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Cross-regulation of signaling pathways: an example of nuclear hormone receptors and the canonical Wnt pathway

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Abstract

Predicting the potential physiological outcome(s) of any given molecular pathway is complex because of crosstalk with other pathways. This is particularly evident in the case of the nuclear hormone receptor and canonical Wnt pathways which regulate cell growth and proliferation, differentiation, apoptosis and metastatic potential in numerous tissues. These pathways are known to intersect at many levels: in the intracellular space, at the membrane, in the cytoplasm and within the nucleus. The outcomes of these interactions are important in the control of stem cell differentiation and maintenance, feedback loops, and regulating oncogenic potential. The aim of this review is to demonstrate the importance of considering pathway crosstalk when predicting functional outcomes of signaling, using nuclear hormone receptor/canonical Wnt pathway crosstalk as an example.

Introduction

Nuclear Hormone Receptors

There are 48 known members of the nuclear hormone receptor (NHR) family of transcription factors. They are generally divided into two subtypes: Type I and Type II (Type III and Type IV also exist but they largely composed of orphan receptors, about which little is known). Type I NHRs include the sex steroid receptors such as estrogen receptor α and β (ER-*NR3A1* and *NR3A2*, respectively), progesterone receptor (PR-*NR3C3*) and androgen receptor (AR-*NR3C4*), as well as glucocorticoid receptor (GR-*NR3C1*), among others. These receptors are found in both the cytoplasm and the nucleus and generally bind as homodimers to their DNA recognition sequences. Type II NHRs include the vitamin D receptor (VDR-*NR1I1*), peroxisome proliferator activated receptors α , β and γ (PPAR-*NR1C1*, *NR1C2*, *NR1C3*, respectively) and retinoic acid receptor α , β , and γ (RAR-*NR1B1*, *NR1B2*, *NR1B3*, respectively), among others. These receptors are constitutively nuclear and generally bind as heterodimers, most frequently with the NHR retinoid $\times$ receptor (RXR-*NR2B1*, *NR2B2*, *NR2B3* for α , β , and γ , respectively), to their DNA binding elements.

In the absence of ligand the Type II receptors, recruit a large co-repressor complex. On some occasions ligand dependent repression occurs on specific regulatory sequences such as negative VDR binding elements. Once ligand enters the cell it traverses the nuclear membrane and binds

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to the receptor, inducing a conformational change that allows the sequential recruitment of histone modifying proteins, transcriptional mediator complexes, and, lastly, the basal transcription machinery to activating recognition sequences.

The Wnt Pathway

The Wnt pathway is strongly associated with an oncogenic phenotype in many cancers including cancer of the liver, breast and particularly, the colon. In the absence of canonical Wnts, the GSK3 β /Axin/APC complex induces the phosphorylation of β -catenin, which targets it for degradation by the proteasome [1–3]. The presence of endocrine and paracrine Wnts induces binding of Frizzled (Fzd) receptor to Low-density lipoprotein receptor related proteins 5 or 6 (LRP5/6), which ultimately leads to the inhibition of the GSK3 β /Axin/APC phosphorylation complex and accumulation of unphosphorylated β -catenin.

β -Catenin is a multifunctional protein with important structural and signaling functions. β -Catenin plays a pivotal role in cell-cell adhesion, linking the cytoplasmic domain of the cadherin family of transmembrane proteins to α -catenin and thus connecting the adhesion complex to the actin cytoskeleton. β -Catenin is also an essential component of the Wnt/Wingless signaling pathway that plays important roles in development and pathogenesis. Through this dual role, β -catenin has the potential to allow changes in cell adhesion and junction formation to affect transmembrane signaling and gene expression. The many functions of β -catenin are, derived from three cellular pools of the molecule under strict regulation: a membrane pool of cadherin-associated β -catenin, a cytoplasmic pool and a nuclear pool [4]. β -catenin that is not targeted to the proteasome by the GSK3 β /Axin/APC complex can be found in the nucleus, as ‘activated’ β -catenin. ‘Activated’ β -Catenin is a heterodimeric binding partner of LEF/TCF proteins that contain DNA-binding motifs[5]. Together these proteins form a transcription initiation complex and regulate the expression of many genes involved in cell growth[6,7].

Stabilization of β -catenin is likely the key event in the transduction of a Wnt signal and the cell has developed an elaborate system to regulate its steady-state levels. In addition to the GSK3 β /Axin/APC destruction complex, the Wnt pathway is also controlled by extracellular antagonists. Secreted Frizzled Related Protein (sFRP) and Wnt Inhibitory Factor 1 (WIF-1) are secreted factors that bind Wnts and prevent them from binding their cognate receptors. Dickkopf (DKK) family members and Wise, a context-dependent secreted protein[8], both antagonize the Wnt pathway by binding to LRP5/6 and preventing Wnts from binding. Dysregulation of any of these pathway components can lead to the production of over-abundant activated β -catenin and an oncogenic phenotype.

Crosstalk between these two pathways may have differing effects on oncogenic potential. Activation of β -catenin signaling is a key event in colorectal carcinogenesis where mutations in the tumor suppressor gene *APC* account for up to 80% of cancers [9] In addition, activating β -catenin mutations have been also reported in melanomas, ovarian cancer, medulloblastomas, and endometrial cancer, underscoring the importance of β -catenin-mediated signaling in oncogenesis [10–12]. The transforming potential of *Wnt* genes correlates precisely with their ability to induce elevated β -catenin levels, suggesting that β -catenin is instrumental in Wnt-mediated transformation [13]. Although the aberrantly-activated Wnt pathway is almost exclusively associated with a cancer phenotype, aberrantly-activated NHR pathways have varied outcomes with respect to a cancer phenotype. The sex hormones, AR and ER, are thought to promote cancer, while others such as PPAR γ and VDR are thought to prevent cancer. While methods of crosstalk are not likely to be exactly the same for each NHR, there are some commonalities. The mechanisms of crosstalk and the reported outcomes are reviewed presently in the context of both development and cancer.

Crosstalk between NHR and Wnt pathway components

Extracellular Space

Many NHRs can regulate the transcription of Wnt antagonists. This becomes clear in the context of stem cell differentiation in certain tissues where Wnt expression can drive cell fate. One of the best-characterized processes involving Wnt and NHR signals in stem cell differentiation involves the driving of mesenchymal progenitor cells into an adipocyte or an osteoblast lineage. The commitment of mesenchymal stem cells to an adipogenic or osteogenic cell fate involves an intimate level of cross regulation between the Wnt pathway and several NHRs (Figure 1A). It is generally accepted that an osteogenic cell fate is induced by Wnt/Fzd activity, while an adipogenic cell fate is induced by PPAR γ activity[14]. These two key regulators reciprocally inhibit each other on multiple levels. Wnt promotes osteogenesis by blocking the PPAR γ and C/EBP α induction that would otherwise occur under adipogenic stimuli [15–17]. Orphan NHR, COUP-TFII, which is a direct target of β -catenin, and, thus, downstream of the Wnt pathway, also inhibits PPAR γ expression. It binds to the PPAR γ promoter and recruits SMRT, a NHR co-repressor which acts to inhibit the application of active histone marks[18]. This effectively enhances a closed chromatin structure around the PPAR γ promoter, preventing transcription. Furthermore, Cyclin D1 and *c-myc*, two well-documented Wnt targets, bind directly to and thereby inhibit the action of PPAR γ [19].

