinical and epidemiological studies so far give promise to a repurposing of this well-known drug.

The sentiment thus far is that direct targeting of PCSCs has been either tolerable with modest clinical effect at best, or intolerable. However, most clinical trials have been focused on treating terminal metastatic CRPC patients. Perhaps the utility of PCSC targeting lies in earlier radiosensitisation, as much pre-clinical data suggests. It would be of great value to focus research efforts in this space.

6. Conclusion

The CSC model has established new directions in the paradigms of prognostication and therapeutic intervention in CaP radioresistance. The complex yet crucial role of PCSCs has led to a search to identify the PCSC population, quantify the PCSC proportion for prognostication and efficiently targeting PCSCs to minimise BCR and the inevitable progression to metastatic CRPC. The degree of heterogeneity within CaP renders the idea of single holy grail PCSC marker unlikely. However, a comprehensive panel of selected markers could be the solution. Already, panels of markers are used in research to further purify PCSC populations, such as CD44$^+$ CD133$^+$ α2β1$^+$ by Collins et al. [70]. As more PCSC-related markers are established and identified, further research should be done to optimise a combination for the sensitive and specific identification of PCSCs which would also form the foundation for developing PCSC-specific therapeutic interventions.

Beyond the laboratory and into the clinical setting, reliable identification of PCSCs from tumour biopsies from localised CaP would likely give invaluable insights regarding the likelihood of radioresistance, metastasis and recurrence which would critically inform subsequent management decisions. Saga et al. recently developed a stochastic model accurately predicting RT efficacy *in vitro* based on the proportion of CD133$^+$CD44$^+$ PCSCs [98]. Predicting radioresistance is of particular value in the light of emerging evidence that RT is able to induce EMT and CSC phenotypes, aggravating instead of treating the existing disease [9,99].

Due to the plasticity of PCSCs in response to RT, longitudinal monitoring of PCSC marker expression would be an important consideration guiding treatment decisions. Ultimately, the ability to accurately and reliably identify and characterise PCSCs would open up avenues to personalised therapeutic interventions and treatment timelines based on the PCSC proportion and marker signature of each patient's tumour. Interventions specifically targeting PCSCs would ideally have fewer side effects and increased tolerability as well. Though much remains shrouded in mystery, it is evident that there is great value in unearthing the secrets of PCSCs.

Funding

The authors thank funding support by Cancer Research Trust Fund; Prostate and Breast Cancer Foundation; UNSW Sydney, and China Scholarship Council (CSC).

Conflicts of interest

No declared conflicts of interest.

References

[1] American Cancer Society, Prostate, (2019).
[2] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA A Cancer J. Clin. 68 (2018) 394–424.
[3] G. Calleris, G. Marra, E. Dalmasso, M. Falcone, R.J. Karnes, A. Morlacco, M. Oderda, R. Sanchez-Salas, F. Soria, P. Gontero, Is it worth to perform salvage radical prostatectomy for radio-recurrent prostate cancer? A literature review, World J. Urol. 37 (2019) 1469–1483.
[4] M. Nakabayashi, W. Xie, G. Buckle, G. Bubley, M.S. Ernstoff, W. Walsh, D.E. Morganstern, P.W. Kantoff, M.-E. Taplin, Long-term follow-up of a phase II trial of chemotherapy plus hormone therapy for biochemical relapse after definitive local therapy for prostate cancer, Urology 81 (2013) 611–616.
[5] E.C. Ko, A.L. Michaud, R.K. Valicenti, Postoperative radiation after radical prostatectomy, Semin. Radiat. Oncol. 27 (2017) 50–66.
[6] D. Zhang, D.G. Tang, K. Rycaj, Cancer stem cells: regulation programs, immunological properties and immunotherapy, Semin. Cancer Biol. 52 (2018) 94–106.
[7] R.K. Nimmakayala, S.K. Batra, M.P. Ponnusamy, Unraveling the journey of cancer stem cells from origin to metastasis, Biochim. Biophys. Acta Rev. Canc. 1871 (2019) 50–63.
[8] A. Dubrovska, J. Elliott, R.J. Salamone, S. Kim, L.J. Aimone, J.R. Walker, J. Watson, M. Sauveur-Michel, C. Garcia-Echeverria, C.Y. Cho, V.A. Reddy, P.G. Schultz, Combination therapy targeting both tumor-initiating and differentiated cell populations in prostate carcinoma, Clin. Cancer Res. 16 (2010) 5692–5702.
