Regulatory Role and Mechanisms of Wnt/β-catenin Signaling Pathway in Mammalian Uterine Development Postprint

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Date: 2017-10-11T00:00:00+00:00

Abstract

The programmed and orderly execution of physiological activities within an organism depends on the coordinated interplay among different signal transduction pathways, among which the Wnt signaling pathway (named after the Drosophila wingless gene Wingless and the mouse oncogene int-1) has attracted extensive attention from researchers and has become a research focus in the fields of molecular biology and cell biology. This study investigates the regulatory role and mechanisms of the Wnt/β-catenin signaling pathway in mammalian uterine development, reviews the regulatory mechanisms of glycogen synthase kinase-3β (GSK-3β), adenomatous polyposis coli (APC), Axin, and osteoblast inhibitory factor (Dkk) on the Wnt/β-catenin signaling pathway, as well as the nuclear activation of this pathway, aiming to further elucidate intrauterine regulatory mechanisms and provide insights for the treatment of uterine diseases.

Full Text

Regulation and Mechanism of the Wnt/β-catenin Signaling Pathway in Mammalian Uterine Development

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Abstract: The orderly execution of physiological programs within organisms depends on coordinated interactions among distinct signal transduction pathways. The Wnt signaling pathway—named after the Drosophila Wingless gene and mouse oncogene int-1—has attracted extensive scholarly attention and emerged as a major research focus in molecular and cell biology. This review examines the regulatory roles and mechanisms of the Wnt/β-catenin signaling pathway in mammalian uterine development, focusing on the modulatory effects of glycogen synthase kinase-3β (GSK-3β), adenomatous polyposis coli (APC), scaffold protein Axin, and osteoblast inhibitory factor (Dkk). We also discuss the nuclear activation of Wnt/β-catenin signaling to further elucidate intrauterine regulatory mechanisms and provide insights for treating uterine diseases.

Keywords: Wnt/β-catenin; uterus; GSK-3β/APC/Axin; Dkk; nuclear activation

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The reproductive performance of female animals is manifested through their fecundity, with the uterus serving as the essential organ for embryogenesis and fetal development in mammals. Abnormal uterine development can lead to menstrual disorders, pathological pregnancies, and infertility in females, while in sows it results in reduced fertilization rates, implantation rates, and litter sizes. All physiological activities within an organism depend on the coordinated function of various signaling pathways. Research has demonstrated that Wnt signaling plays crucial roles in regulating cell growth and differentiation, organ development, embryonic implantation, and the pathogenesis of various diseases and tumors. Studies have further revealed that Wnt signaling is vital for embryonic Müllerian duct development, growth, and differentiation, as well as for postnatal development and normal function of the female reproductive tract. The pathway regulates embryo implantation and maintains uterine homeostasis, while also mediating estrogen activation and progesterone inhibition during the menstrual cycle. Elevated endometrial estrogen levels or unopposed estrogen signaling due to pathological conditions can activate Wnt signaling, triggering endometrial hyperplasia and potentially leading to endometrial cancer. Although signaling pathways represent one of the most active frontiers in life sciences research, the molecular mechanisms of Wnt signaling remain incompletely understood, with many questions requiring urgent investigation. Therefore, reevaluating and actively exploring the regulatory mechanisms of the canonical Wnt/β-catenin signaling pathway has become a research priority for gaining deeper insights into the Wnt family.

1. Wnt/β-catenin Signaling Pathway and Uterine Development

The uterus provides the site for embryonic formation and development in mammals. During embryogenesis, the uterus undergoes cyclical changes under the influence of various reproductive hormones, with concurrent alterations in endometrial and stromal tissues during pregnancy. This complex developmental process is regulated by numerous intra- and extra-uterine factors. Extensive research has established that Wnt signaling is intimately associated with cell differentiation and plays a pivotal role in organismal development, where spatiotemporal dysregulation or aberrant activation of any pathway component can cause developmental abnormalities. Wnt signaling is equally important in reproductive system development, with the canonical Wnt/β-catenin pathway being most relevant to reproduction.

