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Cross-Regulation Between Wnt and NF-κB Signaling Pathways

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Abstract

Cross-regulation between the Wnt and nuclear factor (NF)-κB signaling pathways has emerged as an important area for the regulation of a diverse array of genes and pathways active in chronic inflammation, immunity, development, and tumorigenesis. The ligands, kinases, transcription factors, and products of their target gene expression are involved in cross-regulation of these two signaling pathways. Both β-catenin and NF-κB activate inducible nitric oxide synthase (iNOS) gene expression; however, β-catenin also exerts an inhibitory effect on NF-κB-mediated transcriptional activation, including iNOS. The recent discovery of functional cross-regulation between these two pathways has shown complex roles for Wnt/β-catenin and NF-κB signaling in the pathogenesis of certain cancers and other diseases. This review focuses on the molecular mechanisms of cross-regulation between Wnt/β-catenin and NF-κB signaling pathways in cancer cells.

Keywords

Wnt signaling pathway; NF-κB signaling pathway; cross-regulation; β-catenin

I. INTRODUCTION

Cross-regulation of several cellular signaling pathways has been shown to play important roles in modifying the biological effects of gene expression. Wnt/β-catenin and nuclear factor (NF)-κB are independent pathways involving the regulation of many physiological and pathological effects related to the areas of development, immune function, inflammation, tumorigenesis, tumor invasion, and metastasis, as well as cardiovascular and bone diseases. However, the activity and signaling consequences are also regulated by direct interactions between these two pathways, which results in diversity and complexity.

Many reports demonstrate that these two pathways independently initiate oncogenesis in colon, liver, and other organs. A few recent studies have shed light on the cross-regulation between these two pathways that influences development and carcinogenesis. Because these two pathways are involved in the regulation of gene expression and activation, transcription factors are highly active in most cancer cells, and thus are ideally suited for development of anticancer drug therapies. Several transcription factors, including β-catenin/Tcf and NF-κB, are promising targets for cancer therapeutics.[1] It is now evident that strategies targeting the cross-regulation between these pathways may be a promising direction for future cancer therapeutics.

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The molecular basis of the cross-regulation between the Wnt and NF-κB signaling pathways is an essential issue for fully understanding potential therapeutic mechanisms. In order to clearly describe the interplay between Wnt and NF-κB pathways, the mode for cross-regulation between the two was recently reviewed by Guo and Wang,[2] who applied three basic modes of signaling cross-regulation to elucidate the commonalities. A cross-regulation exists between designated pathways A and B when both of the following criteria are met: i) functionally, the combinatorial signal from A and B must produce a different response than that triggered by A or B alone; and ii) mechanistically, A and B pathways must be connected in at least one of three ways: (a) components of the two pathways physically interact, (b) components of one pathway are enzymatic or transcriptional targets of the other, and (c) one signal modulates or competes for a key modulator or mediator of the other. Moreover, we will also consider the dynamic manner in which canonical or non-canonical Wnt signaling is reciprocally regulated with the NF-κB signaling pathway. Although many commonalities exist by which Wnt signaling interplays with the NF-κB pathway, the analyses of cell line- and tissue-specific differences are still wanting.[2]

In this review, we summarize the mechanisms of cross-regulation between Wnt and NF-κB signal transduction pathways focusing on cross-modulation and reciprocal modulation. For the functional cross-regulation between Wnt and NF-κB signaling pathways, in addition to the marquis proteins β-catenin and NF-κB, we will also address the roles of E-cadherin, Wnt proteins, Wnt antagonists, glycogen synthase (GSK)-3β, inhibitor of NF-κB (IκB)/IκB kinase (IKK), and β-transducin repeat-containing protein (β-TrCP). These components are involved in the regulation of transcriptional activity or degradation cascades of these two pathways.

II. WNT SIGNALING PATHWAY

Currently, Wnt signaling is composed of Wnt/β-catenin (also referred to as canonical Wnt) and non-canonical Wnt signaling is based on the absence of β-catenin involvement. In response to Wnt protein binding to its receptor complex, signal transduction is triggered under physiologic conditions. Constitutively activated β-catenin signaling due to β-catenin mutation or adenomatous polyposis coli (APC) gene deficiency results in evasion of the degradation complex, and has been observed and in colon cancer and other tumors.

II.A. Wnt Proteins

The Wnt proteins are secreted, lipid-modified signaling molecules that control a number of central cellular processes. There are 19 Wnt proteins that have been identified in mammals so far (Wnt homepage: http://www.stanford.edu/~rnusse/wntwindow.html). Some, such as Wnt1 and Wnt3, either activate or inhibit the canonical Wnt signaling pathway, and some, including Wnt5A, Wnt5B, and Wnt11, activate the non-canonical Wnt signaling pathway.[3] The combinations of Wnt proteins (ligands) and their receptors in Wnt signaling have been summarized previously by Kikuchi et al.[4]

