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WNT SIGNALING IN PROSTATE DEVELOPMENT AND CARCINOGENESIS

Gvantsa Kharaishvili[a], Dana Simkova[a], Eka Makharoblidze[b], Katerina Trtkova[a], Zdenek Kolar[a], Jan Bouchal[a]*

[a] *Laboratory of Molecular Pathology of Institute of Pathology, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic*
[b] *Centre for Clinical and Pathological Diagnostics, Alexandre Natishvili Institute of Morphology, Tbilisi, Georgia*
E-mail: jan.bouchal@gmail.com

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Background. The Wnt signaling pathway is crucial for cell fate decisions, stem cell renewal, regulation of cell proliferation and differentiation. Deregulated Wnt signaling is also implicated in a number of hereditary and degenerative diseases and cancer.

Methods and results. This review highlights the role of the Wnt pathway in the regulation of stem/progenitor cell renewal and prostate gland development and how this signaling is altered in prostate cancer. Recent evidence suggests that Wnt signaling regulates androgen activity in prostate cancer cells, enhances androgen receptor expression and promotes the growth of prostate cancer even after androgen ablation therapy. There is also strong evidence that Wnt signaling is enhanced in androgen-ablation resistant tumors and bone metastases.

Conclusions. Further study of the modulators of this pathway will be of therapeutic relevance as inhibition of Wnt signaling may have the potential to reduce the self-renewal and aggressive behaviour of prostate cancer while Wnt signaling activation might enhance stem cell activity when tissue regeneration is required.

INTRODUCTION

The Wnt signaling pathway is crucial in a variety of biological processes including cell fate decisions, neural patterning, planar cell polarity, stem cell self-renewal, cell proliferation, differentiation, migration and apoptosis[1,2]. This has been shown in a number of systems using genetic and biochemical approaches. Deregulated Wnt signaling, on the other hand, is implicated in a number of hereditary and degenerative diseases such as early coronary disease[3], late onset Alzheimer's disease[4], type II diabetes[5], familial tooth agenesis[6], hereditary malignancies such as Wilms tumor[7] and other cancers. Apropos prostate cancer, alterations in Wnt signaling have been recently reviewed[8-12]. This review focuses on normal prostate development and, normal and cancer stem cells.

The term Wnt derives from a contraction of the gene name Wingless, first identified in the development of the fruit fly *Drosophila*, and the proto-oncogene Int-1 (Integration 1) which was first isolated in mammary tumor models in the mouse[13]. Most Wnt genes, of which the human genome has almost twenty, have orthologs throughout the animal kingdom - from metazoa to mammals.

Wnt signaling is currently known to include two major pathways: 1) the canonical or Wnt/ β-catenin pathway (Fig. 1), and 2) the non-canonical pathways which do not involve β-catenin stabilization. There is also a pathway which controls the orientation of mitotic spindles in *Drosophila* and *Caenorhabditis elegans* but this has not yet been found in vertebrates[14].

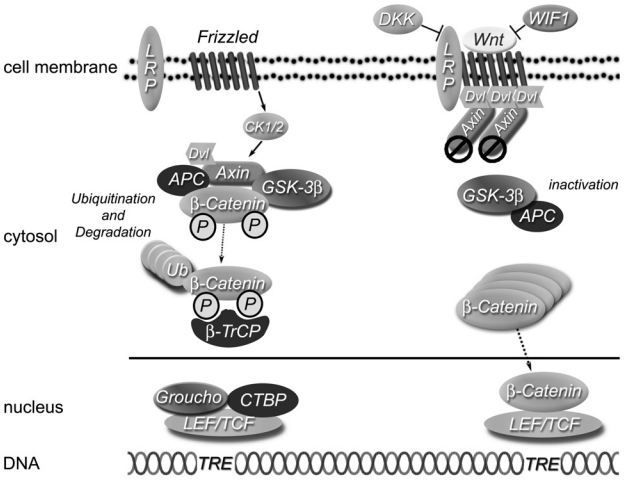

Fig. 1. Canonical Wnt signaling pathway. In the absence of Wnt signal, action of the destruction complex (CKI/2, Dvl, GSK3β, APC, Axin) creates a hyperphosphorylated β-catenin, which is a target for ubiqitination and degradation by the proteasome. Wnt stimulation triggers Dvl recruitment to the plasma membrane by Frizzled (Fz) receptors. Dvl polymers at the membrane serve as a dynamic scaffold for Axin recruitment and inactivation. Overall, binding of Wnt ligand to a Frizzled/LRP-5/6 receptor complex leads to stabilization of hypophosphorylated β-catenin, which interacts with TCF/LEF proteins in the nucleus and activates transcription.