The Wnt/β-catenin pathway is a highly conserved signaling cascade that participates in diverse biochemical processes and represents a critical signaling mechanism during embryonic tissue development and differentiation in higher animals. It regulates cell proliferation, differentiation, polarization, apoptosis, and anti-apoptotic responses through modulation of target gene expression. In uterine development, the pathway operates as follows: When Wnt ligands bind to Frizzled (Fz) receptors and low-density lipoprotein receptor-related proteins (LRP) to form a trimeric complex, the signal is transduced via Fz to Dishevelled (Dsh) in uterine epithelial cells. Through this upstream activation, Dsh inhibits GSK-3β activity, preventing β-catenin phosphorylation and enabling its accumulation in the cytoplasm. β-catenin then translocates into the nucleus, where it activates target genes in concert with T cell-specific transcription factors (TCF) and lymphoid enhancer-binding factors (LEF). In the absence of Wnt signaling, a multiprotein complex comprising APC, Axin, and protein phosphatase 2A (PP2A) facilitates GSK-3β-mediated phosphorylation of β-catenin. Phosphorylated β-catenin undergoes covalent modification by the ubiquitin-proteasome system and is subsequently degraded, thereby terminating Wnt/β-catenin signal transduction [Figure 1: see original paper].

Figure 1. Wnt/β-catenin signaling pathway. Frizzled: Fz receptor; LRP: low-density lipoprotein receptor-related protein; APC: adenomatous polyposis coli; GSK-3β: glycogen synthase kinase-3β; Axin: scaffold protein; Dsh: Dishevelled; Wnt: named after Drosophila Wingless and mouse int-1 oncogene; TCF: T cell-specific transcription factor; β-catenin: β-catenin. [15]

2. Regulatory Mechanisms of the Wnt/β-catenin Signaling Pathway

The prevailing model posits that the GSK-3β/APC/Axin ternary complex directly phosphorylates intracellular β-catenin to regulate its stability, maintaining low cytoplasmic β-catenin levels in the absence of Wnt stimulation.

2.1. GSK-3β Regulation of Wnt/β-catenin Signaling

In the intact Wnt/β-catenin pathway, GSK-3β functions by phosphorylating Axin, β-catenin, and APC, thereby directly inhibiting Dsh to achieve negative regulation of the pathway. In the absence of Wnt signals, GSK-3β forms a complex with β-catenin, facilitated by Axin and APC, to phosphorylate serine/threonine residues at the β-catenin N-terminus. The β-transducin repeat-containing protein (β-TrCP) recognizes N-terminally phosphorylated β-catenin and initiates ubiquitin-mediated degradation. Upon Wnt stimulation, GSK-3β activity is suppressed, allowing β-catenin to escape proteolysis and accumulate in the cytoplasm. Notably, whereas early studies suggested GSK-3β could directly phosphorylate β-catenin, recent evidence indicates that serine-45 of β-catenin must first be phosphorylated by casein kinase-1α (CK1α) before forming a complex with GSK-3β. Studies have confirmed that constitutive β-catenin activation in the Wnt/β-catenin pathway causes endometrial hyperplasia and stromal cell tumors in mice, while conditional β-catenin knockout results in squamous changes in the endometrium. These findings suggest that GSK-3β deficiency or dysfunction may contribute to endometrial hyperplasia and endocrine disorders.

2.2. APC Regulation of Wnt/β-catenin Signaling

APC functions as a scaffold protein in the Wnt/β-catenin pathway, facilitating GSK-3β-mediated phosphorylation of β-catenin. Some studies suggest that APC, which possesses a nuclear export signal, can bind β-catenin and shuttle it from the nucleus to the cytoplasm for subsequent degradation, although β-catenin transport in tumor cells appears APC-independent. Additional research indicates that APC is the regulatory factor most closely associated with apoptosis in the Wnt/β-catenin pathway. APC exhibits a high mutation frequency, and mutant APC can bind to the anti-apoptotic Bcl-2 protein, significantly enhancing Bcl-2 activity.

2.3. Axin Regulation of Wnt/β-catenin Signaling

Initially identified as a tumor suppressor, Axin encodes a protein that critically regulates Wnt/β-catenin signaling. As a scaffold protein, Axin contains binding sites for APC, Dsh, PP2A, GSK-3β, β-catenin, and CK1α, enabling diverse biological functions. Axin negatively regulates the Wnt/β-catenin pathway by controlling intracellular β-catenin levels. Axin mutations have been detected in ovarian endometrioid adenocarcinomas, suggesting that abnormal Axin expression may be related to its pro-apoptotic functions. Studies show that Axin induces apoptosis through activation of the c-Jun N-terminal kinase (JNK) pathway or by reducing intracellular β-catenin levels. When Axin mutations prevent JNK activation, its pro-apoptotic capacity diminishes; when mutations abolish β-catenin binding while preserving JNK activation, the reduction in apoptotic capacity is even more pronounced. Concurrent high expression of both β-catenin and Axin also attenuates Axin-induced apoptosis.