Wnt-mediated osteogenesis may be controlled by a negative feedback mechanism, as Wnt7a and Fzd9 can increase PPAR γ activity and E-cadherin expression in non-small cell lung cancer cells; however, this has not yet been shown in mesenchymal precursors [20].

Conversely, PPAR γ can antagonize Wnt-mediated osteogenesis via several mechanisms. In response to adipogenic stimuli, PPAR γ expression is increased, and nuclear β -catenin is dramatically decreased[17]. Furthermore, PPAR γ overexpression reverses Wnt-1 induced blockade of adipogenesis[17]. This effect may be partially mediated by several mechanisms including a context-dependent decrease in β -catenin transcription, an increase in both GSK3 β -dependent and -independent β -catenin degradation, an increase in Wnt antagonist DKK1 and concomitant decrease in the LRP5/6 co-receptors, and a suppression of Wnt expression such as Wnt 10b[17,21–26].

Glucocorticoids (GC), such as dexamethasone, also antagonize the Wnt pathway during differentiation of mesenchymal stem cells and are key components in inducing adipogenic differentiation. GCs increase expression of DKK1, sFRP-1 and Axin-2[27–29] in osteoblastic precursors. In a transgenic mouse model with decreased GC expression targeted to mature osteoblasts, resultant decreased paracrine Wnt signaling from osteoblasts was ameliorated by ectopic Wnt3A expression during cranial bone development[30]. In mammary epithelial cells GC may also antagonize the Wnt pathway via re-routing β -catenin from the nucleus to the cell membrane, possibly by affecting TGF α -dependent, GSK3 β -mediated phosphorylation of β -catenin[31]. This GC-dependent modification of differentiation down an adipogenic lineage may underlie the reversible osteoporotic side effects of long-term GC treatment of rheumatoid arthritis[29].

VDR may also play a role in deciding the fate of mesenchymal stem cells. VDR knockout mice demonstrate a modestly increased induction of PPAR γ mRNA in response to adipogenic differentiation conditions[32]. This modest PPAR γ induction translates to dramatic derepression of late-stage adipogenic markers[32]. The repression of adipogenesis signals in VDR-sufficient systems may be partially due to the ability of vitamin D to strongly decrease expression Wnt antagonists DKK1 and sFRP2 and as well as activate expression of LRP5 leading to osteogenesis[33].

Vitamin D modulates secreted Wnt antagonists in situations other than cell differentiation, namely in colorectal cancer cells, where vitamin D is known to be anti-tumorigenic. In contrast to mesenchymal stem cells, DKK1 is *increased* by vitamin D via an indirect mechanism in colon cancer cells[34]. Surprisingly a vitamin D-mediated *decrease* in DKK4 expression in both colorectal and breast cancer cell lines was also observed[35]. This is counter-intuitive since DKK4 is thought to antagonize the oncogenic Wnt pathway that is over-active in a vast majority of colorectal cancers. In this study, however, DKK4-enhanced malignant morphology and vascular tubule-like structures in the DLD-1 colon cancer cell line[35].

Retinoic Acid (RA) is a classic differentiating agent that also modulates extracellular Wnt antagonists. RA-increased DKK1 expression is at least partially responsible for RA-dependent neural differentiation of embryonic stem cells[36]. RA is also a key player in early development. RA-mediated inhibition of Wnt signaling is required for body axis extension in mice, and mice deficient in RA demonstrate abnormal expression of Wnt8A and Wnt3A[30]. RA-mediated disruption of Wnt-3A signaling in the tail bud leads to caudal atresia in mouse embryos[37]. The central nervous system, which also expresses Wnt-3A is unaffected by RA treatment, possibly having to do with differential expression of RARs between the central nervous system and the tail bud[37]. A similar phenomenon is responsible for head formation in *Xenopus*; Wnt signaling leads to regulation of genes important for dorsal development and RA inhibits this signaling, possibly via a protein-protein interaction between RAR and β -catenin, a mechanism that will be discussed later [38]. The Wnt pathway can also modulate RA signaling and neuronal differentiation, as all-trans-retinoic-acid (ATRA), the ligand for RAR, increased Fzd-4 and Fzd-10 expression in human embryonic tumor cells and caused them to differentiate into neuronal cells[39]. Furthermore, Wnt suppresses CYP26, an enzyme that is responsible for degrading RA into inactive metabolites, possibly functioning in a negative feedback mechanism[40]. RA differentially regulates a host of genes on the oncogenic Wnt-1 background compared to a normal background implying intimate crosstalk between these two pathways[41]. These interactions are depicted in Figure 1B. While the individual roles of Wnt and RA in development are conserved, their crosstalk appears to be restricted to vertebrates, as it remains undetected in amphioxus, a primitive chordate[40].

Progesterone can also positively modulate DKK1 expression in human endometrial cells [42]. Because progesterone levels reach their peak during this phase, progesterone control of DKK1 expression may play a role in pregnancy and development. Progesterone and estrogen, both ligands for NHRs, are also important in the development of the mammary gland. PR-null animals, whose mammary ducts fail to form side-branches, are rescued by ectopic expression of Wnt-1, indicating that Wnt is acting downstream of progesterone[43]. In the same study, it was shown that estrogen and progesterone induced Wnt-4 expression *in vivo* in the mammary epithelium. Wnt crosstalk with NHRs in the mammary gland is not only responsible for normal development, but may also contribute to abnormal pathology, as is seen in the example of spontaneous mammary tumorigenesis in ER-knockout mice with ectopic MMTV-driven Wnt-1 expression[44].

Thyroid hormone (T_3) increases β -catenin activity in the colon. This effect, however, was puzzling associated with a concomitant increase in sFRP2 expression, which in conjunction with several Fzds was responsible for increasing β -catenin activity in this system[45].

At the membrane

Cell-cell adhesion is considered a marker of a differentiated phenotype and its reduction can promote cancer cell metastasis. Thus, it is not entirely surprising that the NHRs that have in anti-cancer properties can affect cadherins, which are key, calcium-sensitive participants in cell-cell adhesion. The first major evidence of this phenomenon comes from RA-induced increase in the strength of adherens junctions in a poorly-adhesive breast cancer cell line[46,

47]. In these studies, β -catenin was recruited to the membrane in a calcium-dependent manner, implicating a cadherin as the mediator molecule. This effect is likely partially-responsible for RA- (and to a smaller degree, vitamin D-) induced decreases in β -catenin activity in breast and colon cancer cells, as free β -catenin is recruited to the cell membrane, preventing it from entering the nucleus for transactivation of gene expression[48]. A robust vitamin D-mediated increase in E-cadherin expression does occur in a colon cancer cell-line that expresses high levels of VDR[49]. PPAR γ ligands, like RA and vitamin D, also induces differentiation and G1 arrest in pancreatic cancer cells in-part by inducing E-cadherin expression[50]. In contrast, a decrease in E-cadherin expression occurs in hepatocytes treated with PPAR γ ligand [24].