II.B. Wnt/β-Catenin Signaling

In a cell, β-catenin is localized to the transmembrane-, cytoplasmic-, or nuclear-β-catenin pools. The first two pools determine the physiological role in development and homeostasis associating with the cell-cell adherence protein E-cadherin, while the nuclear-β-catenin fraction is involved in oncogenesis associated with the tumor suppressor gene product APC. Wnt/β-catenin signaling is mediated by β-catenin, which plays a dual role as a transcription factor and as a molecule of cell adherence junctions interacting with the cadherins. Wnt/β-catenin signaling has diverse functions in regulating cellular processes such as proliferation, differentiation, migration, and survival, whereas non-canonical Wnt signaling controls tissue

polarity and movement. In the absence of Wnt, cytosolic β-catenin protein is constantly degraded by the β-catenin destruction complex, which is composed of Axin, APC, GSK-3β, and casein kinase 1 (CKI). In the absence of Wnt, CKI and GSK-3β sequentially phosphorylate the amino-terminal region of β-catenin, leading to recognition by β-TrCP, an E3 ubiquitin ligase subunit resulting in poly-ubiquitination and degradation. This continual turnover of β-catenin silences the Wnt pathway. When the Wnt ligand binds to its receptor, Frizzled (Fz), and its co-receptor, low-density lipoprotein receptor-related protein 5/6 (LRP5/6), Wnt, Fz, and LRP6 form a complex, together with an intracellular protein Dishevelled (Dvl), and in turn phosphorylate LRP6. These molecular events prevent β-catenin phosphorylation and degradation. The stabilized β-catenin is accumulated in the cytoplasm and travels to the nucleus, where β-catenin binds the Tcf/Lef (T-cell transcription factor/lymphocyte enhancer factor) family of transcription factors and activates the Wnt target gene expression. In a cancer cell, a component of Wnt signaling such as APC or β-catenin is mutated. In this case, β-catenin can be stabilized in the cytoplasm and works as a co-activator of Wnt/β-catenin signaling involved in many aspects of tumorigenesis, cancer development, and progression.[5–7]

II.C. Non-canonical Wnt Signaling

When Wnt proteins bind to their receptor, there are two branches of the non-canonical Wnt signaling pathway. The first is generally called as the Wnt/c-Jun N-terminal kinase (JNK) pathway, which activates small GTPases such as Rac, Rho, and CDC42 and, more downstream, Rho-kinase (ROCK) or JNK. The other Wnt-mediated non-canonical signaling pathway stimulates the intracellular increase in Ca^{2+}, possibly mediated by G-proteins. This pathway activates several downstream targets, including protein kinase C (PKC), and Ca-calmodulin kinase II (CaMKII). The elevated levels of Ca^{2+} can activate the phosphatase calcineurin, which induces the dephosphorylation of the transcription factor nuclear factor of activated T-cells (NFAT), resulting in an accumulation of NFAT in the nucleus and an activation of target genes. The effects of non-canonical Wnt signaling are in tissue polarity control and cell migration.[8,9]

II.D. Wnt Antagonists

Several secreted protein families inhibit or mediate Wnt signaling: i) secreted Fz-related proteins (sREPs) and Wnt inhibitory protein (WIF) bind Wnt or Fz as inhibitors of canonical and non-canonical Wnt signaling; ii) the Dickkopf (DKK) and the WISE/SOST families are LRP5/6 ligands/antagonists: DKK1 inhibits Wnt signaling via inducing LRP6 internalization/degradation through transmembrane kremen protein, and SOST is able to disrupt Wnt-induced Fz-LRP6 complex in vitro; iii) Shisa proteins trap Fz proteins in the endoplasmic reticulum and prevent Fz from reaching the cell surface; and iv) *Xenopus cerberus* and Nodal and bone morphogenetic protein (BMP) binds to and inhibits Wnt signaling.[7]

III. NF-KB SIGNALING PATHWAY

The NF-κB transcription factors are generally retained in the cytoplasm of resting cells, and when activated bind to a large array of enhancer sequences (over 150 genes) that are present in most (if not all) cells. Mammalian NF-κB transcription factors consist of five homologous subunits (RelA/p65, c-Rel, RelB, p50/ NF-κB1, and p52/ NF-κB2) that dimerize and are held in the cytoplasm by specific proteins, the IκBs. Immediately upstream from the IκB-bound NF-κB dimers is the IKK complex, comprised of two catalytic (IKKα and IKKβ) and one regulatory (IKKγ/ NF-κB essential modulator [NEMO]) subunits. Several pathways of cell stimulation converge to activate the IKK complex, which then phosphorylates NF-κB-bound IκB proteins that targets the IκB protein for ubiquitination and degradation by the 26S

proteasome by creating a binding site for Skp1-Cullin1-F-box protein (SCF)/β-TrCP ubiquitin ligase complex. The liberated NF-κB translocates into the nucleus and engages transcriptional programs. For activation of NF-κB signaling, the two most recognized pathways are the so-called "classical" and "alternative" pathways. The former depends on NEMO, IKKβ activation, and nuclear localization of RelA/p50 dimers, and is associated with inflammation, while the latter depends on IKKα activation, probably via the upstream NF-κB-inducing kinase (NIK) and nuclear localization of p52/RelB heterodimers, and is important in lymphoid organogenesis. Both pathways of NF-κB activation have now been implicated in carcinogenesis.[10,11]

IV. CROSS-REGULATION OF WNT AND NF-KB PATHWAYS

IV.A. Wnts and Cross-regulation Between Wnt and NF-κB Pathways

In recent years, research studies have shown important roles for Wnt5A. The functions of Wnt5 signaling are in bridging innate and adaptive immunity to infections, and Wnt5A is a cancer-related gene involving in invasion and metastasis of many cancers.[12] Wnt5A transcription is regulated by many proteins, included NF-κB. A conserved NF-κB-binding site within the Wnt5A promoter B region elucidates the mechanisms by which tumor necrosis factor-alpha (TNFα) and Toll-like receptor (TLR) signals up-regulate Wnt5A via MAP3K7 signals. SNAI1 (Snail), CD44, G3BP2, and YAP1 are Wnt5A signaling target genes.[13,14] Following stimulation of macrophages with different mycobacterial species and conserved bacterial structures, Wnt5A is expressed, which involves the activation of TLR signaling and NF-κB. Induction of Fz5, the Wnt5A receptor, has also been reported in human peripheral-blood mononuclear cells. Binding to its receptor, Wnt5A activates canonical and non-canonical Wnt signaling pathways and plays key roles in a variety of cellular processes during development and carcinogenesis. Importantly, the expression of Wnt5A protein is controlled by the NF-κB signaling pathway, which may be implicated as an essential mediator not only for infection, but also for cancer development.

