Wnt signaling in castration-resistant prostate cancer: implications for therapy

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Abstract

Increasing evidence has indicated that Wnt signaling plays complex roles in castration resistant prostate cancer (CRPC). Although not all data were consistent, β-catenin nuclear localization and its co-localization with androgen receptor (AR) were more frequently observed in CRPC compared to hormone naïve prostate cancer. This direct interaction between AR and β-catenin seemed to elicit a specific expression of a set of target genes in low androgen conditions in CRPC. Paracrine Wnt signaling also was shown to aid resistance to chemotherapy and androgen deprivation therapy. Results from the next generation sequencing studies (i.e. RNA-seq and whole exosome sequcing) of CRPC specimens have identified the Wnt pathway as one of the top signaling pathways with significant genomic alterations in CRPC, whereas, Wnt pathway alterations were virtually absent in hormone naïve primary prostate cancer. Furthermore, Wnt signaling has been suggested to play an important role in cancer stem cell functions in prostate cancer recurrence and resistance to androgen deprivation therapy. Therefore, in this review we have summarized existing knowledge regarding potential roles of Wnt signaling in CRPC and underline Wnt signaling as a potential therapeutic target for CRPC. Further understanding of Wnt signaling in castration resistance may eventually contribute new insights into possible treatment options for this incurable disease.

Keywords

Wnt signaling; castration-resistant prostate cancer; targeted therapy

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Introduction

Prostate cancer is a biologically diverse disease. A significant proportion of elderly men with this disease are asymptomatic [1, 2]. Most of these asymptomatic cases are prostate cancers which may be managed by watchful waiting and active surveillance. Prostate cancer patients who have clinically localized disease at diagnosis often receive curative treatments through radical prostatectomy or radiotherapy. However, between 20% and 40% of these patients eventually experience biochemical recurrence [3, 4]. Patients with biochemically recurrent disease, metastatic prostate cancer, and locally advanced prostate cancer are then mainly treated with androgen deprivation therapy (ADT). Since the 1940s, ADT consists of surgical or medical castration method to reduce circulating androgens [5]. Unfortunately, all patients eventually develop resistance to ADT called castration-resistant prostate cancer (CRPC) [6]. Recently, two new FDA approved drugs (i.e. Abiraterone Acetate and Enzalutamide) more effectively block androgen synthesis and to prevent activation of androgen receptor (AR) [7, 8]. Although these AR targeting agents extend life of CRPC patients by a few months, resistance to these treatments remains common and currently there is no cure for CRPC [9-18]. Therefore, understanding the molecular mechanisms leading to CRPC and identifying alternative targets are important in developing more effective treatment for CRPC.

Much new evidence summarized here indicates that the Wnt signaling pathway is one of the major pathways that are involved in developing CRPC. The Wnt pathway plays a central role in the development of many tissues and organs [19]. Aberrant activation of the Wnt/β-catenin pathway contributes to the progression of several major human cancers, including colorectal, liver and prostate cancer [19]. Evidence has accumulated that the Wnt/β-catenin pathway plays an important role in CRPC by interacting with AR signaling [20-33]. Recent studies using next-generation sequencing of CRPC tumors have revealed significant genomic alterations in multiple components of the Wnt pathway, whereas, alterations of the Wnt pathway in hormone treatment naïve prostate cancer were viturally undetectable [34, 35]. Moreover, it has been reported that abberant paracrine Wnt signaling from prostatic stroma can also contribute to the resistance to ADT before or after chemotherapy [36, 37]. Hence, the main goal of this review is to summarize the important role of the Wnt/β-catenin in progression to CRPC and discuss potential therapeutic approaches for targeting the Wnt pathway for treatment of CRPC. Proposed mechanisms for elevated Wnt signaling in CRPC are summarized in Table 1.

Nuclear β-catenin expression in CRPC

The Wnt (wingless-type) signaling transduction pathway has three different pathways, the canonical Wnt/β-catenin, non-canonical planar cell polarity, and the non-canonical Wnt/ Calcium pathway; essentially separating but interacting pathways (Figure 1) [19, 38, 39]. The best-studied Wnt signaling pathway is the canonical Wnt/β-catenin pathway in which Wnt ligands form a complex with Frizzled (FZD) receptor and coreceptors such as the low-density lipoprotein receptor-related protein 5 (LRP5) or LRP6. Upon receptor activation, the "destruction complex" which includes adenomatous polyposis coli (APC) protein and Axin is inhibited thereby blocking the phosphorylation of β-catenin by both casein kinase Iα and

glycogen synthase kinase-3β (GSK-3β). Phosphorylation of β-catenin normally targets β-catenin for degradation. This inhibition results in cytoplasmic β-catenin stabilization and accummulation which facilitates its translocation into the nucleus. Nuclear β-catenin acts as a transcriptional coactivator and interacts with transcriptions factors such as T-cell factor (TCF) and lymphoid enhancer factor (LEF) and leads to increased transcription of target genes, such as MMP7, c-Myc, cyclin D1, c-Jun, Fra and other members of the c-Fos family. In addition, Wnt signaling can also regulate other noncononical pathways such as the JNK pathway independent of β-catenin leading to changes in cell polarity, movement, and survival [19, 38, 39].