CANONICAL WNT SIGNALING PATHWAY

The canonical Wnt pathway regulates cellular responses through β-catenin. In the absence of Wnt ligand binding (Wnt signal), β-catenin is phosphorylated by CK1γ (casein kinase 1γ) and a multicomponent destruction complex [containing scaffolding proteins GSK3β (glycogen synthase kinase 3 beta), AXIN and adenomatosis polyposis coli (APC) protein]. Phosphorylated β-catenin is recognized by the E3 ubiquitin ligase β-TRCP (β-transducin repeat-containing protein) and targeted to rapid degradation in the cytoplasm through the ubiquitin proteasome pathway. Low nuclear levels of β-catenin are maintained by nuclear exporters APC and AXIN which shuttle β-catenin from nucleus back to the cytoplasm[1]. Without the AXIN-based scaffold, β-catenin escapes capture, phosphorylation and ubiquitination[8,15].

Wnt signaling is initiated at the plasma membrane where Wnt ligands form ternary complexes with their respective frizzled receptors and single-pass transmembrane coreceptors LRP5 and LRP6 (low-density lipoprotein receptor-related proteins 5 and 6). Co-factors such as R-spondin and Wise also take part in Wnt-receptor complex activity. Signaling from Wnt receptors proceeds through the protein Dishevelled (Dvl). It is recruited to the plasma membrane, interacts with frizzled receptors and polymerizes with other Dishevelled molecules[16,17]. Phosphorylation of the cytoplasmic tail of LRP5 or LRP6 and the formation of the Dishevelled polymer serve as mediators for the translocation of AXIN to the plasma membrane and inactivation of the destruction complex. Thus β-catenin gradually accumulates in cytoplasm and enters the nucleus where it forms a complex with the TCF/LEF family of transcription factors.

Before Wnt signal activation, TCF/LEFs are bound to DNA promoter and enhancer regions of target genes, and along with Groucho and C-terminal binding protein (CtBP), often repress gene expression. Repression by LEF1 requires histone deacetylase (HDAC) activity. Binding of β-catenin to transcription factors transactivates downstream target genes, such as c-myc, cyclin D1, urokinase-type plasminogen activator (uPA), MMP-7, CD44, survivin, endothelin-1, Cox-2 and -9, versican, periostin, fibronectin, the androgen receptor gene and others. These influence cell cycle regulation, invasion and metastasis[18–23].

β-catenin–TCF activity can be modified by two kinases: 1) transforming growth factor β-activated kinase (TAK1) and 2) NEMO-like kinase (NLK)(ref.[24]). These are mammalian mitogen activated protein (MAP) kinase pathway components. TAK1 activates NLK and the latter phosphorylates members of the TCF family. Phosphorylation alters the DNA-binding properties of the β-catenin–TCF complex and in this way blocks Wnt target gene activation. Input from the MAP kinase pathway can thus negatively regulate the Wnt pathway in mammalian cells. Besides TCF/LEF, Wnt signaling can modify other transcription factors, e. g. β-catenin acts as a binary switch to simultaneously activate expression of NFκB.

Corresponding to its dual functions in the cells, β-catenin is localized in two cellular pools. A smaller pool of β-catenin is located in the nucleus and cytoplasm where it mediates Wnt signaling. Most of the β-catenin is located in the cell membrane where it is associated with the cytoplasmic region of E-cadherin, a transmembrane protein involved in homotypic cell-cell contacts – adherens junctions[25–27].

NONCANONICAL (β-CATENIN-INDEPENDENT) WNT SIGNALING PATHWAY

Wnts such as Wnt4, Wnt5a, and Wnt11 do not liberate β-catenin but signal noncanonically. The best characterized of these "noncanonical" pathways are the Wnt/Ca2+ pathway which was first described in vertebrates[28], and the planar polarity pathway which was first identified in *Drosophila*[29]. Other noncanonical pathways include Wnt/Jnk and Wnt/ Rho signaling[30].