Binding with its receptor, Wnt-11 signaling is sufficient to inhibit not only the canonical Wnt but also JNK/activator protein-1 (AP-1) and NF-κB signaling in Chinese hamster ovary (CHO) cells, thus serving as a non-canonical Wnt ligand in this system and leading to the promotion of cell viability.[3]

WntD is a member of the *Drosophila* Wnt family. Toll/NF-κB signaling has an evolutionarily conserved role in regulating innate immunity. WntD acts as a feedback inhibitor of the NF-κB homolog *Dorsal* during both embryonic patterning and in the innate immune response to infection. WntD expression is under the control of *Toll/Dorsal* signaling, and increased levels of WntD block *Dorsal* nuclear accumulation, even in the absence of the IκB homolog Cactus. Thus, the WntD signal is independent of the common Wnt signaling component Armadillo (β-catenin), and WntD serves as a feedback antagonist of *Toll* signaling and maintaining low basal levels of *Toll/Dorsal* signaling in the fly. Moreover, WntD mutants show defects in embryonic *Dorsal* regulation and in the adult innate immune system.[15]

IV.B. Wnt Antagonists Affect the Cross-regulation Between Wnt and NF-κB Pathways

DKK1 is a secreted Wnt antagonist whose transcription is mediated by canonical Wnt signaling.[16–18] The activation of Wnt signaling and overexpression of DKK1 have been observed in breast cancer. It is interesting that human breast cancer cell lines that preferentially form osteolytic bone metastasis exhibited increased levels of Wnt signaling and DKK1 expression. Breast cancer cell-produced DKK1 blocks Wnt3A-induced osteroblastic differentiation and osteroprotegerin (OPG) expression, and Wnt3A-induced NF-κB ligand reduction. These results suggest that breast cancer-produced DKK1 may be an

important mechanistic link between primary breast tumors and secondary osteolytic bone metastases.[19] In postnatal and adult life, osteoblasts and osteoclasts play opposite roles for bone matrix formation and resorption. The interaction of these two cell types determines bone density. Numerous lines of evidence from genetic studies show that Wnt/β-catenin signaling regulates bone mass and bone diseases.[5] However, Wnt/β-catenin signaling promotes the activity of osteoblasts. It is clear that the decreased activity of osteoblasts contributes to osteolytic lesions in multiple myeloma. The production of DKK1 by multiple melanoma cells inhibits osteoblast activity. However, a neutralizing antibody (BHQ880) to DKK1 up-regulates the β-catenin level while down-regulating NF-κB activity in bone marrow stromal cells (BMSCs), and inhibits multiple myeloma cell growth in the severe combined immunodefi ciency (SCID)-hu murine model. These results confi rm DKK1 as an important therapeutic target in myeloma, and provide the rationale for clinical evaluation of BHQ880 to improve bone disease and to inhibit multiple myeloma growth.[20]

IV.C. E-Cadherin Mediates the Cross-regulation Between Wnt and NF-κB Pathways

E-cadherin plays a dual role in cells: in addition to its structural role in adherens junctions, E-cadherin mediates the dynamic of β-catenin, which acts as a transcription factor in the nucleus by serving as a coactivator of the Tcf/Lef family of DNA-binding proteins. On the other hand, E-cadherin, a target gene of Wnt/β-catenin signaling,[21] is involved in the negative regulation of canonical Wnt signaling. Because the molecular basis for the interaction of β-catenin with cadherins and Tcf/Lef family members is mediated by the same domain on the β-catenin molecule (the so-called arm repeat), these interactions are mutually exclusive. Thus, recruitment of β-catenin into adherens junctions by elevating the expression of cadherin can decrease its nuclear pool and antagonize β-catenin–Tcf/Lef transactivation.[22]

Functional cross-regulation between Wnt and NF-κB pathways also occurs during epithelial-mesenchymal transition (EMT) mediated by E-cadherin and its transcriptional repressor Snail. Expression of Snail promotes the conversion of epithelial cells to mesenchymal cells, and occurs concomitantly with the down-regulation of E-cadherin and the up-regulation of expression of mesenchymal genes, such as those encoding fibronectin and Lef1.[23] E-cadherin overexpression decreased the transcriptional activity of the fibronectin promoter and reduced the interaction of β-catenin and NF-κB with this promoter. Fibronectin is a target gene of Wnt signaling.[24] Fibronectin and Lef1 gene expressions are dependent on the transcriptional activity of β-catenin and NF-κB. These activities are both controlled by the presence of E-cadherin-dependent cell contacts in epithelial cells. Similar to β-catenin, NF-κB is found to be physically associated with E-cadherin and other cell-adhesion components. Interaction of the NF-κB p65 subunit with E-cadherin or β-catenin is reduced when adherens junctions are disrupted by K-ras overexpression or by E-cadherin depletion using small interfering RNA (siRNA). E-cadherin, as a Wnt target gene, not only controls the transcriptional activity of β-catenin, but also that of NF-κB during EMT. Binding of NF-κB to the adherens-junctional complex prevents the transcription of mesenchymal genes.[25] The major route for signal transduction by E-cadherin involves the negative-feedback regulation of β-catenin-Tcf signaling and down-regulation of NF-κB. It is expected that NF-κB transcriptional activity is mainly inhibited by the adherens-junction-associated pool of β-catenin.[25] Furthermore, malignant transformation of melanocytes frequently coincides with the loss of E-cadherin expression. Melanoma cells show constitutively active NF-κB, where as no such activity is found in primary melanocytes. The mechanism for loss of E-cadherin leading to induction of NF-κB activity in melanoma cell lines has been proposed to be due to cytoplasmic β-catenin inducing p38-mediated NF-κB activation in malignant melanoma.[26]