In the nucleus

Thyroid hormone (T_3) plays divergent roles in the intestine with regards to intersecting with the β -catenin pathway that may be, in part, explained by the three TR isoforms: TR α 1, TR β 1, and TR β 2. T_3 increased β -catenin activity in the intestinal epithelium of mice by not only regulating sFRP2, but also enhancing β -catenin expression, the effects of which may play a role in development of gut crypts[45,51]. The regulation of both sFRP2 and β -catenin expression involves TR α 1 but not TR β 1. When T_3 is bound to TR β 1, it dose-dependently *suppresses* the β -catenin-mediated induction of cyclin D1 expression in SW480 colorectal cancer cells and HEK 293T human embryonic kidney cells[52]. The mechanism of this latter inhibition is likely due to TR β 1 binding to the TCF-response-element in the cyclin D1 promoter. This inhibits binding of TCF/LEF family members for transactivation of the gene in a β -catenin-dependent manner. In contrast to the colon epithelium, rat pituitary cells that endogenously express all three TRs respond to T_3 by *decreasing* β -catenin mRNA and activity despite a paradoxical increase in proliferation[53]. In this study, the decrease in β -catenin activity is much-prolonged in comparison to the decrease in β -catenin mRNA, and may be at least partially explained by a simultaneous, but longer-lived, increase in Axin2 mRNA and protein expression. The mechanism whereby a decrease in β -catenin activity corresponds with an increase in proliferation in these cells remains unanswered[53].

GC and PPAR γ both modulate β -catenin expression in differentiating mesenchymal stem cells via a number of mechanisms including degradation. ATRA can also induce proteasomal degradation of β -catenin via RXR α in HEK293 and SW480 cells[54]. Upon further exploration, the mechanism of RXR-induced β -catenin degradation seems to involve neither the GSK3 β /APC/Axin- nor the p53/Siah pathways, implicating a novel degradation pathway in ATRA-resistant colon cancer cell lines[55,56]. Contrary to these data, RA has also been shown to *increase* β -catenin stabilization in tracheobronchial epithelial cells, however, this does not correlate with increases in β -catenin activity, rather, a decrease, possibly due to the concomitant decrease in nuclear localization[57].

An interaction between ER and cyclin D1, a primary β -catenin target gene, seems to be unique to this NHR. ER can be directly activated by all three cyclin D isoforms in the absence of ligand [58,59]. Cyclin D is able to form a bridge between ER and the Src family of NHR co-activators via its analogous LxxLL motif [60]. ER has also been shown to inhibit GSK3 β in the hippocampus[61]. The PI3K/AKT pathway, which also inhibits GSK3 β , can, for this reason, be considered a central component in NHR/Wnt pathway crosstalk, and these interactions are nicely reviewed by Mulholland *et al*[62].

Crosstalk between the VDR and Wnt pathways has recently been identified as the root of the hairless phenotype of VDR-null mice (Figure 1C) [63]. Keratinocyte stem cells can differentiate down three lineages: sebocyte, epidermal keratinocyte and follicle keratinocyte (interfollicular epidermis). Defective Wnt signaling drives the stem cells down a sebocyte and epidermal keratinocyte lineage instead of a follicle keratinocyte lineage. This defect causes dermal cysts that are filled with sebocytes and epidermal keratinocytes, a phenotype that is

also evident in the abundant lipid-laden dermal cysts seen in VDR-null animals. Although ectopic expression of activated β -catenin targeted to keratinocytes does not rescue the defective hair cycling that results in progressive alopecia in VDR-null mice, the synergistic activation of Lef-1 activity by β -catenin requires the presence of the VDR in the same complex and can occur in the absence of ligand, though it is ligand-enhanced[64,65]. VDR may also act independently of TCF/LEF as a Wnt pathway effector[65]. These interactions drive keratinocyte stem cells to differentiate into follicle keratinocytes. The presence of constitutively-active β -catenin induces tumors in the mouse epidermis, however, the presence or absence of VDR dictates which type of tumor develops. VDR-sufficient mice develop trichofolliculomas, which are similar to human pilomatricoma. In the absence of VDR, however, prolonged β -catenin activation induces basal cell carcinomas[65], underscoring the potential importance of their interaction in the epidermis.

The best-characterized interaction between NHRs and the canonical Wnt pathway stems from the discovery that RAR binds directly to β -catenin in both breast and colon cancer cells[48]. Since that initial discovery, similar interactions have been discovered for VDR, PPAR γ , AR, RXR, LXR α and β and ER, although the last one may be particularly sensitive to the cellular milieu because it has gone undetected in several other studies [17,24,48,49,54,66–73]. Of note, GR, PR, or TR have not yet been shown to interact directly with β -catenin [66,69]. Although this interaction is relatively conserved among NHRs, they differ in some aspects. Some NHRs, such as VDR, PPAR γ and AR, are found in a complex with both β -catenin and TCF/LEFs, while some NHRs, such as ER and AR and can be found in a complex with TCF/LEFs in the absence of β -catenin[64,71,74–76]. The functional outcomes of these protein-protein interactions can also differ, depending on the NHR. Generally speaking, β -catenin synergistically activates NHR activity, and the liganded NHR reciprocally deactivates or even represses β -catenin activity. This effect is attributed to a number of mechanisms of action (Figure 2). E-cadherin expression is dramatically up-regulated in response to some NHR agonists which redirects β -catenin from the nucleus to the plasma membrane[20,47–50]. Although this is a latent effect, immediate effects can be accounted for by a competition between TCF/LEFs and NHRs for binding to β -catenin and/or p300[48,49,70]. In addition to trans-repression of Wnt-response genes, active repression is accomplished by liganded NHRs binding directly to TCF/LEF family members and recruiting co-repressors such as TLE, NCoR and SMRT. Once these co-repressors are bound, they are not easily displaced by β -catenin, leading to long-term repression[74].

These interactions are highly dependent on the cellular context and can create divergent effects as we have already summarized for development. For example, PPAR γ , whose expression and activity is negatively regulated by Wnts in mesenchymal stem cells, undergoes positive regulation of expression and activity in colon cancer cells in response to a Wnt signal[71]. A similar phenomenon is seen with VDR, which super-activates β -catenin activity in keratinocytes[64], but attenuates it in colorectal cancer cells[48,49,70].

Many of these mechanisms have been extensively studied in the context of AR and prostate cancer. It is generally accepted that unlike colon cancer in which a majority of cases are attributable to inactivating mutations in *APC*, no *APC* mutations are found in prostate cancer [77]. However, the genetic locus of *APC* is lost in some prostate cancer tissues [78]. Men from families with *APC* mutations have an increased risk of prostate cancer [79]. *APC* methylation is commonly found in early stage prostate cancer and correlates with early relapse and poor prognosis [80,81].

Disruption of the β -catenin signaling pathway has been found in prostate cancers [82]. An analysis of primary prostate tumors showed that 5% had activating β -catenin mutations [83, 84]. Although this is a low frequency of mutations, the level is comparable with the frequency

of β -catenin mutations in colon cancer and one might speculate that another component of the Wnt signaling pathway, besides β -catenin or APC, might be targeted for mutation in prostate cancer.

AR and β -catenin each affect the others' transcriptional activation of promoters with cognate target sequences that lie downstream in the signaling cascade. β -Catenin enhances AR-mediated transcription and androgen causes translocation of β -catenin to the nucleus in cells that express both proteins [85–88]. The recruitment of β -catenin to AR is ligand-dependent and is favored by ligand-bound conformation of AR [89,90]. This is particularly important because the same conditions that favor AR association with β -catenin also favor association with p160 coactivator molecules that bind not only to AR, but also to β -catenin itself, implying that there may be a three-way interaction in the AR transcriptional complex [91,92]. β -Catenin is affected by a variety of growth and survival pathways including receptor tyrosine kinases like IGF-1 receptor that signal through PI3 kinase (PI3K) and activate GSK3 β . PI3K-mediated pathways can affect β -catenin interaction with AR, consistent with the notion that receptor tyrosine kinase activation can affect AR [93,94].