[9] R. Leão, C. Domingos, A. Figueiredo, R. Hamilton, U. Tabori, P. Castelo-Branco, Cancer stem cells in prostate cancer: implications for targeted therapy, Urol. Int. 99 (2017) 125–136.
[10] S. Yadav, C.M. Kowolik, M. Lin, D. Zuro, S.K. Hui, A.D. Riggs, D.A. Horne, SMC1A is associated with radioresistance in prostate cancer and acts by regulating epithelial-mesenchymal transition and cancer stem-like properties, Mol. Carcinog. 58 (2019) 113–125.
[11] Z. Cai, Y. Cao, Y. Luo, H. Hu, H. Ling, Signalling mechanism(s) of epithelial-mesenchymal transition and cancer stem cells in tumour therapeutic resistance, Clinica chimica acta; international journal of clinical chemistry 483 (2018) 156–163.
[12] R. Diaz, P.A. Nguewa, M. Redrado, I. Manrique, A. Calvo, Sunitinib reduces tumor hypoxia and angiogenesis, and radiosensitizes prostate cancer stem-like cells, The Prostate 75 (2015) 1137–1149.
[13] D.T. Marie-Egyptienne, I. Lohse, R.P. Hill, Cancer stem cells, the epithelial to mesenchymal transition (EMT) and radioresistance: potential role of hypoxia, Cancer Lett. 341 (2013) 63–72.
[14] T.M. Pawlik, K. Keyomarsi, Role of cell cycle in mediating sensitivity to radiotherapy, Int. J. Radiat. Oncol. Biol. Phys. 59 (2004) 928–942.
[15] M. Cojoc, C. Peitzsch, I. Kurth, F. Trautmann, L.A. Kunz-Schughart, G.D. Telegeev, E.A. Stakhovsky, J.R. Walker, K. Simin, S. Lyle, S. Fuessel, K. Erdmann, M.P. Wirth, M. Krause, M. Baumann, A. Dubrovska, Aldehyde dehydrogenase is regulated by β-catenin/TCF and promotes radioresistance in prostate cancer progenitor cells, Cancer Res. 75 (2015) 1482–1494.
[16] L. Chang, P.H. Graham, J. Hao, J. Ni, J. Bucci, P.J. Cozzi, J.H. Kearsley, Y. Li, Acquisition of epithelial–mesenchymal transition and cancer stem cell phenotypes is associated with activation of the PI3K/Akt/mTOR pathway in prostate cancer radioresistance, Cell Death Dis. 4 (2013) e875–e875.
[17] S. Fulda, Inhibitor of Apoptosis (IAP) proteins as therapeutic targets for radiosensitization of human cancers, Cancer Treat Rev. 38 (2012) 760–766.
[18] J. Ni, P.J. Cozzi, J.L. Hao, J. Beretov, L. Chang, W. Duan, S. Shigdar, W.J. Delprado, P.H. Graham, J. Bucci, J.H. Kearsley, Y. Li, CD44 variant 6 is associated with prostate cancer metastasis and chemo-/radioresistance, The Prostate 74 (2014) 602–617.
[19] F.A.E. Kruyt, J.J. Schuringa, Apoptosis and cancer stem cells: implications for apoptosis targeted therapy, Biochem. Pharmacol. 80 (2010) 423–430.
[20] X. Jia, X. Li, Y. Xu, S. Zhang, W. Mou, Y. Liu, Y. Liu, D. Lv, C.-H. Liu, X. Tan, R. Xiang, N. Li, SOX2 promotes tumorigenesis and increases the anti-apoptotic property of human prostate cancer cell, J. Mol. Cell Biol. 3 (2011) 230–238.
[21] D. Kumar, S. Shankar, R.K. Srivastava, Rottlerin induces autophagy and apoptosis in prostate cancer stem cells via PI3K/Akt/mTOR signaling pathway, Cancer Lett. 343 (2014) 179–189.
[22] R. Ojha, S. Bhattacharyya, S. Singh, Autophagy in cancer stem cells: a potential link between chemoresistance, recurrence, and metastasis, BioResearch Open Access 4 (2015) 97–108.
[23] Y. Lei, D. Zhang, J. Yu, H. Dong, J. Zhang, S. Yang, Targeting autophagy in cancer stem cells as an anticancer therapy, Cancer Lett. 393 (2017) 33–39.
[24] S. Paglin, T. Hollister, T. Delohery, N. Hackett, M. McMahill, E. Sphicas, D. Domingo, J. Yahalom, A novel response of cancer cells to radiation involves autophagy and formation of acidic vesicles, Cancer Res. 61 (2001) 439–444.