β-Catenin is a dual function protein and it can regulate cell-cell adhesion and gene activation [33]. A hallmark of canonical Wnt signaling is the stabilization and nuclear localization of β-catenin [19]. Therefore, various groups have studied β-catenin localization in CRPC specimens compared to hormone-naïve prostate cancer (Table 2). Chesire et al. [40] reported that about 24% (5/21) of metastatic tumors from various anatomical sites of autopsy samples from CRPC patients were positive for β-catenin nuclear localization. de la Taille et al. [41] evaluated β-catenin expression by immunohistochemistry staining in 212 prostate cancer specimens, including 122 localized prostate cancer from prostectomy specimens and 90 from CRPC specimens from transurethral resections of the prostate due to bladder obstruction. Abnormal β-catenin expression was defined as cytoplasmic and/or nuclear staining. In this study, about 23% of radical prostatectomy specimens exhibited abnormal β-catenin expression compared to 38.8% of metastatic CRPC cases with statistically significant result of $p = 0.042$. Likewise, Patriarca et al. [42] found that β-catenin levels were also elevated in 20 acinar prostatic adenocarcinomas after anti-androgen therapy in prostatectomy specimens compared to that of pretreatment biopsies of the same patient group and high Gleason grade, matched and untreated controls. Wan et al. [32] also found nuclear localization of β-catenin in 11 of 27 (40.7%) CRPC bone metastases. Eight (29.6%) of 27 specimens exhibited both β-catenin and androgren receptor positive staining in the nuclei compared to only 3 (11.1%) of the 27 specimens exhibited β-catenin nuclear staining positive when AR was undetectable indicating that more nuclear β-catenin was present in AR-positive nuclei in CRPC tissues. Rajan et al. [35] detected β-catenin protein over-expression in 16 CRPC of 29 matched pairs of hormone-naïve PCa (HNPC) and CRPC; they also found a statistically significant correlation between β-catenin and nuclear AR protein expression in CRPC but not in HNPC. Chen et al. [43] observed 55% (34 out of 62) of primary prostate and 85% (20 out of 23) of prostate carcinoma metastases to lymph nodes and bone specimens with cytoplasmic and nuclear β-catenin expression, respectively. Additionally, Aaltomaa et al. [44] showed that only 18% (39 out of 186) of prostate cance specimens from radical prostatectomy were positive nuclear β-catenin. Finally, Jung et al. [45] reported that ADT-treated patients who exhibited short times to PSA progression expressed higher levels of MMP-7. The expression of cytoplasmic β-catenin, MMP-7, and AR was positively correlated.

In the above section, we summarized studies that reported increased expression of nuclear β-catenin in CRPC; however, there were other groups that observed an opposite trend. Assikis et al. [46] examined β-catenin expression on a tissue microarray from 16 patients who

underwent salvage surgery for symptomatic, locally aggressive androgen-independent prostate cancer and found no nuclear β-catenin expression in these specimens. Whitaker et al. [47] also examined for nuclear β-catenin staining in 17 specimens from patients before hormone therapy and 13 from patients after hormone therapy, and also found no significant differences in the nuclear β-catenin staining between hormone sensitive and hormone relapsed tissues. In prostate cancer specimens that were most likely from hormone naïve patients, Bismar et al. did not detect any nuclear β-catenin staining in 101 prostatic adenocarcinomas, including 72 acinar and 29 ductal, and 16 cases of high-grade prostatic intraepithelial neoplasia (HPIN) using identical immunostaining procedures [48]. Membranous β-catenin act primarily as a cell adhesion molecule. The loss of membranous β-catenin in a small fraction of prostatic adenocarcinomas with higher Gleason scores was found in this study compared to normal prostatic epithelium. This result suggests a differential mechanism of β-catenin from its role as a cofactor for TCF/ LEF or AR. Jaggi et al. [49] also examined 17 samples of prostate cancer specimens and found a significant down-regulation of membranous β-catenin expression in prostate cancer compared to benign prostatic glands and an associatation with increasing Gleason grade ($p = 0.025$). In a prostate cancer prognosis study Horvath et al. [50] showed 64% (149 out of 232) of prostate cancer specimens with more than 10% of cells expressing nuclear β-catenin. However, those patients who had less than 10% of cells expressing β-catenin in the nucleus had decreased biochemical relapse-free survival times. In this study only 17.7% (41 cases) of these prostate cancer specimens were from patients with androgen deprivation therapy, therefore, this study did not separate hormone treatment naïve specimens from CRPC specimens.

In summary, the evidence suggests that there may be a role for nuclear localized β-catenin in CRPC specimens. However, the results remain conflicting. Perhaps the sample sizes and variations in the specimen processing and immunohistochemical staining methods may be contributing to the variations in the reported results with respect to β-catenin nuclear localization in CRPC.

β-catenin and androgen receptor interaction

β-Catenin contains 12 armadillo repeats in a highly conserved central region of the protein [33]. These armadillo repeats not only form a single structural unit to provide the interaction sites with APC, E-cadherin, and TCF/LEFs, but also can adopt an α-helical conformation for nuclear receptor binding proteins [33]. Using a yeast two-hybrid system, Yang et al. [26] demonstrated that β-catenin preferentially and directly bound to the ligand binding domain of AR in the presence of dyhydrotestosterone (DHT) over several other steroid hormone receptors which included estrogen receptor α, progesterone receptor β, and glucocorticoid receptor. This study indicated that the NH(2) terminus and the first 6 armadillo repeats of β-catenin were required components for the AR interaction. The interaction between β-catenin and AR was further confirmed by several other studies showing that β-catenin bound to the activation function 2 region of the AR ligand binding domain and modulated the transcriptional effects of the transcriptional intermediary factor 2 (TIF2) and the AR N-terminal domain. Importantly, a single AR lysine (K720) has been shown to be necessary for the AR/β-catenin and TIF2 interactions [25, 30, 33].

The interactions between β-catenin and AR can be modulated by other cofactors through different signaling pathways in prostate cancer cells as well. β-Catenin interacts with AR in close proximity to the binding groove for p160 coactivators such as TIF2/glucocorticoid receptor interacting protein-1 (GRIP1) [51-54]. TIF2/GRIP1 is one of the three p160 primary coactivator proteins, which serves as a scaffold to recruit a variety of secondary coactivators, including the protein acetyltransferases p300, CBP, and coactivator-associated arginine methyltransferase (CARM1). p300/CBP and CARM1 are reruited by the p160 complex to remodel chromatin through acetylation and methylation of histones and then functions in synergy with β-catenin as coactivators for AR and TCF/LEFs [53, 54]. The methyltransferase activity of CARM1 has also been shown to be necessary for its synergistic coactivator function with β-catenin to activate AR mediated transcription [53].