Vertebrate noncanonical Wnt signaling requires frizzled receptors. Wnt ligand binding to frizzled can increase levels of intracellular calcium and activate two Ca2+-sensitive kinases: 1) calcium/calmodulin-dependent protein kinase II (CAMK2) and 2) protein kinase C (PKC). G-proteins and *Drosophila* dishevelled are also involved in signal transduction by Wnt/Ca2+ pathway and signaling specificity may be achieved via co-receptors, such as Knypek and Ror2 (ref.[31]). This pathway has been implicated in cell movement processes required for embryonic patterning[32–34]. It also functions in promoting ventral cell fate and antagonizing dorsal cell fate during early *Xenopus* development, in regulating gastrulation movements or heart and muscle development[35].

Noncanonical Wnt signaling, transduced to a variety of Dvl- or Ca^{2+}-dependent cascades, can also overlap with the planar cell polarity (PCP) signaling pathway. Wnt5a and Wnt11 that are involved in PCP signaling can also activate calcium signaling[36]. Some PCP proteins, including *flamingo* (CELSR2), become localized to both the proximal and distal sides of the cell. Others, however, including *frizzled*, *dishevelled* and Rho, become localized specifically to the distal side, whereas *prickle homolog 1* (PRICKLE1) and *strabismus* (STBMS1) become localized to the proximal side. The function of all of these proteins is required to ensure both correct segregation into proximal and distal domains and the subsequent development of correct planar polarity[30]. In vertebrates, this pathway requires Wnt ligands, such as *silberblick* (Wnt11 precursor) and *pipe tail* (Wnt5b), whereas no Wnt ligand is known to be involved in *Drosophila* PCP signaling. In vertebrates, the PCP pathway regulates many aspects of development including neural tube closure[30,37], inner ear development and hair orientation[38,39].

Sometimes Wnt-receptor interaction requires recruitment of additional co-factors. For example, secreted collagen glycoprotein, CTHRC1 can promote the formation of a Wnt-frizzled-Ror2 complex, leading to activation of the PCP pathway[40,41]. The Wnt-frizzled interaction may

also be enhanced by proteoglycans, such as protein Dally in *Drosophila*, or inhibited by secreted proteins including *dickkopf 1* (DKK1), *cerberus* (CER1) and SFRPs (secreted frizzled-related proteins).

In some instances, noncanonical Wnt pathway can inhibit canonical Wnt signaling. One example is competition for Dishevelled molecules, that are shared between the two pathways[30]. Another example involves the Wnt5a-induced transcriptional upregulation of Siah2 which can stimulate β-catenin degradation[42].

ROLE OF WNT SIGNALING IN PROSTATE DEVELOPMENT

Prostate development, growth and function is androgen dependent, however, other steroid receptors, such as estrogen receptors (ER) and retinoid receptors (RARs and RXRs), also contribute to prostate morphogenesis and differentiation[43]. Prostate development begins prenatally and lasts until the end of puberty. At 10 weeks of gestation, androgens produced by the fetal testes induce morphogenesis of the endodermal prostatic buds which grow into the surrounding urogenital sinus mesenchyme (UGM), lengthen and arborize to form a complex ductal network. Prostatic buds arise from different parts of the endodermal urogenital sinus and form various prostatic lobes whose ductal branching patterns are unique for each prostatic lobe. The immature prostatic acini and ducts are lined with multiple layers of immature cells that express cytokeratins[44]. During prenatal development, the UGM expresses high levels of androgen receptor (AR) which are initially undetectable in the epithelium of the developing male urogenital tract but are expressed postnatally, several days before the epithelium initiates production of tissue-specific secretory proteins[43]. The pubertal period is marked by androgen-driven increase in gland size, further branching and differentiation of immature prostatic epithelium into the adult-type (outer cuboidal basal and inner cylindrical secretory cells). Mature luminal cells constitute the exocrine part of the prostate and secrete PSA (prostate specific antigen) and PAP (prostate acid phosphatase) while AR appears in the secretory cell layer. In the adult prostate, androgens act on stromal and secretory epithelial cells, except for basal cells. These are relatively undifferentiated, express low or undetectable levels of AR and are androgen-independent.