[25] L. Chang, P.H. Graham, J. Hao, J. Ni, J. Bucci, P.J. Cozzi, J.H. Kearsley, Y. Li, PI3K/Akt/mTOR pathway inhibitors enhance radiosensitivity in radioresistant prostate cancer cells through inducing apoptosis, reducing autophagy, suppressing NHEJ and HR repair pathways, Cell Death Dis. 5 (2014) e1437–e1437.
[26] B. Yao, B. Liu, L. Shi, X. Li, C. Ren, M. Cai, W. Wang, J. Li, Y. Sun, Y. Wu, J. Wen, PAFR selectively mediates radioresistance and irradiation-induced autophagy suppression in prostate cancer cells, Oncotarget 8 (2017) 13846–13854.
[27] J. Theys, B. Jutten, R. Habets, K. Paesmans, A.J. Groot, P. Lambin, B.G. Wouters, G. Lammering, M. Vooijs, E-Cadherin loss associated with EMT promotes radioresistance in human tumor cells, Radiother. Oncol. 99 (2011) 392–397.
[28] A. Gonnissen, S. Isebaert, K. Haustermans, Hedgehog signaling in prostate cancer and its therapeutic implication, Int. J. Mol. Sci. 14 (2013) 13979–14007.
[29] T. Li, Y. Su, Y. Mei, Q. Leng, B. Leng, Z. Liu, S.A. Stass, F. Jiang, ALDH1A1 is a marker for malignant prostate stem cells and predictor of prostate cancer patients' outcome, Lab. Investig. 90 (2010) 234–244.
[30] C. Le Magnen, L. Bubendorf, C.A. Rentsch, C. Mengus, J. Gsponer, T. Zellweger, M. Rieken, G.N. Thalmann, M.G. Cecchini, M. Germann, A. Bachmann, S. Wyler, M. Heberer, G.C. Spagnoli, Characterization and clinical relevance of ALDHbright populations in prostate cancer, Clin. Cancer Res. 19 (2013) 5361–5371.
[31] E. Kalantari, F.H. Saadi, M. Asgari, A. Shariftabrizi, R. Roudi, Z. Madjd, Increased expression of ALDH1A1 in prostate cancer is correlated with tumor aggressiveness: a tissue microarray study of Iranian, Patients 25 (2017) 592–598.
[32] H.F. Huwait, A.M. Nassir, H.M. Abd Elmone, H.F.M. Kamel, N.D. Toni, N.A. Babtain, A.S. Barhamain, A.B.S. Malibari, S.F. Munassar, R.S. Rawa, A.Z. Kufiah, Clinical significance and potential utility of cancer stem cell markers: ALDH1A1 and CD133 in prostate tumors, Int. J. Cancer Res. 14 (2018) 39–51.
[33] D.W. Clark, K. Palle, Aldehyde dehydrogenases in cancer stem cells: potential as therapeutic targets, Ann. Transl. Med. 4 (2016) 518-518.
[34] P. Marcato, C.A. Dean, C.A. Giacomantonio, P.W.K. Lee, Aldehyde dehydrogenase: its role as a cancer stem cell marker comes down to the specific isoform, Cell Cycle 10 (2011) 1378–1384.
[35] A.C. Kimble-Hill, B. Parajuli, C.-H. Chen, D. Mochly-Rosen, T.D. Hurley, Development of selective inhibitors for aldehyde dehydrogenases based on substituted indole-2,3-diones, J. Med. Chem. 57 (2014) 714–722.
[36] L. Patrawala, T. Calhoun, R. Schneider-Broussard, H. Li, B. Bhatia, S. Tang, J.G. Reilly, D. Chandra, J. Zhou, K. Claypool, L. Coghlan, D.G. Tang, Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells, Oncogene 25 (2006) 1696–1708.
[37] E.M. Hurt, B.T. Kawasaki, G.J. Klarmann, S.B. Thomas, W.L. Farrar, CD44+CD24+ prostate cells are early cancer progenitor/stem cells that provide a model for patients with poor prognosis, Br. J. Canc. 98 (2008) 756–765.
[38] W. Xiao, P.H. Graham, C.A. Power, J. Hao, J.H. Kearsley, Y. Li, CD44 is a biomarker associated with human prostate cancer radiation sensitivity, Clin. Exp. Metastasis 29 (2011) 1–9.
[39] J.-w. Ma, X. Wang, L. Chang, X.-y. Zhong, H. Jing, X. Zhu, S. Wang, W. Xiao, CD44 collaborates with ERBB2 mediate radiation resistance via p38 phosphorylation and DNA homologous recombination pathway in prostate cancer, Exp. Cell Res. 370 (2018) 58–67.