AR, in contrast, has an inhibitory effect on TCF/LEF-mediated transcription and can compete with TCF/LEF molecules for β -catenin binding [95–97]. Moreover, AR interacts with TCF/LEF by binding directly to TCF4 [98]. The interaction of AR and the β -catenin/TCF4 complex is also affected by PIN1, a peptidyl-prolyl cis/trans isomerase that stabilizes β -catenin by inhibiting its binding to APC. PIN1 is over expressed in advanced prostate cancer. In cells with intact PTEN, PIN1 may enhance AR signaling by increasing β -catenin levels. In PTEN-deficient cells PIN1 expression may decrease the interaction of AR and β -catenin, thus enhancing TCF4-mediated transcription [99]. We speculate, although it has not been shown, that the putative differentiating and growth attenuation effects of AR in certain cell lines may be due, in part, to inhibition of β -catenin/TCF/LEF transcriptional activation [100].

The relevance of β -catenin to AR in castration-resistant prostate cancer was expanded by the finding that increased levels of the calpain protease in advanced prostate cancer can cleave the N-terminus of β -catenin and generate constitutively active p75 β -catenin [101]. Not only could activated β -catenin affect AR activity, but also potentially recruit TIF2, a NHR co-activator, to the transcriptional complex so that cancer cells would not need other mechanisms of increasing TIF2 expression [102]. Activation of β -catenin in advanced prostate cancer may be more important to cell proliferation than expression of nuclear β -catenin which is actually inversely correlated with tumor progression and preoperative serum PSA levels and directly related to progression-free survival [103].

Concluding Remarks

In summary, the interactions between NHRs and the Wnt pathway are myriad, complex, and still under active investigation and may offer therapeutic targets for many endocrine diseases and also help define the etiology and potential preventative measures for these diseases. However, the functional outcome of this crosstalk depends upon cellular context. Understanding the contexts under which NHRs and the Wnt pathway reciprocally affect each other are critical for the exploitation of these therapeutic targets, as can be seen from the examples of PPAR γ and VDR, which activate or de-activate the β -catenin pathway in different cellular contexts. Much of the work described here has been done *in vitro*, but several studies have demonstrated interactions *in vivo*, which has already lead to a greatly-enhanced understanding of control of stem cell differentiation and development. The intersection of the canonical Wnt pathway with NHRs is surely one of many cases that exemplify the importance of pathway crosstalk. We can look forward to the discovery of new, meaningful interactions that are relevant *in vivo*, and the refinement of currently recognized interactions that will lead to the understanding of development and the identification promising new drug targets.

Reference List

1. Munemitsu S, Albert I, Souza B, Rubinfeld B, Polakis P. Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. *Proc Natl Acad Sci USA* 1995;92:3046–3050. [PubMed: 7708772]
2. Rubinfeld B, Albert I, Porfiri E, Fiol C, Munemitsu S, Polakis P. Binding of GSK3 beta to the APC-beta-catenin complex and regulation of complex assembly. *Science* 1996;272:1023–1026. [PubMed: 8638126]
3. Orford K, Crockett C, Jensen JP, Weissman AM, Byers SW. Serine phosphorylation-regulated ubiquitination and degradation of beta-catenin. *J Biol Chem* 1997;272:24735–24738. [PubMed: 9312064]
4. Barth AI, Nathke IS, Nelson WJ. Cadherins, catenins and APC protein: interplay between cytoskeletal complexes and signaling pathways. *Curr Opin Cell Biol* 1997;9:683–690. [PubMed: 9330872]
5. Behrens J, von Kries JP, Kuhl M, Bruhn L, Wedlich D, Grosschedl R, Birchmeier W. Functional interaction of b-catenin with the transcription factor LEF-1. *Nature* 1996;382:638–642. [PubMed: 8757136]
6. Cong F, Schweizer L, Chamorro M, Varmus H. Requirement for a nuclear function of beta-catenin in Wnt signaling. *Mol Cell Biol* 2003;23:8462–8470. [PubMed: 14612392]
7. Tolwinski NS, Wieschaus E. A nuclear function for armadillo/beta-catenin. *PLoS Biol* 2004;2:E95. [PubMed: 15024404]
8. Itasaki N, Jones CM, Mercurio S, Rowe A, Domingos PM, Smith JC, Krumlauf R. Wise, a context-dependent activator and inhibitor of Wnt signalling. *Development* 2003;130:4295–4305. [PubMed: 12900447]
9. Kinzler KW, Nilbert MC, Vogelstein B, et al. Identification of a chromosome 5q21 gene that is mutated in colorectal cancers. *Science* 1991;251:1366–1370. [PubMed: 1848370]
10. Rubinfeld B, Robbins P, El-Gamil M, Albert I, Porfiri E, Polakis P. Stabilization of beta-catenin by genetic defects in melanoma cell lines. *Science* 1997;275:1790–1792. [PubMed: 9065403]
11. Palacios J, Gamallo C. Mutations in the beta-catenin gene (CTNNB1) in endometrioid ovarian carcinomas. *Cancer Res* 1998;58:1344–1347. [PubMed: 9537226]
12. Zurawel RH, Chiappa SA, Allen C, Raffel C. Sporadic medulloblastomas contain oncogenic beta-catenin mutations. *Cancer Res* 1998;58:896–899. [PubMed: 9500446]
13. Porfiri E, Rubinfeld B, Albert I, Hovanes K, Waterman M, Polakis P. Induction of a beta-catenin-LEF-1 complex by wnt-1 and transforming mutants of beta-catenin. *Oncogene* 1997;15:2833–2839. [PubMed: 9419974]
14. Kennell JA, MacDougald OA. Wnt signaling inhibits adipogenesis through beta-catenin-dependent and -independent mechanisms. *J Biol Chem* 2005;280:24004–24010. [PubMed: 15849360]
15. Bennett CN, Longo KA, Wright WS, Suva LJ, Lane TF, Hankenson KD, MacDougald OA. Regulation of osteoblastogenesis and bone mass by Wnt10b. *Proc Natl Acad Sci US A* 2005;102:3324–3329.
16. Kang S, Bennett CN, Gerin I, Rapp LA, Hankenson KD, MacDougald OA. Wnt signaling stimulates osteoblastogenesis of mesenchymal precursors by suppressing CCAAT/enhancer-binding protein alpha and peroxisome proliferator-activated receptor gamma. *J Biol Chem* 2007;282:14515–14524. [PubMed: 17351296]
17. Moldes M, Zuo Y, Morrison RF, Silva D, Park BH, Liu JJ, Farmer SR. Peroxisome-proliferator-activated receptor gamma suppresses Wnt/beta-catenin signalling during adipogenesis. *Biochemical Journal* 2003;376:607–613. [PubMed: 12954078]
18. Okamura M, Kudo H, Wakabayashi K, Tanaka T, Nonaka A, Uchida A, Tsutsumi S, Sakakibara I, Naito M, Osborne TF, Hamakubo T, Ito S, Aburatani H, Yanagisawa M, Kodama T, Sakai J. COUP-TFII acts downstream of Wnt/beta-catenin signal to silence PPAR gamma gene expression and repress adipogenesis. *Proceedings of the National Academy of Sciences of the United States of America* 2009;106:5819–5824. [PubMed: 19307559]
19. Wang CG, Pattabiraman N, Zhou JN, Fu MF, Sakamaki T, Albanese C, Li ZP, Wu KM, Hulit J, Neumeister P, Novikoff PM, Brownlee M, Scherer PE, Jones JG, Whitney KD, Donehower LA, Harris EL, Rohan T, Johns DC, Pestell RG. Cyclin D1 repression of peroxisome proliferator-activated