ICAT (β-catenin-interacting protein 1), an inhibitor of β-catenin and TCF, can inhibit the canonical Wnt/β-catenin signaling pathway by binding to β-catenin [27]. Expression of ICAT was observed in human prostate cancer tissues and found to be elevated in xenograft tumors in castrated mice [27]. Zhou et al. [27] showed that ICAT and AR can form a ternary complex with β-catenin and stabilize the β-catenin-AR complex, which resulted in enhanced AR-mediated transcription and cell growth. The DEAD box RNA helicase p68 (Ddx5) is often over expressed in prostate cancer tissues compared with benign tissue and studies have shown that Ddx5 is also a transcriptional coactivator of AR [55]. Interestingly, Clark et al. [56] demonstrated that the interaction between Ddx5 and β-catenin required the presence of androgens in androgen-sensitive LNCaP cells as wells as other cell lines such as LNCap AI (a CRPC derivative of LNCaP cell line) when the cells are grown in the absence of androgen. Therefore, the function of Ddx5 was shown to be required for recruitment of AR and β-catenin to the promoter regions of androgen responsive genes for AR mediated transcription.

An AR variant [N-terminal truncated isoform of AR (AR45)], with an altered N-terminal domain with a replacement by a unique, short, seven amino-acid-long stretch, has been identified [57]. Overexpression of AR45 was shown to interact with the full-length AR and inhibit AR transcriptional activity and inhibit the growth of LNCaP cells [58]. However, under the conditions of β-catenin overexpression, AR45 increased dihydrotestosterone mediated AR promoter activity [58]. This result suggested that AR splicing variants may have differential effect on prostate cancer cell growth under β-catenin overexpression or overactivity.

Recent success in clinical trials of second generation of the anti-androgen drugs Abiraterone and Enzalutamide strongly support that the aberrant activation of the AR pathway in the absence of high circulating levels of androgen plays a critical role in CRPC [7, 8]. It has been suggested that AR signaling in CRPC is sustained by development of AR amplication, mutation, alternate spilcing, and several alternative molecular mechanisms [6, 10]. When prostate cancer cells have been adapted to the low androgen environment, β-catenin has been shown to act as a coactivator of AR to enhance AR transcriptional activity not only in the presence of DHT, but also in the presence of androstenedione, a weaker adrenal androgen remaining present in CRPC patients [26, 30, 33]. β-Catenin is also one of the three AR coactivators (other two AR specific coactivators are ARA70 and ARA55) that can enhance

AR transcriptional activity in LNCaP cells when treated with 17β-estradiol [26, 30, 33]. In addition, β-catenin can function as a coactivator with altered ARs with mutations W741C and T877A in prostate cancer cell lines [33]. These AR mutations were detected in CRPC patients that have been treated with bicalutamide leading to the W741C mutation and also in CRPC patients with lymph node metastatic lesions containing the T877A mutation [33]. In rodent studies, Chesire et al. [22] reported that castrated mice receiving androgen treatment exhibited nuclear co-localization of AR and β-catenin in normal prostatic epithelium. Nuclear β-catenin localization was found to occur concomitantly with androgen-induced regrowth of normal rat prostate from androgen deprivation induced regression. Furthermore, Wang et al. [20] observed increased expression and nuclear co-localization of AR and β-catenin as well as the interaction between endogenous AR and β-catenin in CRPC from castrated mice. However, they found no interaction or colocalization of AR and β-catenin in xenografts from noncastrated mice. Mutations of β-catenin are uncommon in prostate cancer (< 5%) [59, 60].

Taken together, these results suggested that β-catenin plays an integral role in formation of the androgen-receptor transcriptional complex in CRPC. Based on available information, we proposed a simiplied relationship between Wnt and AR signaling during prostate cancr development and progression as summarized in Figure 2. AR and Wnt signaling may reinforce each other to elicit specific target genes for promoting androgen-independent growth and progression. As such, β-catenin/AR interactions could have distinct clinical relevance and be a potential therapeutic target for treatment of CRPC, especially working best at low androgens.

The cross-talk between β-catenin and multiple pathways in prostate cancer

Nuclear β-catenin can also arise through other mechanisms besides alterations in the canonical Wnt signaling pathway. In this section we consider examples of the accumulation of β-catenin through other mechanisms. Constitutive protein kinase B (also known as AKT) activation in prostate cancer due to loss of PTEN can inhibit GSK-3β activity leading to stabilization and nuclear accumulation of β-catenin [61]. Liu et al. [62] demonstrated that addition of H2-relaxin to LNCaP cells resulted in increased phosphorylation of protein kinase B (Akt) and phosphorylation of glycogen synthase kinase-3β (GSK-3β) with subsequent cytoplasmic accumulation of β-catenin. This is followed by nuclear translocation, formation of the β-catenin/AR complex and increased AR transcriptional activity in LNCaP cells. Paradoxically, in neuronal cells, Pawlowski et al. [63] demonstrated that ligand-bound AR promoted the accumulation of β-catenin in the nucleus, The nuclear co-localization of AR and β-catenin was independent of the GSK-3β, p42/44 ERK mitogen-activated protein kinase, and phosphatidylinositol 3-kinase pathways. Other groups have shown that GSK-3β can phosphorylate AR and suppress its ability to activate transcription under certain conditions. Wnt pathway activation can stimulate Akt activity which promotes an MDM-2-mediated degradation process that reduces AR protein levels in Wnt-stimulated prostate cancer cells [64, 65].