[40] J. Ni, P. Cozzi, J. Beretov, J. Bucci, P. Graham, Y. Li, Abstract 1999: study of CD44 variant 6 (CD44v6) in prostate cancer chemo-/radio resistance in vivo, Cancer Res. 78 (2018) 1999-1999.
[41] K. Korski, A. Malicka-Durczak, J. Bręborowicz, Expression of stem cell marker CD44 in prostate cancer biopsies predicts cancer grade in radical prostatectomy specimens, Pol. J. Pathol. : Off. J. Pol. Soc. Pathol. 65 (2014) 291–295.
[42] A.M. De Marzo, C. Bradshaw, J. Sauvageot, J.I. Epstein, G.J. Miller, CD44 and CD44v6 downregulation in clinical prostatic carcinoma: relation to Gleason grade and cytoarchitecture, The Prostate 34 (1998) 162–168.
[43] A.N. Vis, M.A. Noordzij, K. Fitoz, M.F. Wildhagen, F.H. Schroder, T.H. van der Kwast, Prognostic value of cell cycle proteins p27kip1 and MIB-1, and the cell adhesion protein CD44s in surgically treated patients with prostate cancer, J. Urol. 164 (2000) 2156–2161.
[44] R. Makarewicz, A. Zyromska, H. Andrusewicz, Comparative analysis of biological profiles of benign prostate hyperplasia and prostate cancer as potential diagnostic, prognostic and predictive indicators, Folia Histochem. Cytobiol. 49 (2011) 452–457.
[45] A.V. Ugolkov, L.J. Eisengart, C. Luan, X.J. Yang, Expression analysis of putative stem cell markers in human benign and malignant prostate, The Prostate 71 (2011) 18–25.
[46] C. Liu, K. Kelnar, B. Liu, X. Chen, T. Calhoun-Davis, H. Li, L. Patrawala, H. Yan,

C. Jeter, S. Honorio, J.F. Wiggins, A.G. Bader, R. Fagin, D. Brown, D.G. Tang, The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44, Nat. Med. 17 (2011) 211–215.
[47] J. Hao, Investigating the Roles of CD44 and CD147 in Prostate Cancer Metastasis and Drug-Resistance, (2012).
[48] J.R. Marthick, J.L. Dickinson, Emerging Putative Biomarkers: the Role of Alpha 2 and 6 Integrins in Susceptibility, Treatment, and Prognosis, Prostate cancer, 2012, pp. 298732–298739 2012.
[49] C.-L. Philippe, Therapeutic value of an integrin antagonist in prostate cancer, Curr. Drug Targets 17 (2016) 321–327.
[50] T. Wang, J. Huang, M. Vue, M.R. Alavian, H.L. Goel, D.C. Altieri, L.R. Languino, T.J. FitzGerald, αvβ3 integrin mediates radioresistance of prostate cancer cells through regulation of Survivin, Mol. Cancer Res. 17 (2019) 398–408.
[51] G. van der Horst, C. van den Hoogen, J.T. Buijs, H. Cheung, H. Bloys, R.C.M. Pelger, G. Lorenzon, B. Heckmann, J. Feyen, P. Pujuguet, R. Blanque, P. Clement-Lacroix, G. van der Pluijm, Targeting of α(v)-integrins in stem/progenitor cells and supportive microenvironment impairs bone metastasis in human prostate cancer, Neoplasia 13 (2011) 516–525.
[52] K.J. Reeves, J.E. Hurrell, M. Cecchini, G. van der Pluijm, J.M. Down, C.L. Eaton, F. Hamdy, P. Clement-Lacroix, N.J. Brown, Prostate cancer cells home to bone using a novel in vivo model: modulation by the integrin antagonist GLPG0187, Int. J. Cancer 136 (2015) 1731–1740.
[53] A. Abdollahi, D.W. Griggs, H. Zieher, A. Roth, K.E. Lipson, R. Saffrich, H.-J. Gröne, D.E. Hallahan, R.A. Reisfeld, J. Debus, A.G. Niethammer, P.E. Huber, Inhibition of alpha(v)beta3 integrin survival signaling enhances antiangiogenic and antitumor effects of radiotherapy, Clin. Cancer Res. 11 (2005) 6270–6279.