IV.D. GSK-3β Mediates the Cross-regulation Between Wnt and NF-κB Pathways

GSK-3β has emerged as one of the most attractive therapeutic targets for the treatment of many diseases and disorders. GSK-3β plays dual roles in the APC-β-catenin destruction complex in regulating Wnt signaling and as a critical regulator of NF-κB activity, including gene transcription, cell cycle, apoptosis, inflammation, glucose metabolism, stem-cell renewal, and differentiation.[27] Targeting GSK-3β is a promising approach for cancer therapy.[28] Deregulated GSK-3β activity in colorectal cancer is associated with tumor cell survival and proliferation. The inhibition of GSK-3β has been observed in many tumors,[29] and activates canonical Wnt and NF-κB signaling pathways. Specifically, GSK-3β controls the degradation of β-catenin by phosphorylating β-catenin at Ser^{37} and Ser^{33}. These phosphorylations provide a binding site for the E3 ubiquitin ligase β-TrCP, leading β-catenin to the proteasome complex for degradation. In this respect, GSK-3β is a negative regulator of Wnt signaling. On the other hand, GSK-3β positively regulates NF-κB by mediating the degradation of IκB, a central inhibitor of NF-κB.[30,31] Inhibition of GSK-3β differentially modulates NF-κB, cAMP response element-binding protein (CREB), AP-1, and β-catenin signaling in mouse primary hepatocytes, but fails to promote TNFα-induced apoptosis. Stimulation of canonical Wnt signaling and CREB activity led to up-regulated levels of anti-apoptotic factor.[32] These observations indicate a complex cross-regulation between NF-κB and β-catenin pathways.

In collaboration with Perwez Hussain, we have shown that the human inducible nitric oxide synthase (iNOS or NOSII) gene is a target of the Wnt signaling pathway.[33] Two functional Tcf-4 binding elements (TBE1 and TBE2) were identified upstream in the human iNOS promoter. Overexpression of β-catenin and Tcf4 significantly increased both basal and cytokine-induced human iNOS promoter activity, and the induction was dependent on intact TBE sites. Furthermore, overexpression of β-catenin or TCF4 increased iNOS mRNA and protein expression in HCT-116 cells. Lithium chloride, an inhibitor of GSK-3β, increased the cytosolic β-catenin level, iNOS expression, and nitric oxide (NO) production in primary human and rat hepatocytes and cancer cell lines. In vivo, lithium chloride also increased hepatic β-catenin level in a dose-dependent manner with simultaneous increase in iNOS expression. These fi ndings support the hypothesis that β-catenin up-regulates iNOS, and suggests a novel mechanism by which the Wnt/β-catenin signaling pathway contributes to cancer by increasing NO production (Fig. 1).

Other research has shown that E-cadherin disassembly and concomitant inactivation of GSK-3β that induces β-catenin triggered NF-κB-dependent up-regulation of iNOS in hepatocytes.[34] GSK-3β/APC may also regulate NF-κB activity with an inverse correlation in vitro and in vivo through β-catenin by cross-regulating with NF-κB signaling pathway.[35]

A study of the associations between these two pathways during trans-differentiation was conducted. The β-catenin/Tcf4/p300 signaling loops play an important role in trans-differentiation toward the morular phenotype of endometrial carcinomas. Cross-regulation between NF-κB/p65 and β-catenin/Tcf4/p300 signaling pathways through alterations in GSK-3β expression during trans-differentiation of endometrial carcinoma cells was noted. These fi ndings provide evidence that a shift from NF-κB to β-catenin signaling pathways through alterations in GSK-3β expression may be essential for the induction of trans-differentiation of endometrial carcinoma cells.[36]

Signaling pathway cross-regulation does not just occur inside cells, but is also an intercellular event. Macrophage cells have a critical role in intestinal tumorigenesis. Because the activated macrophages caused by *Helicobacter* infection produce NF-κB-dependent TNFα, which phosphorylates GSK-3β. The inactivated GSK-3β results in the stabilization of β-catenin, and promotes Wnt/β-catenin signaling in gastric cancer cells. This model is

consistent with the observation that β-catenin nuclear accumulation in macrophage-infiltrated dysplastic mucosa of the K19-Wnt1 mouse stomach.[37] This study provides strong evidence that TNFα is a link between the chronic inflammation and promotion of preexisting Wnt/β-catenin signaling during tumorigenesis of gastric cancers.[37,38]