Adenomatous polyposis coli (APC) is a major regulator of β-catenin protein level through phosphorylation of β-catenin which signals the degradation of β-catenin. Inactivation of

APC gene by hypermethylation was detected in prostate cancer but not in normal prostate tissues [66, 67] thereby favoring the accumulation of β-catenin. However, Mulholland et al. [68] reported that AR can promote β-catenin nuclear translocation independently of APC. Pin1 is a peptidyl-prolyl cis/trans isomerase that stabilizes β-catenin by inhibiting its binding to the APC gene product and subsequent GSK-3β-dependent degradation. The expression of Pin1 in radical prostatectomy specimens is strongly correlated with the incidence of recurrence and metastasis [69]. Pin1 stabilizes β-catenin and abrogates the β-catenin and AR interaction, leading to increased β-catenin/TCF-4 signaling and increased expression of the WNT target genes c-Myc and TCF-4 istself [69]. The result is exclusive to PTEN-deficient LNCaP cells. Protocadherin-PC, localized on the human Y chromosome, was shown to be selectively expressed in apoptosis-resistant and hormone-resistant human prostate cancer cells and tissues [70]. Expression of cytoplasmic protocadherin-PC can induce expression of Wnt target genes through interacting and stabilizing β-catenin [70]. These mechanisms illustrate different means of directing gene regulation involving β-catenin.

Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor that plays an essential role in cellular reponse to hypoxia. HIF-1α is known to enhance β-catenin activated AR transactivation in hypoxia [71]. During cellular hypoxia, increased expression of complexes composed of HIF-1α, AR and β-catenin in the nucleus were observed and activated androgen responsive genes. Knockdown of HIF-1α attenuated the recruitment of AR and β-catenin to the androgen response elements (AREs) and deactivated activation of androgen-responsive genes [71]. β-Catenin can also interact with FOXO transcriptional factors in response to oxidative stress to promote cells exit from the cell cycle and entry into cell quiescence [72]. FOXA1 was shown to be overexpressed in advanced prostate cancer and metastases, and over expression of FOXA1 led to enrichment of the Wnt signaling pathway [73]. Heterogeneous nuclear ribonucleoprotein K (HnRNP K) was also found to be overexpressed in prostate cancer tissues and overexpression of HnRNP K positively associated with high Gleason score and poor prognosis [74, 75]. HnRNP K can bind to the β-catenin/TCF-4 complex for regulation of pre-mRNA splicing and some types of alternative splicing have been suggested to promote prostate cancer progression [74, 75]. Finally, β-catenin can also influence the metastatic potential of prostate cancer cells by suppression of transcription of a metastasis suppressor, KAI1, through formation of a β-catenin-reptin chromatin remodeling complex [76].

TCF family memebers in prostate cancer

Despite extensive research, the crosstalks between AR and Wnt/β-catenin signaling pathways remain complex and conflicting. The canonical Wnt pathway is mediated by TCF/LEF-1 transcription factor family members which include TCF-1, TCF-3, TCF-4 and LEF-1 [38]. The human AR gene itself has been shown to be a Wnt target gene [65]. Activation of Wnt/β-catenin signaling by Wnt stimulation can increase AR expression *via* TCF/LEF-1-binding sites on the AR promoter, leading to upregulation of AR target genes [65]. On the contrary, AR can also compete with TCF/LEF-1 for β-catenin binding and thus inhibit TCF/LEF-1 mediated transcription [77]. Chesire et al. showed that anti-androgens alleviated the AR mediated suppression of TCF transcriptional activity and activation of TCF/LEF-1 inhibited the expression of AR-regulated genes [40, 78]. Other studies demonstrated that

there is a direct interaction between the AR DNA binding domain and TCF-4 and that endogenous AR is bound to the TCF responsive element in the *MYC* promoter [79]. Ectopic expression of TCF-4 by transfection repressed the transcriptional activity of full-length AR, which was only partially attenuated by β-catenin transfection [79]. AR activator of 55 kDa (ARA-55), also named hydrogen peroxide-induced clone 5 (HIC-5), belongs to the paxillin family of LIM proteins and is also a component of the focal adhesion complex [80, 81]. ARA-55 can bind to AR resulting in an increase of AR transcriptional activity and in turn alter ligand specificity of AR [80, 81]. The LIM domain-containing C-terminal half of ARA-55 binds to a conserved alternatively spliced exon in LEF/TCF transcription factor and functions as negative regulator of a subset of LEF/TCF family members [80, 81]. Possible function significance of these different LEF/TCF transcription complexes requires further investigation.

The Lymphoid Enhancer Factor 1 (LEF-1) is another member of TCF/LEF family and also a Wnt target gene [82]. Li and Lee et al. reported that *LEF1* mRNA levels are up 100 fold higher in LNCaP AI cells (a CRPC derivative of LNCaP cells) compared to the parental LNCaP cells as determined by a microarray gene expression profiling analysis [82]. LEF-1 expression was associated with increased cell proliferation, migration, and invasion as well as AR expression [82, 83]. LEF-1 is usually expressed in the basal epithelial layer of the urogenital sinus in the human fetal prostate and in the urogenital mesenchyme and the basal epithelial layer of the urogenital sinus in mouse prostate development. The survival of LEF-1-expressing basal cells was not affected by treatment with the anti-androgen bicalutamide. Moreover LEF-1 can repopulate the luminal compartment following bicalutamide-induced regression of branching morphogenesis in the absence of androgen signaling [84]. This result suggested that LEF-1 may be involved in producing an androgen-independent population of prostate progenitors. TMPRSS2-ERG fusion protein has recently been shown to be reactivated in CRPC [85, 86]. Wu et al. [87] provided evidence that LEF-1 is a critical transcriptional target of the ETS-family transcription factor ERG and that LEF-1 expression is selectively upregulated in TMPRSS2-ERG fusion positive prostate cancer as observed by microarray profiling analysis. The combined results suggested that LEF-1 and Wnt signaling may provide a good novel targets for treatment of prostate cancer patients with TMPRSS2-ERG fusion expression.