[54] A. Heidenreich, S.K. Rawal, K. Szkarlat, N. Bogdanova, L. Dirix, A. Stenzl, M. Welslau, G. Wang, F. Dawkins, C.J. de Boer, D. Schrijvers, A randomized, double-blind, multicenter, phase 2 study of a human monoclonal antibody to human αν integrins (intetumumab) in combination with docetaxel and prednisone for the first-line treatment of patients with metastatic castration-resistant prostate cancer, Ann. Oncol. 24 (2013) 329–336.
[55] F.M. Chu, J. Picus, P.M. Fracasso, R. Dreicer, Z. Lang, B. Foster, A phase 1, multicenter, open-label study of the safety of two dose levels of a human monoclonal antibody to human α(v) integrins, intetumumab, in combination with docetaxel and prednisone in patients with castrate-resistant metastatic prostate cancer, Investig. New Drugs 29 (2011) 674–679.
[56] A. Alva, S. Slovin, S. Daignault, M. Carducci, R. DiPaola, K. Pienta, D. Agus, K. Cooney, A. Chen, D.C. Smith, M. Hussain, Phase II study of Cilengitide (EMD 121974, NSC 707544) in patients with non-metastatic castration resistant prostate cancer, NCI-6735. A study by the DOD/PCF prostate cancer clinical trials consortium, Investig. New Drugs 30 (2010) 749–757.
[57] M.A. Rosenthal, P. Davidson, F. Rolland, M. Campone, L. Xue, T.H. Han, A. Mehta, Y. Berd, W. He, A. Lombardi, Evaluation of the safety, pharmacokinetics and treatment effects of an alpha(nu)beta(3) integrin inhibitor on bone turnover and disease activity in men with hormone-refractory prostate cancer and bone metastases, Asia Pac. J. Clin. Oncol. 6 (2010) 42–48.
[58] A. Andriu, J. Crockett, S. Dall'Angelo, M. Piras, M. Zanda, I.N. Fleming, Binding of αvβ3 integrin-specific radiotracers is modulated by both integrin expression level and activation status., molecular imaging and biology : MIB, Off. Publ.Acad.Mol. Imaging 20 (2018) 27–36.
[59] A.J. Beer, S.M. Schwarzenböck, N. Zantl, M. Souvatzoglou, T. Maurer, P. Watzlowik, H. Kessler, H.-J. Wester, M. Schwaiger, B.J. Krause, Non-invasive assessment of inter-and intrapatient variability of integrin expression in metastasized prostate cancer by PET, Oncotarget 7 (2016) 28151–28159.
[60] Y. Luo, X. Cui, J. Zhao, Y. Han, M. Li, Y. Lin, Y. Jiang, L. Lan, Cells susceptible to epithelial-mesenchymal transition are enriched in stem-like side population cells from prostate cancer, Oncol. Rep. 31 (2014) 874–884.
[61] T.W. Stark, P.J. Hensley, A. Spear, H. Pu, S.S. Strup, N. Kyprianou, Predictive value of epithelial-mesenchymal-transition (EMT) signature and PARP-1 in prostate cancer radioresistance, The Prostate 77 (2017) 1583–1591.
[62] H.M. Behnsawy, H. Miyake, K.-i. Harada, M. Fujisawa, Expression patterns of epithelial-mesenchymal transition markers in localized prostate cancer: significance in clinicopathological outcomes following radical prostatectomy, BJU Int. 111 (2013) 30–37.
[63] N. Harner-Foreman, J. Vadakekolathu, S.A. Laversin, M.G. Mathieu, S. Reeder, A.G. Pockley, R.C. Rees, D.J. Boocock, A novel spontaneous model of epithelial-mesenchymal transition (EMT) using a primary prostate cancer derived cell line demonstrating distinct stem-like characteristics, Sci. Rep. 7 (2017) 40633.
[64] C.R. Jeter, B. Liu, X. Liu, X. Chen, C. Liu, T. Calhoun-Davis, J. Repass, H. Zaehres, J.J. Shen, D.G. Tang, NANOG promotes cancer stem cell characteristics and prostate cancer resistance to androgen deprivation, Oncogene 30 (2011) 3833–3845.
[65] S.A. Mani, W. Guo, M.-J. Liao, E.N. Eaton, A. Ayyanan, A.Y. Zhou, M. Brooks, F. Reinhard, C.C. Zhang, M. Shipitsin, L.L. Campbell, K. Polyak, C. Brisken, J. Yang, R.A. Weinberg, The epithelial-mesenchymal transition generates cells with properties of stem cells, Cell 133 (2008) 704–715.