IV.E. IKKs/IκB and the Cross-regulation Between Wnt and NF-κB Pathways

Two kinases, IKKα and IKKβ, are critical activators of the NF-κB pathway. They are also important in the regulation of β-catenin function. IKKβ decreases β-catenin-dependent transcriptional activation, while IKKα increases β-catenin-dependent transcriptional activity in IKKα- and IKKβ-deficient mouse embryo fibroblasts[39] and in human multiple myeloma.[40] IKKα and IKKβ interacting with and phosphory-lating β-catenin may in part be responsible for regulating β-catenin protein levels and cellular localization and integrating signaling events between the NF-κB and Wnt pathways.[39] Even if some multiple melanoma cell lines have constitutive classical NF-κB activity, and a subset of multiple melanoma cell lines shows alternative NF-κB activity, only IKKα down-regulation decreases the expression of β-catenin and aurora-A, which are known to mediate multiple melanoma cell growth and survival.[40] IKKα plays a pivotal role in the regulation of β-catenin signaling through different mechanisms. First, IKKα can inhibit β-catenin degradation mediated not only by the Axin/APC/GSK-3β complex, but also by the Siah-1 pathway. Consistently, IKKα abolished the inhibition of β-catenin/Tcf-dependent transcription by Siah-1. Furthermore, IKKα interacted with β-catenin and mediated β-catenin stabilization by inhibiting β-catenin ubiquitination, which in turn stimulated β-catenin/Tcf-dependent transcription[41] Second, IKKα but not IKKβ, induces CyclinD1 expression, which is identified as a target of Wnt/β-catenin signaling pathway, also through Tcf activity. The CyclinD1 gene functions as a point of convergence between the Wnt/β-catenin and IκB pathways in mitogenic signaling. Mitogenic induction of G(1)-S phase progression and CyclinD1 expression is PI3K dependent, and CyclinD1($^{-/-}$) cells show reduced PI3K-dependent S-phase entry. PI3K-dependent induction of CyclinD1 is blocked by inhibitors of PI3K/Akt/IκB/IKKα or β-catenin signaling. A single Tcf site in the CyclinD1 promoter is required for induction by PI3K or IKKα.[42]

RelA (p65) also is involved in the down-regulation of the Wnt/β-catenin pathway. This suppression does not depend on the transacting transcriptional ability of RelA. Furthermore, RelA affects neither the nuclear import of β-catenin nor the DNA-binding ability of the β-catenin/Tcf complex, suggesting that NF-κB modifies this signaling pathway after the binding of the β-catenin/Tcf complex with target DNA.[43]

Previous studies have shown that NF-κB activation plays certain roles in mediating proliferation and anti-apoptosis in response to progastrin on pancreatic cancer cells[44] and on proximal colonic crypts of FAbp-PG mice.[45] β-catenin expression can be activated in colonic crypts of mice in response to chronic (Fabp-PG mice) and acute (wild-type FVB/N mice) progastrin stimulation.[46] Significant increases are observed in the relative levels of cellular and nuclear β-catenin and pbeta-cat45 in proximal colonic crypts of Fabp-PG mice compared with that in wild-type littermates. IKKα/β/NF-κB activates β-catenin signaling, because treatment of Fabp-PG mice with the NEMO peptide (an inhibitor of IKKα/β/NF-κB activation) significantly blocks increases in cellular/nuclear levels of total β-catenin, pβ-catenin Ser^{45}, and pβ-catenin Ser^{552} in proximal colons. Cellular levels of pβ-catenin Ser^{33,37} and Thr^{41}, however, increase in the proximal colon in response to NEMO, probably due to a signifi cant increase in pGSK-3β Tyr^{216}, facilitating degradation of β-catenin. Distal colonic crypts were less responsive.[46,47] This suggests a functional cross-regulation between the NF-κB and β-catenin pathways, and that the activation of β-catenin may contribute to the hyperproliferative effects of progastrin on proximal colonic crypts.[46]

IV.F. Physical Interaction of β-Catenin and NF-κB Components and the Cross-regulation of Wnt and NF-κB Pathways

1. β-Catenin Physically Interacts with NF-κB Components and Inhibits NF-κB Target Gene Function—Gene transcription activity can be activated or inhibited by signal-induced β-catenin and NF-κB interaction between transcription factors on regulatory elements positioned near their target genes. β-catenin as a coactivator of canonical Wnt signaling has been intensively studied, including cross-regulation with the NF-κB pathway. A direct interaction between Wnt and NF-κB signaling pathways was reported in a pioneering study finding that β-catenin can physically complex with NF-κB, resulting in a reduction of NF-κB DNA binding, transactivation activity, and target gene expression in some cancer cells.[48] It is interesting that repressed NF-κB activity was observed in human colon cancer cells in which β-catenin is activated. Importantly, activated β-catenin was found to inhibit the expression of NF-κB target genes, including *Fas* and *Traf1*. Furthermore, a strong inverse correlation was identified between the expression levels of β-catenin and Fas in colon and breast tumor tissues, suggesting that β-catenin regulates NF-κB and its targets in vivo. These findings led to the suggestion that β-catenin may play an important role in oncogenesis through the cross-regulation of NF-κB.[48]

Another example of the cross-regulation of these two pathways is the fine-tuned regulation of human iNOS gene expression. Our studies demonstrate that the hiNOS gene is regulated and targeted by NF-κB[49] and the Wnt/β-catenin signaling pathway.[33] Recently, we also reported that Wnt/β-catenin signaling regulates cytokine- or TNFα-induced hiNOS expression through interaction with the NF-κB pathway. Our in vitro (colon and liver cancer cell models) and in vivo (hepatocellular carcinoma tissues) data show that β-catenin signaling inversely correlates with cytokine-induced hiNOS and other NF-κB-dependent gene expression (*Fas* and *Traf1*). These fi ndings underscore the complex role of Wnt/β-catenin, NF-κB, and iNOS signaling in the pathophysiology of infl ammation-associated carcinogenesis (Fig. 1).[50] However, a different study demonstrated that not all NF-κB target genes are repressed by the increased expression of β-catenin.[51] This reveals distinct and gene-selective molecular strategies for the down-regulation of NF-κB target genes by β-catenin.