Expression profiling and second generation seqencing identifies activation of the Wnt pathway in CRPC

Recent gene expression profiling analyses have allowed for the identification specific molecular signatures that are associated with CRPC. Wang et al. [20] performed affymetrix genechip analysis using LNCaP xenografts and hollow fiber models to identify global changes in gene expression profiling associated with CRPC. They found that the Wnt/β-catenin signaling pathway was one of the major pathways activated in CRPC. Rajan et al. [35] performed RNA sequencing (RNA-seq) profiling on tumour-rich, targeted prostatic biopsies from 7 patients with locally advanced or metastatic prostate cancer before and approximately 22 weeks after ADT. The results showed that 29 (e.g. FZD4, FZD7, JUN, and MMP7) out of 150 genes in the Wnt signalling pathway were upregulated after ADT,

which was the top pathway with significantly upregulated genes. In addition, 14 of these upregulated genes were reported in previously published studies on ADT-driven gene expression changes [88, 89]. These data suggested that the Wnt signaling pathway is predominantly upregulated in CRPC.

By using whole-exome sequencing technology to compare castration-resistant and androgen-sensitive matched pairs of prostate cancer xenografts derived from the same site of origin, Kumar et al. [90] found that 86 gene mutations were unique to CRPCs. Among them, there was a significant enrichment of mutations in the components of the Wnt pathway in CRPC tumors, including FZD6, GSK3B and WNT6. Furthermore, Grasso et al. [34] performed whole-exome sequencing on 50 metastatic CRPCs obtained at rapid autopsy (including three different foci from the same patient) and 11 treatment-naïve, high-grade localized prostate cancers. They also identified components of the Wnt signaling pathway to be significantly mutated (57 somatic mutations in 38 samples) in CRPC, on the other hand, Wnt pathway alterations were virtually absent in hormone naïve primary prostate cancer. In conclusion, these results suggested that mutations in the Wnt pathway likely emerge during progression or the development of resistance after ADT in prostate cancer.

Wnt paracrine signaling from prostatic stroma affects prostate epithelium in the settings of resistance to ADT or chemotherapy

There are emerging studies indicating that Wnt paracrine signaling from neigboring prostatic stroma cells could affect prostate epithlium during prostate cancer initiation and development of resistance to ADT or chemotherapy [36, 37, 91]. Using a tissue recombination method, Zong et al. [92] showed that high-mobility group AT-hook 2 (HMGA2) overexpressing urogenital sinus mesenchymal (UGSM) promoted mutifoci prostatic intraepithlial neoplasia (PIN) in the neighboring naïve epithelium. Co-overexpression of HMGA2 and AR in UGSM cells synergistically induced poorly differentiated prostate adenocarcinoma. Wnt ligands (*i.e.* WNT2, 4 and 9A) were shown to be the major paracrine factors from HMGA2 overexpressing UGSM. HMGA2-induced PIN formation was strongly inhibited by overexpression of the Wnt antagonists DKK1 and SFRP2, both secreted products. This study suggested that Wnt paracrine signaling may play an important stroma contribution to prostate cancer initiation and progression. In a related study, Li et al. [36] demonstrated that stroma-specific knockout mice for the TGF-β type II receptor expression (Tgfbr2^{fspKO}) increased the expression of Wnt3a to promote PIN formation and tumorigenesis, and that systemic treatment with Wnt3a neutralizing antibodies inhibited growth of LNCaP/Tg fbr2^{fspKO} xenografts. Placencio et al. [91] from the same group further demonstrated that the prostates of Tgfbr2^{fspKO} mice had constitutively active Wnt signaling regardless of androgen status. The prostates of Tgfbr2^{fspKO} as well as Tgfbr2^{fspKO} prostatic stromal cells/wild-type or SV40 large T antigen expressing epithelia recombinants were resistant to androgen deprivation-mediated regression. These results suggested that the paracrine Wnt signaling from Tgfbr2^{fspKO} prostate stroma cells not only facilitated the progression of PIN lesions to adenocarcinoma, but also conferred resistance to the epithelial component to androgen deprivation.

Liu et al. [93] studied the effect of dihydrotestosterone (DHT) on the interactions between preosteoblasts MC3T3 cells and bone metastasis cell line MDA-PCa-2b. They found that DHT exerted more potent growth stimulating effect on MDA-PCa-2b cells *via* upregulation of Wnt activity in bone cells. The effect was enhanced when the prostate cancer cells were cocultured with preosteoblasts compared to DHT treatment of MDA-PCa-2b cells alone. The enhanced growth of MDA-PCa-2b cells by DHT in this coculture experiment can be blocked by exogenous Wnt antagonists such as DKK-1 and SFRP-1 recombinant proteins. This result indicated a potential role of paracrine Wnt factors from bone cells on prostate cancer cell growth at bone metastatic sites. Sun et al. [37] showed that mitoxantrone and docetaxel therapy induced expression of stromal WNT16B and the elevated expression levels of WNT16B in prostatectomy tissue samples were associated with high risk of cancer recurrence. WNT16B expression in the stroma can also promote epithelial-to-mesenchymal transition (EMT) to increase tumor invasiveness and tumor growth. These findings provided a new mechanism of acquired resistance to chemotherapy drugs that is based on the properties of the tumor microenvironment.