[66] T. Celià-Terrassa, O. Meca-Cortés, F. Mateo, A. Martínez de Paz, N. Rubio, A. Arnal-Estapé, B.J. Ell, R. Bermudo, A. Díaz, M. Guerra-Rebollo, J.J. Lozano, C. Estarás, C. Ulloa, D. Álvarez-Simón, J. Milà, R. Vilella, R. Paciucci, M. Martínez-Balbás, A.G. de Herreros, R.R. Gomis, Y. Kang, J. Blanco, P.L. Fernández, T.M. Thomson, Epithelial-mesenchymal transition can suppress major attributes of human epithelial tumor-initiating cells, J. Clin. Investig. 122 (2012) 1849–1868.
[67] H. Tanaka, E. Kono, C.P. Tran, H. Miyazaki, J. Yamashiro, T. Shimomura, L. Fazli, R. Wada, J. Huang, R.L. Vessella, J. An, S. Horvath, M. Gleave, M.B. Rettig, Z.A. Wainberg, R.E. Reiter, Monoclonal antibody targeting of N-cadherin inhibits prostate cancer growth, metastasis and castration resistance, Nat. Med. 16 (2010) 1414–1420.
[68] A. Dubrovska, J. Elliott, R.J. Salamone, G.D. Telegeev, A.E. Stakhovsky, I.B. Schepotin, F. Yan, Y. Wang, L.C. Bouchez, S.A. Kularatne, J. Watson, C. Trussell, V.A. Reddy, C.Y. Cho, P.G. Schultz, CXCR4 expression in prostate cancer progenitor cells, PLoS One 7 (2012) e31226.
[69] M. Trerotola, S. Rathore, H.L. Goel, J. Li, S. Alberti, M. Piantelli, D. Adams, Z. Jiang, L.R. Languino, CD133, Trop-2 and alpha2beta1 integrin surface receptors as markers of putative human prostate cancer stem cells, Am. J. Tourism Res. 2 (2010) 135–144.
[70] A.T. Collins, P.A. Berry, C. Hyde, M.J. Stower, N.J. Maitland, Prospective identification of tumorigenic prostate cancer stem cells, Cancer Res. 65 (2005) 10946–10951.
[71] A.A. Merchant, W. Matsui, Targeting hedgehog — a cancer stem cell pathway, Clin. Cancer Res. 16 (2010) 3130–3140.
[72] M. Lauth, Å. Bergström, T. Shimokawa, R. Toftgård, Inhibition of GLI-mediated transcription and tumor cell growth by small-molecule antagonists, Proc. Natl. Acad. Sci. 104 (2007) 8455–8460.
[73] R. Nanta, D. Kumar, D. Meeker, M. Rodova, P.J. Van Veldhuizen, S. Shankar, R.K. Srivastava, NVP-LDE-225 (Erismodegib) inhibits epithelial-mesenchymal transition and human prostate cancer stem cell growth in NOD/SCID IL2Rγ null mice by regulating Bmi-1 and microRNA-128, Oncogenesis 2 (2013) e42-e42.
[74] A.E. Ross, R.M. Hughes, S. Glavaris, K. Ghabili, P. He, N.M. Anders, R. Harb, J.J. Tosoian, L. Marchionni, E.M. Schaeffer, A.W. Partin, M.E. Allaf, T.J. Bivalacqua, C. Chapman, T. O'Neal, A.M. DeMarzo, P.J. Hurley, M.A. Rudek, E.S. Antonarakis, Pharmacodynamic and pharmacokinetic neoadjuvant study of hedgehog pathway inhibitor Sonidegib (LDE-225) in men with high-risk localized prostate cancer undergoing prostatectomy, Oncotarget 8 (2017) 104182–104192.
[75] P.M. LoRusso, C.M. Rudin, J.C. Reddy, R. Tibes, G.J. Weiss, M.J. Borad, C.L. Hann, J.R. Brahmer, I. Chang, W.C. Darbonne, R.A. Graham, K.L. Zerivitz, J.A. Low, D.D. Von Hoff, Phase I trial of hedgehog pathway inhibitor vismodegib (GDC-0449) in patients with refractory, locally advanced or metastatic solid tumors, Clin. Cancer Res. 17 (2011) 2502–2511.
[76] B.L. Maughan, D.L. Suzman, B. Luber, H. Wang, S. Glavaris, R. Hughes, R. Sullivan, R. Harb, K. Boudadi, C. Paller, M. Eisenberger, A. Demarzo, A. Ross, E.S. Antonarakis, Pharmacodynamic study of the oral hedgehog pathway inhibitor, vismodegib, in patients with metastatic castration-resistant prostate cancer, Cancer Chemother. Pharmacol. 78 (2016) 1297–1304.