2. β-Catenin Physically Interacts with NF-κB Components and Synergizes NF-κB Target Gene Function—In an analysis of the expression of TNFα-induced C-reactive protein (CRP), the p50 subunit of NF-κB as a positive regulator was found to be responsible for the transcriptional activation of CRP, and β-catenin to enhance the expression of a CRP mRNA in concert with p50. This protein-protein interaction is required for CRP expression. Therefore, CRP is mediated by the cross-regulation between Wnt/β-catenin and NF-κB signaling pathways via β-catenin binding with the NF-κB p50 subunit.[52] It is interesting that this interaction can be disrupted by the β-catenin-binding RNA aptamer as a tool for studying protein-protein interaction within the transcription complex and for modulating the expression of a target gene. The RNA aptamer binds Armadillo repeats of β-catenin, is effective in disrupting protein-protein interaction between β-catenin and NF-κB (p50), and effectively reduces TNFα-induced transcription from the promoter of CRP regulated by β-catenin and NF-κB p50.[53]

3. β-Catenin/Reptin Complex Physically Interacts with NF-κB Components and Controls NF–κB Target Gene Function—In a study of the transcriptional regulation of the metastasis suppressor gene *KAI1* (Kangai 1), which is a target gene of NF-κB signaling,[54] *KAI1* expression was found to be regulated by the interaction between β-catenin or Tip60 with the NF-κB pathway at the *KAI1* promoter. β-catenin expression functionally inhibits KAI1 expression by β-catenin complexed with reptin, which displaces the Tip60

coactivator complex. Down-regulation of KAI1 in prostate cancer cells involves the inhibitory actions of β-catenin, along with a reptin chromatin remodeling complex. This inhibitory function of β-catenin-reptin requires both increased β-catenin expression and recruitment of histone deacetylase activity. The coordinated actions of β-catenin-reptin components that mediate the repressive state serve to antagonize a Tip60 coactivator complex that is required for activation; the balance of these opposing complexes controls the expression of KAI1 and metastatic potential. The molecular mechanisms underlying the antagonistic regulation of β-catenin-reptin involve the binding of NF-κB p50/p50 with β-catenin and the reptin complex at the *KAI1* promoter in metastatic cells. This is a typical β-catenin interaction with NF-κB and down-regulates its target gene, *KAI1* expression occurring on the *KAI1* promoter.[51]

IV.G. Cross-regulation of Wnt and NF-κB Pathways Mediated by Transcriptional Complex

1. NLK Mediates the Cross-regulation Between Wnt/β-Catenin and NF-κB Pathways—A very important kinase, NEMO-like kinase (NLK), is involved in the cross-regulation of Wnt and NF-κB pathways. NLK can be activated by Wnt1 and non-canonical Wnt signaling,[55] and is a serine/threonine kinase. NLK suppresses not only the transcription activity of the β-catenin/Tcf complex through phosphorylation of Tcf,[56] which establishes a negative feedback mechanism for the regulation of Wnt signaling, but also the transcription co-activators of NF-κB, such as CREB binding protein (CBP)/p300, rather than NF-κB itself.[57] These results suggest that NLK is a key player in the cross-regulation between Wnt and NF-κB signaling pathways, and may suppress a wide range of gene expression, possibly through NLK phosphorylating the C-terminal domain of CBP.[57]

2. NF-κB Signaling Affects β–catenin Transcriptional Activity—Lef1 is coactivator of the β-catenin transcription factor complex. A conserved NF-κB-binding site between mouse and human was selected through a bioinformatics analysis and mapped to 14 kb upstream of *Lef1* transcription initiation site. Overexpression of Lef1 in cartilage tissue of osteoarthritic patients has been observed, along with NF-κB-mediated *Lef1* gene regulation in chondrocytes. Treatment of IL-1β augments *Lef1* up-regulation and nuclear translocation of NF-κB in chondrocytes. Lef1 expression was synergistically up-regulated by interactions of NF-kB with Lef1/β-catenin in the same cells. This implicates a pivotal role for NF-κB in Lef1 expression in arthritic chondrocytes and in cartilage degeneration.[58]

In a study of non-steroidal anti-inflammatory drugs (NSAIDs) repressing CRT (β-catenin/Tcf4-regulated transcription) in colorectal cancer, the NSAID diclofenac and a methanol extract of *Polysiphonia japonica* inhibited Wnt/β-catenin signaling without altering the level of β-catenin protein, and reduced the expression of β-catenin/Tcf-dependent genes. Diclofenac and the *P. japonica* extract, on the other hand, induced degradation of IκBα, which increased free NF-κB in cells. Also, the ectopic expression of p65, which is a component of NF-κB, suppressed CRT. These findings suggest that diclofenac inhibits Wnt/β-catenin signaling via activation of NF-κB in colon cancer cells.[52,59]

IV.H. Target Gene Product Involves Cross-Regulation Between Wnt and NF-κB Pathways

Thyroid cancer-1 (TC1 or C8orf4) is a small protein present in vertebrates. TC1 is a novel endothelial inflammatory regulator enhancing NF-κB activity[60] that up-regulates heat-shock proteins.[61] TC1 is also a target gene of NF-κB signaling and up-regulates the Wnt/β-catenin pathway by relieving the antagonistic activity of Chibby (Cby), a nuclear β-catenin-associated antagonist of the Wnt/wingless pathway[62] for β-catenin-mediated transcription.[63] Cby is associated with inflammation and aggressive behavior in cancer with poor survival. Upon coexpression in mammalian cells, TC1 redistributes from the nucleolus to nuclear speckles, where it co-localizes with Cby. TC1 also up-regulates the expression of β-catenin

target genes that are implicated in invasiveness and aggressive behavior of cancers, such as metalloproteinases, laminin gamma2, and others.[63]