In the developing prostate model, Wnt signaling regulates prostatic epithelial branching morphogenesis, luminal epithelial cell differentiation and proliferation of prostate epithelial progenitor cells. Wnt5a mRNA is expressed at the distal tips and along the centro-distal periductal UGM during branching morphogenesis and is focally upregulated as buds emerge from anterior, dorsolateral and ventral UGS regions. Abnormal UGS morphology, bud patterns and decrease in prostatic bud number have been observed in Wnt5a null recombinant fetuses[45]. Prostate buds were decreased in Wnt5a-/- mice but this was secondary to testosterone deficiency as Wnt5a-/- UGS cultured in DHT grew normally. During prostate growth, Wnt5a may be secreted from the mesenchyme and act as an inhibitor of UGE growth as addition of ectopic Wnt5a to wild-type UGS in culture inhibits growth of the ventral prostate. Wnt5a can also modulate canonical Wnt signaling through downregulation of WIF1 in neonatal rat prostate[46].

Rat ventral prostate cultures on postnatal day 2 (P2) treated with 50 nM of Wnt ligand Wnt3a showed blunted and enlarged ductal tips at 7th day, whereas control cultures displayed extensive branching with primary, secondary and tertiary ducts and reduced number of fine branches. DKK1 treated prostates showed poor epithelial branching and in contrast to Wnt3a-treated prostates, they lacked enlarged ductal tips[47]. The highest level of AXIN2 is on P2, consistent with a higher progenitor cell population and declines over time according to prostate maturation when the majority of the epithelial cells are terminally differentiated luminal cells[47]. Other Wnt genes were also observed to be highly expressed on P3 in ventral lobes including three canonical Wnts (Wnt2, Wnt2b and Wnt7b), three non-canonical Wnts (Wnt4, Wnt5a and Wnt11), Fzd2 and 4 and Dvl. Except for Wnt7b, all genes showed high expression at birth with levels declining during and after the completion of morphogenesis[48]. Along with spatially restricted expression, these dynamic temporal expression profiles suggest important roles for these morphogens during prostate gland development.

Expression analysis of SFRP1 shows high signal in developing mesenchyme-stroma of the prostate. SFRP1 null prostates exhibited multiple developmental defects in the epithelium such as reduced branching morphogenesis, delayed proliferation, and increased expression of genes encoding prostate-specific secretory proteins. In contrast, over-expression of SFRP1 in the adult prostates of transgenic mice showed prolonged epithelial proliferation and decreased expression of the secretory genes[49]. Another antagonist of the Wnt pathway, SFRP2, was shown to be expressed on embryonic day 16.5 UGS and downregulated according to prostate maturation[50]. Embryonic regulation of prostate development was also studied by Schaeffer et al.[51]. These authors comprehensively profiled androgen-induced gene expression changes in both pharmacologically virilized female UGS after injection with dihydrotestosterone and in physiologic male prostate development at embryonic day 17.5 after the onset of androgen-induced transcriptional changes[51]. Wnt pathway was among the highly regulated processes, however, functional experiments are needed for clarification of the role of particular genes.

Better understanding of the complex regulation of prostate development has been provided by tissue recombination techniques using TGF-β type II receptor conditional knockout mouse with loss of TGF-β responsivity in the mesenchymal compartment ($Tgfbr2^{KO}$)(ref.[52]). UGM from $Tgfbr2^{KO}$ or control mice was recombined with wild-type adult mice bladder urothelial cells. The urothelium associated with $Tgfbr2^{+}$ UGM was instructively differentiated into prostatic epithelium, as expected. In contrast, the urothelium associated with $Tgfbr2^{KO}$ UGM permissively maintained the phenotype of bladder

epithelial cells. Microarray analysis of UGM tissues suggested down-regulation of multiple Wnt ligands and up-regulation of the Wnt antagonist, WIF-1, in the Tgfbr2^{KO} UGM compared to Tgfbr2^{+} UGM. Furthermore, WIF-1 lentivirus overexpression in the wild-type UGM resulted in the inhibition of prostate epithelium induction. These results suggested that paracrine Wnt signaling mediates the role of TGF-β in the inductive effects of the UGM on the adjacent epithelium[52].

WNT SIGNALING AND PROSTATE STEM CELLS

Human prostate stem cells (PSCs) reside within the basal cell compartment of the gland[53]. This idea is supported by an experiment where mice null for the basal marker p63 are born without prostate or mammary gland[54]. Several other molecular markers of the prostate stem cells have recently been proposed, including α2β1 integrin, CD133 and Sca-1 (Stem cell antigen 1). Human α2β1^{hi}/CD133^{+} enriched cells, established and maintained prostate epithelium when transplanted into an immunodeficient mouse, although entire functional prostate did not develop[55]. More recently, Lawson et al.[56,57] enriched murine prostate epithelial stem cells with Lin(CD45/CD31/Ter119)$^{-}$Sca-1^{+}CD49f1^{+} cell surface profile by FACS (fluorescence-activated cell sorting). These cells showed low levels of luminal cell markers (NKX3.1, cytokeratins 8 and 18) but expressed high levels of CK5, CK14 and p63 and possessed basal-like phenotype. They both formed spheres *in vitro* and developed ductal structures *in vivo* prostate regeneration assay. The cells were also further used for testing their oncogenic potential following genetic manipulation (see below).