- receptor gamma expression and transactivation. *Molecular and Cellular Biology* 2003;23:6159–6173. [PubMed: 12917338]
20. Winn RA, Van Scoyk M, Hammond M, Rodriguez K, Crossno JT, Heasley LE, Nemenoff RA. Antitumorigenic effect of Wnt 7a and Fzd 9 in non-small cell lung cancer cells is mediated through ERK-5-dependent activation of peroxisome proliferator-activated receptor gamma. *Journal of Biological Chemistry* 2006;281:26943–26950. [PubMed: 16835228]
 21. Gerhold DL, Liu F, Jiang G, Li Z, Xu J, Lu M, Sachs JR, Bagchi A, Fridman A, Holder DJ, Doebber TW, Berger J, Elbrecht A, Moller DE, Zhang BB. Gene expression profile of adipocyte differentiation and its regulation by peroxisome proliferator-activated receptor-gamma agonists. *Endocrinology* 2002;143:2106–2118. [PubMed: 12021175]
 22. Liu JJ, Farmer SR. Regulating the balance between peroxisome proliferator-activated receptor gamma and beta-catenin signaling during adipogenesis - A glycogen synthase kinase 3 beta phosphorylation-defective mutant of beta-catenin inhibits expression of a subset of adipogenic genes. *Journal of Biological Chemistry* 2004;279:45020–45027. [PubMed: 15308623]
 23. Ross SE, Hemati N, Longo KA, Bennett CN, Lucas PC, Erickson RL, MacDougald OA. Inhibition of adipogenesis by Wnt signaling. *Science* 2000;289:950–953. [PubMed: 10937998]
 24. Sharma C, Pradeep A, Wong L, Rana A, Rana B. Peroxisome proliferator-activated receptor gamma activation can regulate beta-catenin levels via a proteasome-mediated and adenomatous polyposis coli-independent pathway. *Journal of Biological Chemistry* 2004;279:35583–35594. [PubMed: 15190077]
 25. Christodoulides C, Laudes M, Cawthorn WP, Schinner S, Soos M, O’Rahilly S, Sethi JK, Vidal-Puig A. The Wnt antagonist Dickkopf-1 and its receptors are coordinately regulated during early human adipogenesis. *Journal of Cell Science* 2006;119:2613–2620. [PubMed: 16763196]
 26. Bennett CN, Ross SE, Longo KA, Bajnok L, Hemati N, Johnson KW, Harrison SD, MacDougald OA. Regulation of Wnt signaling during adipogenesis. *Journal of Biological Chemistry* 2002;277:30998–31004. [PubMed: 12055200]
 27. Ohnaka K, Taniguchi H, Kawate H, Nawata H, Takayanagi R. Glucocorticoid enhances the expression of dickkopf-1 in human osteoblasts: novel mechanism of glucocorticoid-induced osteoporosis. *Biochemical and Biophysical Research Communications* 2004;318:259–264. [PubMed: 15110782]
 28. Wang FS, Lin CL, Chen YJ, Wang CJ, Yang KD, Huang YT, Sun YC, Huang HC. Secreted frizzled-related protein 1 modulates glucocorticoid attenuation of osteogenic activities and bone mass. *Endocrinology* 2005;146:2415–2423. [PubMed: 15677765]
 29. Hayashi K, Yamaguchi T, Yano S, Kanazawa I, Yamauchi M, Yamamoto M, Sugimoto T. BMP/Wnt antagonists are upregulated by dexamethasone in osteoblasts and reversed by alendronate and PTH: Potential therapeutic targets for glucocorticoid-induced osteoporosis. *Biochemical and Biophysical Research Communications* 2009;379:261–266. [PubMed: 19101512]
 30. Zhao X, Duyster G. Effect of retinoic acid signaling on Wnt/beta-catenin and FGF signaling during body axis extension. *Gene Expr Patterns* 2009;9:430–435. [PubMed: 19539783]
 31. Guan Y, Rubenstein NM, Failor KL, Woo PL, Firestone GL. Glucocorticoids control beta-catenin protein expression and localization through distinct pathways that can be uncoupled by disruption of signaling events required for tight junction formation in rat mammary epithelial tumor cells. *Mol Endocrinol* 2004;18:214–227. [PubMed: 14551262]
 32. Cianferotti L, Demay MB. VDR-mediated inhibition of DKK1 and SFRP2 suppresses adipogenic differentiation of murine bone marrow stromal cells. *J Cell Biochem* 2007;101:80–88. [PubMed: 17212358]
 33. Fretz JA, Zella LA, Kim S, Shevde NK, Pike JW. 1,25-Dihydroxyvitamin D-3 regulates the expression of low-density lipoprotein receptor-related protein 5 via deoxyribonucleic acid sequence elements located downstream of the start site of transcription. *Mol Endocrinol* 2006;20:2215–2230. [PubMed: 16613987]
 34. Aguilera O, Pena C, Garcia JM, Larriba MJ, Ordonez-Moran P, Navarro D, Barbachano A, de Silanes IL, Ballestar E, Fraga MF, Esteller M, Gamallo C, Bonilla F, Gonzalez-Sancho JM, Munoz A. The Wnt antagonist DICKKOPF-1 gene is induced by 1 alpha,25-dihydroxyvitamin D-3 associated to the differentiation of human colon cancer cells. *Carcinogenesis* 2007;28:1877–1884. [PubMed: 17449905]