[77] M. Lee, H. Hong, W. Kim, L. Zhang, T.W. Friedlander, L. Fong, A.M. Lin, E.J. Small, X.X. Wei, T.J. Rodvelt, B. Miralda, B. Stocksdale, C.J. Ryan, R. Aggarwal, Itraconazole as a noncastrating treatment for biochemically recurrent prostate cancer: a phase 2 study, Clin. Genitourin. Cancer 17 (2019) e92–e96.
[78] E.S. Antonarakis, E.I. Heath, D.C. Smith, D. Rathkopf, A.L. Blackford, D.C. Danila, S. King, A. Frost, A.S. Ajiboye, M. Zhao, J. Mendonca, S.K. Kachhap, M.A. Rudek, M.A. Carducci, Repurposing itraconazole as a treatment for advanced prostate cancer: a noncomparative randomized phase II trial in men with metastatic castration-resistant prostate cancer, The Oncologist 18 (2013) 163–173.
[79] A. Gonnissen, S. Isebaert, C.M. McKee, R. Dok, K. Haustermans, R.J. Muschel, The hedgehog inhibitor GANT61 sensitizes prostate cancer cells to ionizing radiation both in vitro and in vivo, Oncotarget 7 (2016) 84286–84298.
[80] N. Krishnamurthy, R. Kurzrock, Targeting the Wnt/beta-catenin pathway in cancer: update on effectors and inhibitors, Cancer Treat Rev. 62 (2018) 50–60.
[81] I. Bisson, D.M. Prowse, WNT signaling regulates self-renewal and differentiation of prostate cancer cells with stem cell characteristics, Cell Res. 19 (2009) 683–697.
[82] P.G. Soerensen, T. Andersson, U. Buhl, T. Moelvadgaard, P.B. Jensen, N. Brunner, D. Nielsen, Phase I dose-escalating study to evaluate the safety, tolerability, and pharmacokinetic and pharmacodynamic profiles of Foxy-5 in patients with metastatic breast, colorectal, or prostate cancer, J. Clin. Oncol. 32 (2017) TPS1140-TPS1140.
[83] A.S.S. Khaja, L. Helczynski, A. Edsjö, R. Ehrnström, A. Lindgren, D. Ulmert, T. Andersson, A. Bjartell, Elevated level of Wnt5a protein in localized prostate cancer tissue is associated with better outcome, PLoS One 6 (2011) e26539.
[84] S. Thiele, A. Göbel, T.D. Rachner, S. Fuessel, M. Froehner, M.H. Muders, G.B. Baretton, R. Bernhardt, F. Jakob, C.C. Glüer, M. Bornhäuser, M. Rauner, L.C. Hofbauer, WNT5A has anti-prostate cancer effects in vitro and reduces tumor growth in the skeleton in vivo, J. Bone Miner. Res. :Off. J.Am. Soc.Bone Miner. Res. 30 (2015) 471–480.
[85] F. Chen, X. Chen, Y. Ren, G. Weng, P.C. Keng, Y. Chen, S.O. Lee, Radiation-induced glucocorticoid receptor promotes CD44+ prostate cancer stem cell growth through activation of SGK1-Wnt/β-catenin signaling, J. Mol. Med. 97 (2019) 1169–1182.
[86] E.A. Kleibeuker, M.A. ten Hooven, H.M. Verheul, B.J. Slotman, V.L. Thijssen, Combining radiotherapy with sunitinib: lessons (to be) learned, Angiogenesis 18 (2015) 385–395.
[87] O. Bratt, M. Häggman, G. Ahlgren, O. Nordle, A. Björk, J.-E. Damber, Open-label, clinical phase I studies of tasquinimod in patients with castration-resistant prostate cancer, Br. J. Canc. 101 (2009) 1233–1240.
[88] R. Pili, M. Häggman, W.M. Stadler, J.R. Gingrich, V.J. Assikis, A. Björk, O. Nordle, G. Forsberg, M.A. Carducci, A.J. Armstrong, Phase II randomized, double-blind, placebo-controlled study of tasquinimod in men with minimally symptomatic metastatic castrate-resistant prostate cancer, J. Clin. Oncol. : Off. J. Am. Soc. Clin. Oncol. 29 (2011) 4022–4028.
[89] C. Sternberg, A. Armstrong, R. Pili, S. Ng, R. Huddart, N. Agarwal, D. Khvorostenko, O. Lyulko, A. Brize, N. Vogelzang, R. Delva, M. Harza, A. Thanos, N. James, P. Werbrouck, M. Bögemann, T. Hutson, P. Milecki, S. Chowdhury, E. Gallardo, G. Schwartsmann, J.-C. Pouget, F. Baton, T. Nederman, H. Tuvesson, M. Carducci, Randomized, double-blind, placebo-controlled phase III study of tasquinimod in men with metastatic castration-resistant prostate cancer, J. Clin. Oncol. : Off. J. Am.