It is generally believed that the Wnt with FGF, Notch, Hedgehog, and TGFβ/BMP signaling networks is implicated in the maintenance of tissue homeostasis by regulating self-renewal of normal stem cells as well as proliferation or differentiation of progenitor cells[58,59]. However, evidence of Wnt signaling involvement in prostate morphogenesis is based on limited studies. The immunohistochemical analysis of rat prostate organ cultures using basal (p63) and luminal (CK8) cell markers, showed that modulation of Wnt signaling can influence differentiation of progenitor cells into luminal cells. More p63 positivity was seen in the ductal region of Wnt3a-treated cultures while fewer p63 positive cells were present in DKK1-treated cultures. CK8 immunostaining was complementary. These findings suggest that Wnt signaling regulates the terminal differentiation of basal cells into luminal cells by controlling the proliferation and/or maintenance of epithelial progenitor cells[47]. Array experiments defined more Wnt proteins and ligands for different PSC populations. In order to identify molecules and pathways that are active in primitive prostate populations, Blum et al.[60] determined the transcriptional profiles of four populations of cells: (i) urogenital epithelium from 16-day embryos, representing fetal PSC, (ii) Sca-1^{High} cells, enriched in adult PSC, (iii) Sca-1^{Low}, representing transit-amplifying cells, and (iv) Sca-1^{Neg} cells that represent the most mature population and have almost no regenerative potential. Increased expression of many Wnt signaling molecules was observed in both fetal and adult PSC which means that adult PSC acquire characteristics of self-renewing primitive fetal prostate stem cells which in turn might also be characteristic of oncogenesis[60].

In another work Blum and colleagues[61] generated a list of transcripts differentially expressed in embryonic UGE versus UGM. They used computational gene expression analysis to decipher the potential transcriptional factors and the main biological functionalities that characterize the embryonic prostatic stem cell niche. These authors also established several ligand-receptor interactions relevant in controlling signals in the stem cell niche. The Wnt pathway has the greatest ligand-receptor representation in the prostate stem cell niche. Proposed prostate stem cell niche genes, such as Fzd6, Wnt2 and Wnt4, are also differentially expressed by other primitive niches such as embryonic dermis and epidermis[61].

Epigenetic or genetic alterations of stem cell related genes, give rise to cancer stem cells[56,62]. Although basal stem/progenitor cells have been proposed to represent a cell type of cancer origin, human prostate cancer has a markedly luminal phenotype[56]. Wang et al.[63] showed by genetic lineage-marking that rare luminal cells (CARNs, castration-resistant Nkx3–1 expressing cells) are bipotential and can self-renew *in vivo*. Single-cell transplantation assays also showed that CARNs can reconstitute prostate ducts in renal grafts. Further, inducible deletion of the PTEN tumor suppressor in CARNs resulted in rapid carcinoma formation after androgen-mediated prostate regeneration[63].

On the other hand, Lawson et al.[57], (see above) found basal/stem cells more efficient targets of oncogenic transformation than luminal cells. FGF10 paracrine stimulation of basal but not luminal cells resulted in multifocal adenocarcinoma. Similarly, overexpression of AKT1 or ERG1, the most frequent partner for chromosomal translocations with TMPRSS2 in prostate cancer, in the basal/stem cells resulted in PIN lesions. Importantly, large tumors were generated from basal but not luminal cells upon combined activation of AKT1 and AR[57,64]. These studies pose the question, if prostate cancers indeed originate from different cell types (e.g., the basal or luminal compartment) and the resulting tumors might have different genetic profiles, biologic behavior and therapeutic responses.