The expression of leucine zipper tumor suppressor 2 (lzts2) is positively regulated by NF-κB activity in colon, liver, and breast cancer cells, whereas it is negatively regulated in glioma cells. Through lzts2, NF-κB negatively regulates the Wnt/β-catenin signaling pathway in colon, liver and breast cancer cells, whereas it has an opposite effect on this signaling pathway in glioblastoma. These findings indicate that NF-κB cross-regulates Wnt/β-catenin signaling via lzts2 in various human cancer cells.[64]

IV.I. Wnt-Regulated β-TrCP Mediates Cross-regulation of Wnt and NF-κB Pathways

1. β-TrCP Expression is Associated with Wnt and NF-κB Pathways—The ubiquitin/proteasome pathway is involved in the cross-regulation between Wnt and NF-κB by promoting ubiquitination of IκBα and β-catenin for their degradation. These molecular events lead to positive and negative regulation of the NF-κB and Wnt pathways, respectively. β-TrCP, an E3 ubiquitin ligase receptor, is a component of the ubiquitin ligase complex targeting β-catenin and IκBα for proteasomal degradation by specifically recognizing a 19-amino-acid destruction motif in IκB and β-catenin.[65] With targeted disruption of the β-TrCP gene in knockout mice, IκB and β-catenin degradation can be prevented.[66]

2. β-TrCP Is a Target of the Wnt/β-Catenin Pathway and Up-regulates NF-κB and Down-regulates Wnt/β-Catenin Signaling—Several lines of evidence support a role for β-TrCP in the cross-regulation between Wnt/β-catenin and NF-κB signaling. A very important finding for the mechanism of the cross-regulation is that β-catenin/Tcf signaling elevates the expression of the β-*TrCP* mRNA and protein in a Tcf-dependent manner, which does not require β-TrCP transcription. Induction of β-TrCP expression by the β-catenin/Tcf pathway results in an accelerated degradation of the wild-type β-catenin, suggesting that a negative feedback loop may control the β-catenin/Tcf regulation. This signal also up-regulates NF-κB transactivation without affecting IκB kinase activity. Therefore, the maintenance of the β-TrCP level is important for coordination between β-catenin/Tcf and NF-κB signaling.[67] Endogenous β-TrCP1 expression is regulated through the conserved Wnt cascade. Up-regulation of Wnt1 results in the β-catenin-mediated activation of Tcf-4, leading to increased β-TrCP1 expression and NF-κB activity in vascular smooth muscle cells.[68] The relationship among β-TrCP, β-catenin, and NF-κB in colorectal cancer has shown that increased expression of β-TrCP1 is associated with the activation of both β-catenin and NF-κB, suggesting that integration of these signaling pathways by increased β-TrCP expression may contribute to an inhibition of apoptosis and tumor metastasis.[69,70]

3. Overexpression of CRD-BP Stabilizes β-TrCP mRNA—β-catenin also stabilizes the mRNA encoding for β-TrCP1, and identifies the RNA-binding protein CRD-BP (coding region determinant-binding protein) as a previously unknown target of the β-catenin/Tcf transcription factor.[71] CRD-BP binds to the coding region of β-TrCP1 mRNA. Overexpression of CRD-BP stabilizes β-TrCP1 mRNA and elevates β-TrCP1 levels in vitro and in vivo, resulting in the activation of the SCF (β-TrCP) E3 ubiquitin ligase, and in accelerated turnover of its substrates, including IκB and β-catenin, in colorectal cancer cells.[71] High levels of CRD-BP are found in primary human colorectal tumors and malignant melanomas exhibiting active β-catenin/Tcf signaling, implicating CRD-BP induction in the up-regulation of β-TrCP1, in the activation of dimeric transcription factor NF-κB, and in the suppression of apoptosis in these cancers.[71,72]

IV.J. Epigenetic Modifications Medicate Cross-regulation Between Wnt and NF-κB Pathways

Post-translational modification of Tcf/Lef includes phosphorylation, acetylation, sumoylation, and ubiquitination/degradation.[7] CD44 overexpression and Wnt/β-catenin activation have been observed in colon cancer. The expression of CD44, a cross-membrane protein, and a receptor of hyaluronan (HA), is regulated by the Wnt pathway.[73] HA binding to CD44 up-regulates p300 expression and its acetyltrans-ferase activity, which in turn promotes acetylation of β-catenin and NF-κB-p65, leading to activation of β-catenin-associated Tcf/Lef transcriptional co-activation and NF-κB-specific transcriptional up-regulation, respectively. This interaction can be reversed by activation of the NAD-dependent deacetylase sirtuin-1 (SIRT1) by resveratrol (a natural antioxidant). Resveratrol induces SIRT1-p300 association and acetyltransferase inactivation, leading to deacetylation of HA/ CD44-induced β-catenin and NFκB-p65, inhibition of β-catenin-Tcf/Lef enhancer factor, and NF-κB-specific transcriptional activation.[74] The Wnt target gene product, CD44 triggers the post-translational modification and up-regulates Wnt/β-catenin and NF-κB signaling, as well as MDR and Bcl-xL gene expression, respectively. Through these modifications, breast cancer cells gain anti-apoptosis/survival benefit and chemotherapeutic resistance.[74]

IV.K. Cross-regulation Between Wnt/β-catenin and NF-κB Pathways Indicates the Link Between Chronic Inflammation and Tumorigenesis

Epithelia of the vertebrate intestinal tract characteristically maintain an inflammatory hyporesponsiveness toward the lumenal prokaryotic microflora. The identification of enteric organisms (nonvirulent *Salmonella* strains) whose direct interaction with model human epithelia attenuates synthesis of inflammatory effector molecules elicited by diverse proinflammatory stimuli has been reported. This immunosuppressive effect involves inhibition of the IκB/NF-κB pathway by blockade of IκBα degradation, which prevents subsequent nuclear translocation of the active NF-κB dimer. These data suggest that prokaryotic determinants can be responsible for the unique tolerance of the gastrointestinal mucosa to proinflammatory stimuli.[75]