35. Pendas-Franco N, Aguilera O, Pereira F, Gonzalez-Sancho JM, Munoz A. Vitamin D and Wnt/beta-catenin Pathway in Colon Cancer: Role and Regulation of DICKKOPF Genes. *Anticancer Research* 2008;28:2613–2623. [PubMed: 19035286]
36. Verani R, Cappuccio I, Spinsanti P, Gradini R, Caruso A, Magnotti MC, Motolese M, Nicoletti F, Melchiorri D. Expression of the Wnt inhibitor Dickkopf-1 is required for the induction of neural markers in mouse embryonic stem cells differentiating in response to retinoic acid. *Journal of Neurochemistry* 2007;100:242–250. [PubMed: 17064353]
37. Shum ASW, Poon LLM, Tang WWT, Koide T, Chan BWH, Leung YCG, Shiroishi T, Copp AJ. Retinoic acid induces down-regulation of Wnt-3a, apoptosis and diversion of tail bud cells to a neural fate in the mouse embryo. *Mechanisms of Development* 1999;84:17–30. [PubMed: 10473117]
38. Li SW, Lou X, Wang JH, Liu B, Ma LP, Su ZP, Ding XY. Retinoid signaling can repress blastula Wnt signaling and impair dorsal development in *Xenopus* embryo. *Differentiation* 2008;76:897–907. [PubMed: 18452549]
39. Katoh M. Regulation of WNT signaling molecules by retinoic acid during neuronal differentiation in NT2 cells: Threshold model of WNT action (Review). *International Journal of Molecular Medicine* 2002;10:683–687. [PubMed: 12429992]
40. Onai T, Lin HC, Schubert M, Koop D, Osborne PW, Alvarez S, Alvarez R, Holland ND, Holland LZ. Retinoic acid and Wnt/beta-catenin have complementary roles in anterior/posterior patterning embryos of the basal chordate amphioxus. *Dev Biol* 2009;332:223–233. [PubMed: 19497318]
41. Tice DA, Szeto W, Soloviev I, Rubinfeld B, Fong SE, Dugger DL, Winer J, Williams PM, Wieand D, Smith V, Schwall RH, Pennica D, Polakis P. Synergistic induction of tumor antigens by Wnt-1 signaling and retinoic acid revealed by gene expression profiling. *Journal of Biological Chemistry* 2002;277:14329–14335. [PubMed: 11832495]
42. Tulac S, Overgaard MT, Hamilton AE, Jumbe NL, Suchanek E, Giudice LC. Dickkopf-1, an inhibitor of Wnt signaling, is regulated by progesterone in human endometrial stromal cells. *Journal of Clinical Endocrinology and Metabolism* 2006;91:1453–1461. [PubMed: 16449346]
43. Briskin C, Heineman A, Chavarria T, Elenbaas B, Tan J, Dey SK, McMahon JA, McMahon AP, Weinberg RA. Essential function of Wnt-4 in mammary gland development downstream of progesterone signaling. *Genes & Development* 2000;14:650–654. [PubMed: 10733525]
44. Bocchinfuso WP, Hively WP, Couse JF, Varmus HE, Korach KS. A mouse mammary tumor virus Wnt-1 transgene induces mammary gland hyperplasia and tumorigenesis in mice lacking estrogen receptor-alpha. *Cancer Res* 1999;59:1869–1876. [PubMed: 10213494]
45. Kress E, Rezza A, Nadjar J, Samarut J, Plateroti M. The Frizzled-related sFRP2 Gene Is a Target of Thyroid Hormone Receptor alpha 1 and Activates beta-Catenin Signaling in Mouse Intestine. *Journal of Biological Chemistry* 2009;284:1234–1241. [PubMed: 19001373]
46. Vermeulen SJ, Bruyneel EA, Van Roy FM, Mareel MM, Bracke ME. Activation of the E-cadherin/catenin complex in human MCF-7 breast cancer cells by all-trans-retinoic acid. *Br J Cancer* 1995;72:1447–1453. [PubMed: 8519658]
47. Byers S, Pishvaian M, Crockett C, Peer C, Tozeren A, Sporn M, Anzano M, Lechleider R. Retinoids increase cell-cell adhesion strength, beta-catenin protein stability, and localization to the cell membrane in a breast cancer cell line: a role for serine kinase activity. *Endocrinology* 1996;137:3265–3273. [PubMed: 8754749]
48. Easwaran V, Pishvaian M, Salimuddin, Byers S. Cross-regulation of beta-catenin-LEF/TCF and retinoid signaling pathways. *Curr Biol* 1999;9:1415–1418. [PubMed: 10607566]
49. Palmer HG, Gonzalez-Sancho JM, Espada J, Berciano MT, Puig I, Baulida J, Quintanilla M, Cano A, de Herreros AG, Lafarga M, Munoz A. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. *J Cell Biol* 2001;154:369–387. [PubMed: 11470825]
50. Ohta T, Elnemr A, Yamamoto M, Ninomiya I, Fushida S, Nishimura GI, Fujimura T, Kitagawa H, Kayahara M, Shimizu K, Yi SQ, Miwa K. Thiazolidinedione, a peroxisome proliferator-activated receptor-gamma ligand, modulates the E-cadherin/beta-catenin system in a human pancreatic cancer cell line, BxPC-3. *International Journal of Oncology* 2002;21:37–42. [PubMed: 12063547]

51. Plateroti M, Kress E, Mori JJ, Samarut J. Thyroid hormone receptor alpha 1 directly controls transcription of the beta-catenin gene in intestinal epithelial cells. *Molecular and Cellular Biology* 2006;26:3204–3214. [PubMed: 16581794]
52. Natsume H, Sasaki S, Kitagawa M, Kashiwabara Y, Matsushita A, Nakano K, Nishiyama K, Nagayama K, Misawa H, Masuda H, Nakamura H. beta-Catenin/Tcf-1-mediated transactivation of cyclin D1 promoter is negatively regulated by thyroid hormone. *Biochemical and Biophysical Research Communications* 2003;309:408–413. [PubMed: 12951064]
53. Miller LD, Park KS, Guo QBM, Alkharouf NW, Malek RL, Lee NH, Liu ET, Cheng SY. Silencing of Wnt signaling and activation of multiple metabolic pathways in response to thyroid hormone-stimulated cell proliferation. *Molecular and Cellular Biology* 2001;21:6626–6639. [PubMed: 11533250]
54. Xiao JH, Ghosn C, Hinchman C, Forbes C, Wang J, Snider N, Cordrey A, Zhao Y, Chandraratna RAS. Adenomatous polyposis coli (APC)-independent regulation of beta-catenin degradation via a retinoid X receptor-mediated pathway. *Journal of Biological Chemistry* 2003;278:29954–29962. [PubMed: 12771132]
55. Dillard AC, Lane M. Retinol reduces beta-catenin protein in retinoic acid-resistant colon cancer cells by increasing beta-catenin-RXR-alpha interaction in the nucleus and translocation into the cytosol. *Faseb Journal* 2007;21:A59.
56. Dillard AC, Lane MA. Retinol decreases beta-catenin protein levels in retinoic acid-resistant colon cancer cell lines. *Molecular Carcinogenesis* 2007;46:315–329. [PubMed: 17219422]
57. Li Y, Wu R, Wang X, Chen W. Effects of retinoic acid on the beta-catenin/TCF pathway in cultured porcine tracheobronchial epithelial cells. *J Huazhong Univ Sci Technolog Med Sci* 2004;24:421–3. 432. [PubMed: 15641681]
58. Zwijsen RML, Wientjens E, Klompmaaker R, vanderSman J, Bernards R, Michalides RJAM. CDK-independent activation of estrogen receptor by cyclin D1. *Cell* 1997;88:405–415. [PubMed: 9039267]
59. Neuman E, Ladha MH, Lin N, Upton TM, Miller SJ, DiRenzo J, Pestell RG, Hinds PW, Dowdy SF, Brown M, Ewen ME. Cyclin D1 stimulation of estrogen receptor transcriptional activity independent of cdk4. *Molecular and Cellular Biology* 1997;17:5338–5347. [PubMed: 9271411]
60. Zwijsen RML, Buckle RS, Hijmans EM, Loomans CJM, Bernards R. Ligand-independent recruitment of steroid receptor coactivators to estrogen receptor by cyclin D1. *Genes & Development* 1998;12:3488–3498. [PubMed: 9832502]
61. Cardona-Gomez GP, Mendez P, DonCarlos LL, Azcoitia I, Garcia-Segura LM. Interactions of estrogen and insulin-like growth factor-I in the brain: molecular mechanisms and functional implications. *Journal of Steroid Biochemistry and Molecular Biology* 2002;83:211–217. [PubMed: 12650718]
62. Mulholland DJ, Dedhar S, Coetzee GA, Nelson CC. Interaction of nuclear receptors with the Wnt/beta-catenin/Tcf signaling axis: Wnt you like to know? *Endocr Rev* 2005;26:898–915. [PubMed: 16126938]
63. Li YC, Pirro AE, Amling M, Delling G, Baron R, Bronson R, Demay MB. Targeted ablation of the vitamin D receptor: an animal model of vitamin D-dependent rickets type II with alopecia. *Proc Natl Acad Sci USA* 1997;94:9831–9835. [PubMed: 9275211]
64. Cianferotti L, Cox M, Skoriya K, Demay MB. Vitamin D receptor is essential for normal keratinocyte stem cell function. *Proceedings of the National Academy of Sciences of the United States of America* 2007;104:9428–9433. [PubMed: 17517646]
65. Palmer HG, Anjos-Afonso F, Carmeliet G, Takeda H, Watt FM. The vitamin D receptor is a Wnt effector that controls hair follicle differentiation and specifies tumor type in adult epidermis. *PLoS ONE* 2008;3:e1483. [PubMed: 18213391]
66. Pawlowski JE, Ertel JR, Allen MP, Xu M, Butler C, Wilson EM, Wierman ME. Liganded androgen receptor interaction with beta-catenin - Nuclear co-localization and modulation of transcriptional activity in neuronal cells. *Journal of Biological Chemistry* 2002;277:20702–20710. [PubMed: 11916967]
67. Truica CI, Byers S, Gelmann EP. Beta-catenin affects androgen receptor transcriptional activity and ligand specificity. *Cancer Res* 2000;60:4709–4713. [PubMed: 10987273]