Another study has demonstrated the role of Wnt signaling pathway on the tumorigenic potential of different cell subpopulations[65]. Self-renewing prostaspheres, formed by PCa cell lines expressing proliferation, differentiation and stem cell-associated markers CD44, ABCG2 and CD133, were treated with Wnt inhibitors. Prostaspheres revealed reduction in their size and self-renewal. In contrast, addition of Wnt3a caused increased prostasphere size and self-renewal associated with increased nuclear β-catenin, CK18, CD133 and CD44 expression. LNCaP and C4–2B prostate cancer cell lines expressing androgen receptor were treated by its antagonist bicalutamide which led to reduced prostasphere size and expression of

PSA but did not inhibit prostasphere formation[65]. These effects are concordant with the androgen-independent self-renewal of cells with stem cell characteristics and androgen-dependent proliferation of transit amplifying cells.

WNT SIGNALING ABNORMALITIES IN HUMAN PROSTATE CANCER

Aberrant activation of Wnt signaling in tumorigenesis has frequently been reported. The prime example is colorectal cancer, in which approximately 85% of cases display loss-of-function mutations in the tumor supressor APC gene[26]. The APC protein is an essential component of the APC/AXIN complex that degrades β-catenin and is a negative regulator of Wnt signaling. The "gatekeeper" role of APC in tumorigenesis is largely confined to the colon and although mutations in APC have been reported in other cancers, their incidence is relatively rare. Mutations in β-catenin, however, are much more widespread and the mutated form is more refractory to phosphorylation and degradation. In prostate cancer, mutations of serine or threonine residues at the N-terminus of the β-catenin protein have been detected in 5% of tumors[66]. In another study, over 20% of advanced prostate tumors have elevated levels of β-catenin[67]. Mutations were present focally and therefore might occur during tumor progression suggesting that alteration of β-catenin may represent a late event in prostate cancer. Specific expression of a mutant β-catenin lacking exon3 resulted in development of hyperplasia, squamous cell transdifferentiation and prostate intraepithelial neoplasia (PIN)(ref.[68]). Protein hTRCP which is involved in the degradation of β-catenin, may also be mutated in rare cases[67]. The Wnt pathway may also be disrupted by mutation of the gene encoding Axin. Three new potentially relevant Axin1 mutations and four different Axin polymorphisms were found in PCa cell lines[69]. Apart of the Wnt pathway, Axin acts as a scaffold protein regulating specific protein phosphorylation in TGFβ and SAPK (stress-activated protein kinase) signaling pathways[70].

In prostate cancer, cadherin-catenin interaction in the cell membrane is abnormal. Cadherin-mediated cell-cell adhesion is lost, and consequentially cytoplasmic and nuclear levels of β-catenin increase[71]. Using an E-cadherin-negative prostate cancer cell line, TSU.pr-1 was shown that β-catenin is capable of augmenting AR-mediated transcription, and the effect of β-catenin on AR can be enhanced by the loss of E-cadherin expression[72]. This may promote AR-mediated cell growth in high grade prostate cancer. Despite these data, the degree of E-cadherin expression in prostate cancer remains controversial[73]. Other relationships between Wnt signaling and androgen receptor have been recently reviewed[10,15]. Briefly, AR activity may be affected by GSK-3β kinase activity[74,75] and the AR gene is also a direct target of LEF-1/TCF transcriptional regulation[76]. Wnt inhibitor SFRP1 has recently been reported as a negative regulator of androgen receptor activity[77] (also see below). Interestingly, Wnt signaling is upregulated following castration but downregulated after androgen replacement[47,78]. Last but not least, Wnt signaling regulates the self-renewal of prostate cancer cells with stem cell characteristics independently of androgen receptor activity[65].

The expression levels of several Wnt ligands were found to be altered in advanced prostate cancer cells. High levels of both Wnt1 and β-catenin were associated with advanced, metastatic, hormone-refractory prostate carcinoma whereas normal prostatic tissue failed to exhibit any detectable nuclear staining for β-catenin[79]. Other studies have also reported that Wnt1 is expressed in prostate cancer cell lines and appears to be elevated in some human prostate tumor tissues, in lymph nodes and bone metastases[80]. Overexpression of Wnt ligands such as Wnt2 and Wnt5a was also found in human prostate tumors[81,82]. Wnt5a increased free intracellular calcium and CaMKII elevated in prostate cancer cells lines, indicating that Wnt/Ca2+ pathway operates via CaMKII in PCa. The latest induced cytoskeleton reorganization and increased cell motility[82]. In this sense, overexpression of Wnt5a in human prostate cancer cell lines stimulated their invasion activities[83]. Abnormal expression of Wnt5a was also observed in 28% of prostate cancer by immunohistochemistry and positivity was correlated with high Gleason scores and biochemical relapse of prostate cancer. Another non-canonical Wnt-11 which can be regulated by androgens, was also increased in advanced prostate cancer and bone metastases[84,85]. Further, Wnt-11 induced expression of neuroendocrine differentiation (NED) markers NSE and ASCL1, while silencing of Wnt-11 in androgen-depleted LNCaP and androgen-independent PC3 cells prevented NED and resulted in apoptosis. In addition, silencing of Wnt-11 reduced PC3 cell migration and ectopic expression of Wnt-11 promoted LNCaP cell invasion[85].