Wnt ligands, receptors, secreted Wnt antagonists in prostate cancer

There is accumulating data showing over-expression of Wnt ligands and FZD receptors in prostate cancer potentially form autocrine or paracrine loops that support prostate cancer progression. The expression of WNT1 was detected in prostate cancer cells, tissues, lymph nodes and bone metastases, and the expression positively correlated with high Gleason scores and high serum PSA levels [48]. Elevated expression of Wnt agonists: WNT5A, WNT2, WNT6, and WNT11 have also been detected in prostate cancer tissues versus normal samples [23, 94-96]. Wnt agonists like WNT5A and WNT11 can induce the non-canonical Wnt pathway (e.g. through the JNK pathway) [23]. Interestingly, WNT11 inhibited androgen-dependent but not androgen-independent prostate cancer cell growth [23]; whereas, WNT3A stimulation enhanced AR activity and prostate cancer cell growth in presence of low androgen levels [23]. WNT11 can be regulated by androgens and WNT11 can induce expression of neuroendocrine differentiation markers NSE and ASCL1 as well as promote cell invasion [97]. WNT5A can also activate Wnt/Ca^{2+} pathway via CaMKII [98]. Yamamoto et al. [99] showed that WNT5A overexpression enhanced cell invasion in prostate cancer cell lines (*i.e.* PC3 cells), which required the expression of Wnt receptors Frizzled-2 and Ror2. Abnormal expression of WNT5A was positively correlated with high Gleason scores and biochemical relapse of prostate cancer [99]. Using transgenic mouse models, Takahashi et al. [100] demonstrated that the introduction of the AR with the T877A mutation into epithelial cells of the TRAMP mice resulted in an accelerated onset of tumor formation and tumor growth, moreover, this effect of the AR T877A mutation can be blocked by crossing Wnt-5a haploinsufficient mice with the TRAMP mice. In contrast, Syed Khaja et al. [101] reported that overexpression of WNT5A protein in patients with localized prostate cancer was shown to predict a favorable outcome after surgery. Recombinant WNT5A treatment of 22Rv1 and DU145 cells resulted in a decreased invasion [101]. These results suggested that Wnts may act differentially in a context-dependent maner during prostate cancer progression.

There are several Wnt receptors that are expressed in normal prostate tissues which includes FZD-1, -4, -6, and -10. Out of these receptors, expression of FZD-4 and FZD-6 were found to be increased in prostate tumors [102-105]. Gupta et al. [106] reported that ERG oncogenic transcriptional factor regulated the expression of FZD-4, which mediated epithelial-to-mesenchymal transition in prostate cancer.

Secreted Wnt antagonists, which include the secreted frizzled-related protein (sFRP) family, Dickkopf (DKK) family, and Wnt inhibitory factor-1 (WIF-1), are negative modulators of Wnt signaling [107-109]. Wnts bind to FZDs via cysteine-rich domain (CRD) sequence with high affinity. Expression of CRD alone can inhibit Wnt/β-catenin signaling [107]. All sFRPs contain a CRD sequence and can inhibit Wnt signaling either by sequestering Wnt ligands or by forming nonfunctional complexes with Frizzled receptors [107]. The Dkk family proteins include DKK-1, -2, -3, and -4 in humans [108]. DKK-1 inhibits Wnt signaling by disrupting the binding of LRP6 to the Wnt/FZD ligand-receptor complex [108]. Although WIF-1 does not share any sequence similarity with the CRD sequence of FZDs and sFRPs, it can also bind to Wnts and inhibit signaling [109]. Down-regulation of sFRPs, DKKs and WIF-1 by gene deletion or promoter hypermethylation are frequently detected in many human cancers including prostate cancer [110-114], suggesting their possible role as tumor suppressors. Endogenous Dkk-3 was found to be required to limit cell proliferation both in the developing mouse prostate and in 3D cultures of human prostate epithelial cells [115]. DKK-3 was further shown to regulate the response of normal prostate epithelial cells to transforming growth factor-β (TGF-β) [116]. These studies are consistent with a model in which DKK-3 is required by normal cells to prevent the TGF-β-dependent switch from tumor suppressor to tumor promoter. In contrast, DKK-1 was shown to promote tumor growth and prostate cancer progression in part by suppression of p21 (CIP1/WAF1) through a mechanism independent of canonical Wnt signaling [117]. DKK-1 also inhibited Wnt induced osteoblastic activity [118]. DKK-1 appears to play a role in parathyroid hormone related protein (PTHrP) induced osteolytic activity and in transition from osteoblastic to osteolytic bone lesions [119]. The sFRP family member sFRP1 was also found to be down-regulated both in prostate cancer tissues and prostate cancer cell lines. SFRP1 can function as a negative regulator of the AR [120]. However, this effect of sFRP1 was not associated with Wnt inhibition [120]. Joesting et al. [121] found that sFRP1 was overexpressed in prostate cancer stromal cells and ovexpression of sFRP1 activated JNK pathway, but not the canonical Wnt pathway. Horvath et al. [50] reported that sFRP4 overexpression can decrease cell proliferation, anchorage-independent growth, and invasiveness in PC3 cells. SFRP3/FRZB was the first identied secreted Wnt antagonist during studies of the Spemann's organizer of *Xenopus* [122]. Our group has shown that expression of sFRP3/FRZB or WIF-1 in a CRPC cell line PC3 caused a reversal of epithelial-to-mesenchymal transition and inhibition of tumor growth by inhibition of canonical Wnt pathway [123, 124]. Taken together, these results suggested that the role of Wnt ligands and secreted antagonists work in context-dependent manner in different types of cells or by varied ligand-receptor interactions. Wnt ligands and receptors appear to be important in CRPC.

Wnt signaling in disease models of CRPC

In transgenic mouse models, conditionally deleted exon3 of β-catenin resulted in production of high-grade PIN (HG-PIN) and induction of Foxa2 re-expression in the adult mouse prostate through Wnt/β-catenin signaling as well as promoting prostate growth even under the conditions of androgen deprivation [125]. In mouse models with the SV40 large T-antigen, which inactivates p53 and Rb [126], or in mice expressing mutated K-ras and form invasive carcinoma [127], or in mice with loss of PTEN expression [128], β-catenin overexpression can promote highly invasive prostate cancer and squamous metaplasia, even in the absence of androgens. These findings provided strong evidence for a critical role of the Wnt/β-catenin signaling in prostate cancer development and progression.