68. Chesire DR, Isaacs WB. Ligand-dependent inhibition of beta-catenin/TCF signaling by androgen receptor. *Oncogene* 2002;21:8453–8469. [PubMed: 12466965]
69. Mulholland DJ, Cheng H, Reid K, Rennie PS, Nelson CC. The androgen receptor can promote beta-catenin nuclear translocation independently of adenomatous polyposis coli. *Journal of Biological Chemistry* 2002;277:17933–17943. [PubMed: 11856748]
70. Shah S, Islam MN, Dakshnamurthy S, Rizvi I, Rao M, Herrell R, Zinser G, Valrance M, Aranda A, Moras D, Norman A, Welsh J, Byers SW. The molecular basis of vitamin D receptor and beta-catenin crossregulation. *Mol Cell* 2006;21:799–809. [PubMed: 16543149]
71. Jansson EA, Are A, Greicius G, Kuo IC, Kelly D, Arulampalam V, Pettersson S. The Wnt/beta-catenin signaling pathway targets PPAR gamma activity in colon cancer cells. *Proceedings of the National Academy of Sciences of the United States of America* 2005;102:1460–1465. [PubMed: 15665104]
72. Kouzmenko AP, Takeyama K, Ito S, Furutani T, Sawatsubashi S, Maki A, Suzuki E, Kawasaki Y, Akiyama T, Tabata T, Kato S. Wnt/beta-catenin and estrogen signaling converge in vivo. *Journal of Biological Chemistry* 2004;279:40255–40258. [PubMed: 15304487]
73. Uno S, Endo K, Jeong Y, Kawana K, Miyachi H, Hashimoto Y, Makishima M. Suppression of beta-catenin signaling by liver X receptor ligands. *Biochemical Pharmacology* 2009;77:186–195. [PubMed: 18983830]
74. Amir AL, Barua M, McKnight NC, Cheng ST, Yuan X, Balk SP. A direct beta-catenin-independent interaction between androgen receptor and T cell factor 4. *Journal of Biological Chemistry* 2003;278:30828–30834. [PubMed: 12799378]
75. Song LN, Herrell R, Byers S, Shah S, Wilson EM, Gelmann EP. Beta-catenin binds to the activation function 2 region of the androgen receptor and modulates the effects of the N-terminal domain and TIF2 on ligand-dependent transcription. *Mol Cell Biol* 2003;23:1674–1687. [PubMed: 12588987]
76. El Tanani M, Fernig DG, Barraclough R, Green C, Rudland P. Differential modulation of transcriptional activity of estrogen receptors by direct protein-protein interactions with the T cell factor family of transcription factors. *Journal of Biological Chemistry* 2001;276:41675–41682. [PubMed: 11522780]
77. Watanabe M, Kakiuchi H, Kato H, Shiraishi T, Yatani R, Sugimura T, Nagao M. APC gene mutations in human prostate cancer. *Jpn J Clin Oncol* 1996;26:77–81. [PubMed: 8609698]
78. Phillips SM, Morton DG, Lee SJ, Wallace DM, Neoptolemos JP. Loss of heterozygosity of the retinoblastoma and adenomatous polyposis susceptibility gene loci and in chromosomes 10p, 10q and 16q in human prostate cancer. *Br J Urol* 1994;73:390–395. [PubMed: 7911059]
79. Lehrer S, McCurdy LD, Stock RG, Kornreich R, Stone NN, Eng C. Body mass, age, and the APC I1307K allele in Ashkenazi Jewish prostate cancer patients. *Cancer Genet Cytogenet* 2000;122:131–133. [PubMed: 11106824]
80. Henrique R, Ribeiro FR, Fonseca D, Hoque MO, Carvalho AL, Costa VL, Pinto M, Oliveira J, Teixeira MR, Sidransky D, Jeronimo C. High promoter methylation levels of APC predict poor prognosis in sextant biopsies from prostate cancer patients. *Clin Cancer Res* 2007;13:6122–6129. [PubMed: 17947477]
81. Richiardi L, Fiano V, Vizzini L, De ML, Delsedime L, Akre O, Gillio TA, Merletti F. Promoter Methylation in APC, RUNX3, and GSTP1 and Mortality in Prostate Cancer Patients. *J Clin Oncol* 2009
82. Gerstein AV, Almeida TA, Zhao G, Chess E, Shih I, Buhler K, Pienta K, Rubin MA, Vessella R, Papadopoulos N. APC/CTNNB1 (beta-catenin) pathway alterations in human prostate cancers. *Genes Chromosomes Cancer* 2002;34:9–16. [PubMed: 11921277]
83. Voeller HJ, Truica CI, Gelmann EP. Beta-catenin mutations in human prostate cancer. *Cancer Res* 1998;58:2520–2523. [PubMed: 9635571]
84. Chesire DR, Ewing CM, Sauvageot J, Bova GS, Isaacs WB. Detection and analysis of beta-catenin mutations in prostate cancer. *Prostate* 2000;45:323–334. [PubMed: 11102958]
85. Truica CI, Byers S, Gelmann EP. Beta-catenin affects androgen receptor transcriptional activity and ligand specificity. *Cancer Res* 2000;60:4709–4713. [PubMed: 10987273]
86. Mulholland DJ, Cheng H, Reid K, Rennie PS, Nelson CC. The androgen receptor can promote beta-catenin nuclear translocation independently of adenomatous polyposis coli. *J Biol Chem* 2002;277:17933–17943. [PubMed: 11856748]