Wnt inhibitors are secreted proteins that block Wnt signaling either by binding to Wnts themselves (SFRP family and WIF1) or to the LRP5/6 Wnt co-receptors (Dickkopf family). Down-regulation of the Wnt inhibitors DKKs and SFRPs occurs frequently in human cancers and most reports show that loss of expression of these inhibitors is mainly caused by promoter hypermethylation[86]. WIF1 mRNA appears to be downregulated in a considerable percentage of prostate cancer samples[81]. Functionally, Horvath et al.[87] showed that SFRP4 overexpression was associated with decreased proliferation, decreased anchorage-independent growth and decreased invasiveness of PC3 cells. SFRP1 was reported as downregulated both in prostate cancer and cell lines[88]. Kawano et al.[77] found SFRP1 as a negative regulator of the androgen receptor but this was mediated via neither canonical nor known non-canonical Wnt pathways. A physical interaction of SFRP1 with Frizzled receptors was found and a novel, so far uncharacterized pathway which might be shared with Wnt5a, has been suggested[77,89]. The role of SFRP1 may depend both on cell and receptor specific context as Joesting et al.[90] reported elevated expression of SFRP1 in fibroblasts derived from prostate cancer stroma.

One of the hallmarks of advanced prostate cancer is the development of bone metastases. Autopsy studies have found that approximately 80% of prostate cancer patients

had established macrometastases involving bone, and 90% of these bone lesions had an osteoblastic phenotype[91]. Cancer-mediated modulation of Wnt activity influences bone remodeling and Wnts are being considered as possible bone anabolic agents. In vivo experiments showed that stable DKK1 (the dickkopf homolog 1; inhibitor of Wnt pathway) expression in prostate cancer cells blocked Wnt-induced osteoblastic activity and bone lesions produced by DKK1-transfected C4–2B cells were highly osteolytic[75]. Keller et al.[92] established that advanced prostate cancer cells acquire mesenchymal and even osteoblastic characteristics but they might also be accompanied by osteolytic activity. One of a variety of factors implicated in this process is parathyroid hormone related protein (PTHrP) which has been shown in vivo to enhance the formation of osteolytic metastases presumably through induction of osteoclast activity[93]. Activated osteoclasts in the bone microenvironment, secrete large amounts of PGE_2 (Prostaglandin E_2) that can increase Wnt-inhibitor expression by both PCa and osteoblast-lineage cells in the early, osteolytic phase of PCa bone metastases. Study of dose-dependent effects of PGE_2 on components of the Wnt signaling pathway in pre-osteoblasts and in human PCa cells showed that low dose PGE_2 increased MC3T3 pre-osteoblast proliferation and expression of LRP5/6, β-catenin, RUNX2, BMP-2, EP1 (E series of prostaglandin receptors)-1 and -4. In contrast, higher doses of PGE_2, inhibited MC3T3 cell proliferation and differentiation. Higher doses also inhibited the expression of LRP 5/6, RUNX2, BMP, EP1 and EP2 receptor subtypes and increased the expression of two soluble Wnt inhibitors, Dkk-1 and SFRP-1 (ref.[94]). In addition, other pathways with relevance to bone metastasis can contribute to β-catenin activation. Examples are the receptor tyrosine kinase c-Met[95] and bone morphogenetic proteins which play a role in bone remodeling and are mediators of PCa-induced osteoblastic activity[96].

CONCLUSION

Wnt signaling crosstalks with the androgen receptor and other signaling pathways both in normal prostate development and carcinogenesis. Further study of modulators of this pathway will be of therapeutic relevance as inhibition of Wnt signaling might have the potential to reduce the self-renewal and aggressive behaviour of prostate cancer while Wnt signaling activation might enhance stem cell activity when tissue regeneration is required.

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