Salmonella-epithelial cell interactions are known to activate the proinflammatory NF-κB signaling pathway and have recently been found to also influence the β-catenin-signaling pathway. By using polarized epithelial cell models, the same bacteria-mediated effects were shown to be involved in the molecular cross-regulation between the NF-κB and the β-catenin signaling pathways. Convergence of these two pathways is a result of the direct interaction between the NF-κB p50 subunit and β-catenin.[76] PhoP(c), the avirulent derivative of a wild-type *Salmonella* strain, attenuates NF-κB activity and the expression of its target gene, IL-8, by stabilizing the association of β-catenin with NF-κB. These findings strongly suggest that the cross-regulation between the β-catenin and NF-κB pathways is an important regulator of intestinal inflammation.[76] Moreover, the same research group also found that AvrA, a bacterial effector existing in *Salmonella*, cross-regulates Wnt and NF-κB signaling pathways in colonic epithelial cell inflammation by deubiquitination, leading to increased β-catenin and decreased NF-κB activation.[77] On the other hand, β-catenin also plays an opposing role in the regulation of the NF-κB pathway. Wild-type *Salmonella* infection directly increases GSK-3β activity, which phosphorylates β-catenin, leading to its degradation, and further decreasing the physical association between NF-κB and β-catenin, which consequently increases NF- κB activity.[47] As mentioned previously, *Helicobacter*-infected k-19-wnt1 mouse stomach activates macrophages and causes β-catenin nuclear accumulation. This experimental model provides pivotal evidence that the macrophage-derived TNFα promotes Wnt/β-catenin signaling, which may influence gastrointestinal oncogenic potential. This also supports the observation that malignancy is frequently

preceded by chronic inflammation in individuals harboring activating mutations in the *APC* or *CTNNB1* genes or enhanced activation of Wnt/β-catenin signaling.[37,38]

V. RECIPROCAL REGULATION OF WNT AND NF-ΚB PATHWAYS

V.A. Wnt Signaling Initiates Interdependent Regulation with NF-κB

Using hair follicle induction as a model system, the patterning of dermal Wnt signaling requires epithelial β-catenin activity. Wnt signaling is absolutely required for NF-κB activation, and *Edar* is a direct Wnt target gene.[78] Wnt signaling is initially activated independently of EDA/EDAR/NF-κB activity in primary hair follicle primordia. However, Eda/Edar/NF-κB signaling is required to refine the pattern of Wnt activity and to maintain this activity at later stages of placode development. Maintenance of localized expression of Wnt10b and Wnt10a requires NF-κB signaling, provides a molecular explanation for the latter observation, and identifies Wnt10b as a direct NF-κB target. Moreover, DKK4, a Wnt/β-catenin signaling antagonist,[79] is a target gene of Eda/Edar.[80] NF-κB indirectly limits Wnt activity by activating DKK4, which in turn inhibits β-catenin signaling. These data reveal a complex interplay and interdependence of the Wnt and EDA/EDAR/NF-κB signaling pathways in the initiation and maintenance of primary hair follicle placodes.[78] These studies imply that NF-κB signaling may limit Wnt/β-catenin activity and refine the pattern of hair placode borders by establishing a DKK4-mediated negative-feedback regulation.

V.B. Interplay Between Wnt/β-Catenin and NF-κB Pathway Regulated by lzts2

The modulation of NF-κB activity shows a direct correlation with β-catenin/Tcf pathway in human adipose tissue (hASCs) and bone marrow (hBMSCs)-derived mesenchmal stem cells. Expression of lzts2, which represses β-catenin nuclear translocation and transcription activity, is positively regulated by NF-κB signaling.[81] Interestingly, down-regulation of lzts2 increases β-catenin and NF-κB activity in hASCs, increases the proliferation of hASCs and hBMSCs, and blocks the NF-κB-inhibitor-induced repressive effects on proliferation and Tcf promoter activation. Moreover, the activated NF-κB induced by the down-regulation of lzts2 is accompanied by increased β-TrCP expression and decreased IκB levels. The reciprocal cross-regulation of the β-catenin/Tcf pathway by NF-κB is mediated by lzts2 in hASCs.[81]

VI. CONCLUSIONS

Over the last 20 years since their identification, great progress has been made in understanding the complex role of the Wnt and NF-κB signaling pathways. Wnt and NF-κB signaling exert crucial roles in development, homeostasis, and pathogenesis by regulating the transcription of cell type-specific programs of Tcf and NF-κB target genes. However, the mechanisms and biological effects of the cross-regulation of these two pathways remains an area of intense investigation. The studies of cross-regulation allow detailed analyses of Wnt and NF-κB signaling pathway interactions responsive to either Wnt or NF-κB signals.

Most of models provide evidence for mechanisms of cross-regulation between Wnt and NF-κB pathways either through β-catenin and NF-κB physical interaction or through target gene expression of the pathways for the convergence of the cross-regulation of both cell and animal models. With these notions, it is expected that dissection of the interdependent mechanisms regulating these pathways will demonstrate more insight into the cell biology of a signaling network crucial for development, homeostasis, and carcinogenesis.

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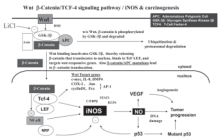

FIGURE 1.

Summary of Wnt/β-catenin, NF-κB, and iNOS pathways. Both β-catenin and NF-κB activate iNOS gene expression, however, β-catenin also exerts an inhibitory effect on NF-κB-mediated transcriptional activation, including iNOS.