87. Pawlowski JE, Ertel JR, Allen MP, Xu M, Butler C, Wilson EM, Wierman ME. Liganded androgen receptor interaction with beta-catenin: nuclear co-localization and modulation of transcriptional activity in neuronal cells. *J Biol Chem* 2002;277:20702–20710. [PubMed: 11916967]
88. Yang F, Li X, Sharma M, Sasaki CY, Longo DL, Lim B, Sun Z. Linking beta-catenin to androgen-signaling pathway. *J Biol Chem* 2002;277:11336–11344. [PubMed: 11792709]
89. Masiello D, Chen SY, Xu Y, Verhoeven MC, Choi E, Hollenberg AN, Balk SP. Recruitment of beta-catenin by wild-type or mutant androgen receptors correlates with ligand-stimulated growth of prostate cancer cells. *Mol Endocrinol* 2004;18:2388–2401. [PubMed: 15256534]
90. Song LN, Herrell R, Byers S, Shah S, Wilson EM, Gelmann EP. Beta-catenin binds to the activation function 2 region of the androgen receptor and modulates the effects of the N-terminal domain and TIF2 on ligand-dependent transcription. *Mol Cell Biol* 2003;23:1674–1687. [PubMed: 12588987]
91. Li H, Kim JH, Koh SS, Stallcup MR. Synergistic effects of coactivators GRIP1 and beta-catenin on gene activation: cross-talk between androgen receptor and Wnt signaling pathways. *J Biol Chem* 2004;279:4212–4220. [PubMed: 14638683]
92. Song LN, Herrell R, Byers S, Shah S, Wilson EM, Gelmann EP. Beta-catenin binds to the activation function 2 region of the androgen receptor and modulates the effects of the N-terminal domain and TIF2 on ligand-dependent transcription. *Mol Cell Biol* 2003;23:1674–1687. [PubMed: 12588987]
93. Sharma M, Chuang WW, Sun Z. Phosphatidylinositol 3-kinase/Akt stimulates androgen pathway through GSK3beta inhibition and nuclear beta-catenin accumulation. *J Biol Chem* 2002;277:30935–30941. [PubMed: 12063252]
94. Verras M, Sun Z. Beta-catenin is involved in insulin-like growth factor 1-mediated transactivation of the androgen receptor. *Mol Endocrinol* 2005;19:391–398. [PubMed: 15514031]
95. Chesire DR, Isaacs WB. Ligand-dependent inhibition of beta-catenin/TCF signaling by androgen receptor. *Oncogene* 2002;21:8453–8469. [PubMed: 12466965]
96. Mulholland DJ, Read JT, Rennie PS, Cox ME, Nelson CC. Functional localization and competition between the androgen receptor and T-cell factor for nuclear beta-catenin: a means for inhibition of the Tcf signaling axis. *Oncogene* 2003;22:5602–5613. [PubMed: 12944908]
97. Song LN, Herrell R, Byers S, Shah S, Wilson EM, Gelmann EP. Beta-catenin binds to the activation function 2 region of the androgen receptor and modulates the effects of the N-terminal domain and TIF2 on ligand-dependent transcription. *Mol Cell Biol* 2003;23:1674–1687. [PubMed: 12588987]
98. Amir AL, Barua M, McKnight NC, Cheng S, Yuan X, Balk SP. A direct beta-catenin-independent interaction between androgen receptor and T cell factor 4. *J Biol Chem* 2003;278:30828–30834. [PubMed: 12799378]
99. Chen SY, Wulf G, Zhou XZ, Rubin MA, Lu KP, Balk SP. Activation of {beta}-Catenin Signaling in Prostate Cancer by Peptidyl-Prolyl Isomerase Pin1-Mediated Abrogation of the Androgen Receptor-{beta}-Catenin Interaction. *Mol Cell Biol* 2006;26:929–939. [PubMed: 16428447]
100. Berger R, Febbo PG, Majumder PK, Zhao JJ, Mukherjee S, Signoretti S, Campbell KT, Sellers WR, Roberts TM, Loda M, Golub TR, Hahn WC. Androgen-induced differentiation and tumorigenicity of human prostate epithelial cells. *Cancer Res* 2004;64:8867–8875. [PubMed: 15604246]
101. Rios-Doria J, Kuefer R, Ethier SP, Day ML. Cleavage of beta-catenin by calpain in prostate and mammary tumor cells. *Cancer Res* 2004;64:7237–7240. [PubMed: 15492240]
102. Linja MJ, Porkka KP, Kang Z, Savinainen KJ, Janne OA, Tammela TL, Vessella RL, Palvimo JJ, Visakorpi T. Expression of androgen receptor coregulators in prostate cancer. *Clin Cancer Res* 2004;10:1032–1040. [PubMed: 14871982]
103. Horvath LG, Henshall SM, Lee CS, Kench JG, Golovsky D, Brenner PC, O'Neill GF, Kooner R, Stricker PD, Grygiel JJ, Sutherland RL. Lower levels of nuclear beta-catenin predict for a poorer prognosis in localized prostate cancer. *Int J Cancer* 2005;113:415–422. [PubMed: 15455387]

Figure 1. Wnt and NHR Crosstalk in Stem Cell Differentiation

(A) Differentiation of mesenchymal stem cells is modulated by the key regulators PPAR γ (adipogenesis) and Wnt (osteogenesis). A host of NHRs play a role in modulating the activity of these two key regulators. Glucocorticoid inhibits Wnt signaling by increasing the expression of Wnt antagonists DKK and sFRP (extracellular) and Axin (intracellular) and also by decreasing the expression of Wnts. PPAR γ is also able to inhibit Wnt signaling by inducing the degradation of β -catenin under specific conditions. VDR can modulate differentiation by inhibiting DKK and sFRP, which pushes the cells towards osteoblast differentiation. Wnt, itself, can propagate osteogenesis by inhibiting PPAR γ activity via the expression of Cyclin D1 and *c-myc*, which bind to PPAR γ and inhibit its activity. β -catenin also increases the

expression COUP-TFII, which represses PPAR γ transcription. (B) Embryonic stem cells differentiate down a neuronal path under the influence of retinoic acid (RA), which is known to inhibit the Wnt pathway. RA activity also induces body axis extension in the developing embryo. This Wnt inhibition is, in part, effected by an RA-mediated increase in Wnt antagonists, Fzd and DKK. Wnts are responsible for caudal development in the mouse and dorsal development in the frog. A potential negative-feedback loop occurs during Wnt-mediated inhibition of CYP26, an enzyme that metabolizes RA. (C) Keratinocyte stem cells differentiate into sebocytes, follicle keratinocytes or epidermal keratinocytes, which comprise the interfollicular epidermis. VDR and β -catenin cooperate to induce differentiation into follicle keratinocytes, however, perturbation of β -catenin or VDR (independently of each other) leads to cysts that contain epidermal keratinocytes and sebocytes.

Figure 2. β-Catenin-Mediated Crosstalk with NHRs in the Nucleus

Degrees of transcriptional activation/repression are indicated on a scale with + being activation, ++ being increased activation, +++ being synergistic activation, and - being inhibition. **(A)** TCF and NHR compete for binding to β-Catenin. In the presence of agonist, activated NHR usurps β-catenin from TCF:β-catenin complexes. This synergistically activates NHR-responsive genes. At the same time, TCF, in the absence of β-catenin, is able to repress its gene promoters. **(B)** TCF and NHR compete for co-activators other than β-catenin. Activated NHR recruits p300 away from TCF:β-catenin complexes, decreasing or inhibiting Wnt responsive genes, while enhancing NHR-regulated genes. **(C)** β-catenin is recruited to adherens junctions. E-cadherin is a transcriptional target of activated NHRs. Once it is translated, it can recruit cytoplasmic β-catenin to the membrane, preventing it from co-activating both Wnt responsive genes and NHR responsive genes. **(D)** NHRs bind TCF and β-catenin. NHRs can bind to TCF/LEF family members and repress genes by recruiting classical NHR co-repressors such as NCoR/SMRT and classical TCF co-repressors such as TLE. β-catenin may, or may not be found in the same complex with TCF/NHR/co-repressors. The transcriptional outcome of β-catenin binding the same complex is still unclear.