Soc. Clin. Oncol. 34 (2016) 2636–2643.
[90] M.D. Michaelson, S. Oudard, Y.-C. Ou, L. Sengeløv, F. Saad, N. Houede, P. Ostler, A. Stenzl, G. Daugaard, R. Jones, F. Laestadius, A. Ullèn, A. Bahl, D. Castellano, J. Gschwend, T. Maurina, E. Chow Maneval, S.-L. Wang, M.J. Lechuga, J. Paolini, I. Chen, Randomized, placebo-controlled, phase III trial of sunitinib plus prednisone versus prednisone alone in progressive, metastatic, castration-resistant prostate cancer, J. Clin. Oncol. : Off. J. Am. Soc. Clin. Oncol. 32 (2014) 76–82.
[91] P.G. Corn, D.Y. Song, E. Heath, J. Maier, R. Meyn, D. Kuban, T.A. DePetrillo, P. Mathew, Sunitinib plus androgen deprivation and radiation therapy for patients with localized high-risk prostate cancer: results from a multi-institutional phase 1 study, Int. J. Radiat. Oncol. Biol. Phys. 86 (2013) 540–545.
[92] S.L. Dalrymple, R.E. Becker, H. Zhou, T.L. DeWeese, J.T. Isaacs, Tasquinimod prevents the angiogenic rebound induced by fractionated radiation resulting in an enhanced therapeutic response of prostate cancer xenografts, The Prostate 72 (2012) 638–648.
[93] C. Brooks, T. Sheu, K. Bridges, K. Mason, D. Kuban, P. Mathew, R. Meyn, Preclinical evaluation of sunitinib, a multi-tyrosine kinase inhibitor, as a radiosensitizer for human prostate cancer, Radiat. Oncol. 7 (2012) 154.
[94] M.J. Mayer, L.H. Klotz, V. Venkateswaran, Metformin and prostate cancer stem cells: a novel therapeutic target, Prostate Cancer Prostatic Dis. 18 (2015) 303–309.
[95] V.E. Zannella, A.D. Pra, H. Muaddi, T.D. McKee, S. Stapleton, J. Sykes, R. Glicksman, S. Chaib, P. Zamiara, M. Milosevic, B.G. Wouters, R.G. Bristow, M. Koritzinsky, Reprogramming metabolism with metformin improves tumor oxygenation and radiotherapy response, Clin. Cancer Res. 19 (2013) 6741–6750.
[96] J. Zhang, C. Shen, L. Wang, Q. Ma, P. Xia, M. Qi, M. Yang, B. Han, Metformin inhibits epithelial–mesenchymal transition in prostate cancer cells: involvement of the tumor suppressor miR30a and its target gene SOX4, Biochem. Biophys. Res. Commun. 452 (2014) 746–752.
[97] H. Yu, L. Yin, X. Jiang, X. Sun, J. Wu, H. Tian, X. Gao, X. He, Effect of metformin on cancer risk and treatment outcome of prostate cancer: a meta-analysis of epidemiological observational studies, PLoS One 9 (2014) e116327.
[98] R. Saga, Y. Matsuya, R. Takahashi, K. Hasegawa, H. Date, Y. Hosokawa, Analysis of the high-dose-range radioresistance of prostate cancer cells, including cancer stem cells, based on a stochastic model, J. Radiat. Res. 60 (2019) 298–307.
[99] K. Murata, R. Saga, S. Monzen, E. Tsuruga, K. Hasegawa, Y. Hosokawa, Understanding the mechanism underlying the acquisition of radioresistance in human prostate cancer cells, Oncol. Lett. 17 (2019) 5830–5838, https://doi.org/10.3892/ol.2019.10219.
[100] M.P. Martin, D. Borchiellini, J. Viotti, A. Guillot, J.B. Paoli, D. Besson, W. Hilgers, C. El Kouri, G. Cavaglione, F. Priou, T. Lharidon, R. Largillier, J.-L. Deville, B. Hoch, R. Schiappa, J.F. Tanti, F. Bost, J.-M. Ferrero, TAXOMET: a French prospective multicenter randomized controlled phase II study comparing docetaxel plus metformin versus docetaxel plus placebo in mCRPC, J. Clin. Oncol. 37 (2019) 5004-5004.