In xenograft mouse models, H2 relaxin (RLN2) was shown to facilitate castrate-resistant growth of prostate cancer cells through AKT phosphorylation-mediated activation of both the Wnt/β-cateinin and the AR pathway [129]. As mentioned before Hic-5/ARA55 is a co-factor for both TCF/LEFs and AR and can inhibit the Wnt/β-catenin pathway [36]. Overexpresssion of HIC-5/ARA55 in LNCaP cells can restore sensitivity of xenograft composed of LNCaP cells and Tgfbr2-KO fibroblasts to androgen deprivation-induced tumor regression [130]. In an orthotopic C4-2B CRPC xenograft mouse model, Placencio et al. [131] found that mesenchymal stem cells (MSCs) were recruited into tumor sites and were associated with enhanced tumor growth. The result occurs with activation of Wnt signaling. When MSCs were used as a targeted delivery vector for the exogenously expressed sFRP2, tumor growth was reduced and the response to androgen deprivation was restored. These animal and tissue culture studies suggested that components of the Wnt signaling pathway may be involved in prostate cancer progression to more invasive phenotype and contributed to castration resistance.

Therapeutic potentials of targeting Wnt/β-catenin in CRPC and future directions

Although, the results remain inconclusive, β-catenin nuclear localization as well as its co-localization with AR has been more frequently observed in CRPC compared to hormone naïve prostate cancer. Alterations in multiple signaling pathways, including PI3K/AKT, HIF-1α, PIN1, *APC* gene silencing and more have been shown to cause nuclear localization of β-catenin and activation of Wnt signaling. Moreover, β-catenin acts as an AR co-factor to enhance androgen-stimulated AR transcriptional activation and increase sensitivity to low levels of androgens and to non-androgen ligands. As a result, the interaction between β-catenin and AR in CRPC may elicit specific target genes for promoting androgen-independent growth and progression. The next-generation sequencing technology (*i.e.* whole-exome and RNA sequencing) has revealed that the Wnt pathway is one of the top signaling pathways that were frequently mutated or genomically altered in lethal CRPC patients. Likewise, paracrine Wnt signaling also contributed to resistance to ADT after chemotherapy and androgen deprivation therapy.

In addition to high expression in cancer cells, Wnt/β-catenin signaling is highly activated in cancer stem cells (CSCs) [132]. Prostate CSCs are suggested to be resistant to androgen

deprivation therapy and responsible for cancer recurrence [133]. Targeting CSCs by inhibition of the Wnt pathway may have the potential to reduce the self-renewal and aggressive behavior of prostate cancer [134]. As a proof of princle, Lee et al. [135] demonstrated that a novel compound that disrupts both β-catenin/TCF and β-catenin/AR protein interactions can inhibit prostate tumor growth in a xenograft model and also blocked bicalutamide-resistant sphere-forming cells. This study indicated the potential of targeting the β-catenin/AR as a good treatment target for CRPC.

As described in this review, the Wnt signaling pathway plays a complex role in CRPC. Given the multiple important roles of Wnt signaling in CRPC, the Wnt signaling pathway can not be ignored as a source of therapeutic targets. Inhibition of the Wnt pathway would allow therapies to target not only epithelial cells but also stromal cells, as well as, CSCs, androgen-dependent, and androgen-independent prostate cancer cells. Future therapies for CRPC would most likely benefit from combination of both anti-androgens and Wnt inhibitors.

Acknowledgments

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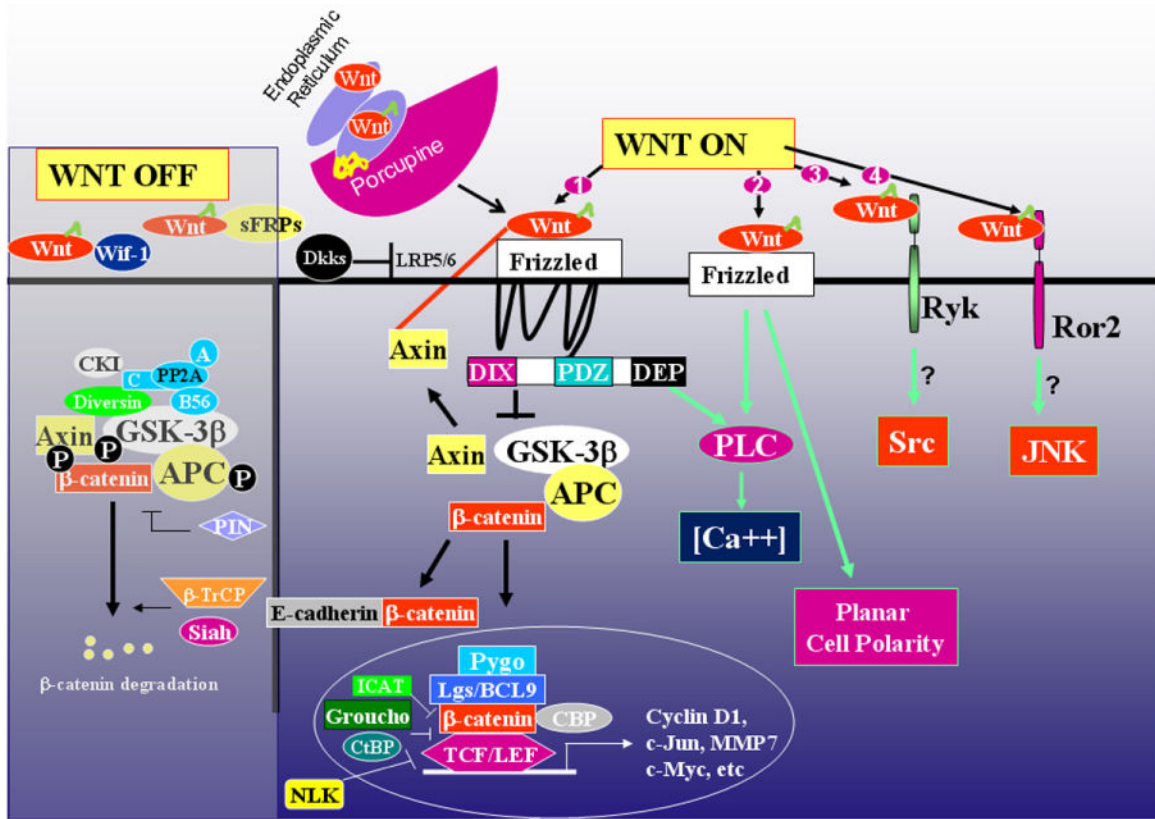
Figure 1.
Overview of the Wnt signaling pathway. In the "Wnt-Off" state, Wnt/receptor interactions are interrupted by secreted Wnt antagonists, including sFRPs, DKKs and WIF1; and β-catenin is degraded by the formation of the "destruction complex" consisting of APC protein, Axin and others. In the "Wnt-On" state, Wnts are are lipid modified by the acyl transferase porcupine in the endoplasmic reticulum, and act in an autocrine and paracrine fashion. The Wnts form a complex with Frizzled receptor and coreceptors LRP5/LRP6. Upon receptor activation, the "destruction complex" which includes APC, Axin and others is inhibited thereby blocking β-catenin phosphorylation for degradation. This inhibition results in cytoplasmic β-catenin stabilization and accummulation which facilitates its translocation into the nucleus. Nuclear β-catenin acts as a transcriptional co-activator for LEF1/TCF, leading gene transcription of Wnt target genes, such as JUN, Cyclin-D1, and MMP7. In addition, Wnts bind to tyrosine-protein kinase transmembrane receptors ROR2 and RYK to activate other non-canonical planar cell polarity, the Wnt/JNK and the Wnt/Calcium pathways.
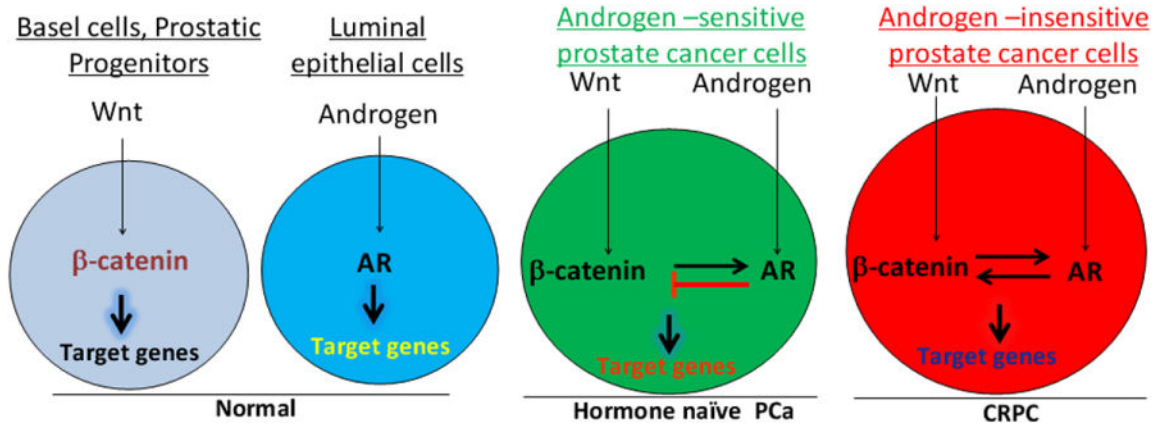
Figure 2.
A simiplified and hypothetical relationship between Wnt and AR signaling during prostate cancer development and progression. In the normal prostate, Wnt signaling maintains prostate progenitor cells through regulation of Wnt target gene transcription, whereas AR signaling only functions in secretory luminal epithelial cells. In hormone treatment naïve prostate cancer cells, Wnt signaling promotes transcription of AR target genes, while androgen signaling inhibits the transcription of Wnt target genes. In CRPC, AR and Wnt signaling reinforces each other to elicit specific target genes for promoting androgen-independent growth and progression.

Table 1

Proposed mechanisms for elevated Wnt signaling in CRPC

Mechanisms	References
Nuclear β-catenin localization	[32, 41]
AR/β-catenin interaction	[26, 32, 35, 68]
LEF1 overexpression	[83]
Crosstalk between β-catenin and AKT, HIF-1α and others	[69-75]
Overexpression of Wnt ligands and receptors	[23, 94-106]
Paracrine Wnt signaling	[36, 37, 91-93]
Loss of secreted Wnt antagonists	[136]
Epithelial to mesenchymal transition	[123, 124, 137]
Cancer stem cells	[134]

Table 2

Nuclear β-catenin localization in CRPC and hormone naïve prostate cancer

Sources	Percentage of positive staing	References
Metastatic tumors from autopsy	24% (5/21), nuclear	[40]
CRPC from TURP	38.8% (35/90), nuclear	[41]
CRPC bone metastases	40.7% (11/27), nuclear	[43]
CRPC matched pairs	55% (16/29), nuclear and cytoplasmic	[35]
Localized PCa from RP	23% (49/212), nuclear	[41]
Localized PCa from RP	18% (39/186), nuclear	[50]

PCa: prostate cancer; RP: radical prostatectomy; CRPC: castration resistant prostate cancer; TURP: transurethral resections of the prostate.