2.4. Dickkopf (Dkk) Regulation of Endometrial Receptivity

Endometrial receptivity—the ability of the endometrium to accept embryos—represents a crucial indicator of reproductive performance. Research indicates that Dkk proteins regulate endometrial receptivity by modulating Wnt/β-catenin signaling. Dkk, a secreted protein expressed spatiotemporally on the endometrial surface, shows increased expression during the implantation period, altering secretory phase receptivity. The Dkk family comprises Dkk1, Dkk2, Dkk3, and Dkk4, with overlapping functional domains and coordinated spatiotemporal regulation in mesodermal co-expression. Dkk1 and Dkk2 are most closely associated with endometrial receptivity changes. Dkk1 inhibits Wnt signaling by binding to transmembrane proteins Kremen1 (Krm1) and Kremen2 (Krm2). Dkk2 similarly inhibits Wnt/β-catenin signaling but can activate the pathway in Krm2-deficient cells, with its activating effect exceeding its inhibitory effect. Studies show that Dkk1 exhibits weak or absent expression during the proliferative phase of normal female mammalian endometrium but increases 13.1-fold during the mid-secretory phase, suggesting a role in mediating successful embryo implantation. Dkk2 expression initiates in uterine glandular epithelium on day 4 of the implantation window and declines by day 6, coinciding with altered uterine receptivity. During uterine decidualization, Dkk2 inhibits Wnt/β-catenin signaling. Since Dkk2 and Krm2 are not co-expressed between days 6–7 of pregnancy, Dkk2-mediated inhibition may occur independently of Krm2, though the precise mechanism requires further investigation.

2.5. Other Regulatory Mechanisms of Wnt/β-catenin Signaling

Emerging research continues to identify novel regulatory factors. For example, presenilin-1 (PS-1), implicated in Alzheimer’ s disease, functions as a negative regulator of Wnt/β-catenin signaling. Recent studies reveal that PS-1 can mediate β-catenin phosphorylation and degradation in an Axin/CK1α-independent manner, similar to Axin’ s function. Prostaglandin F2α receptor (FPB) positively regulates Wnt/β-catenin signaling by activating the phosphatidylinositol-3 kinase (PI3K)/Rho pathway. Axin-associating molecule (Axam), an Axin-binding protein, inhibits Wnt/β-catenin signal transduction. Additionally, cytoplasmic deubiquitinating enzymes can bind β-catenin, promoting its accumulation and nuclear entry. Inhibitory B kinase-α (Ikkα) and Ikkβ both phosphorylate β-catenin, with Ikkα enhancing and Ikkβ suppressing its transcriptional activity.

Previous research focused primarily on serine/threonine phosphorylation, while tyrosine phosphorylation was thought to only regulate cadherin/β-catenin complexes in cell adhesion. However, recent studies demonstrate that tyrosine phosphorylation also plays an indispensable role in Wnt/β-catenin signaling by extending β-catenin half-life, promoting nuclear accumulation, and activating target genes.

2.6. Nuclear Activation of Wnt/β-catenin Signaling

Upon Wnt signaling, β-catenin escapes ubiquitin-mediated degradation, accumulates in the cytoplasm, and translocates into the nucleus to activate target genes with LEF/TCF. Research suggests that LEF/TCF maintains β-catenin nuclear retention, while β-catenin can exit the nucleus independently of chromosome region maintenance-1 (CRM-1) via its own nuclear export signal. In the nucleus, β-catenin binds to DNA-binding proteins TCF/LEF to form a ternary complex with DNA, altering DNA conformation and recruiting additional transcription factors to initiate target gene transcription—a model recently confirmed by Graham et al.' s crystal structure analysis. Numerous nuclear proteins also modulate β-catenin transcriptional activity. Among LIM protein family members, four-and-a-half LIM domain protein 2 (FHL2) cooperates with β-catenin/TCF to activate transcription of target genes such as cyclin D1 and interleukin-8. Notably, the legless (Lgs) protein identified in Drosophila enables pygopus (Pygo) binding to β-catenin, with Pygo' s plant homeodomain (PHD) facilitating β-catenin or TCF access to chromatin for Wnt target gene activation.

3. Summary and Outlook

Although understanding of Wnt/β-catenin signaling in uterine and other reproductive systems continues to deepen, mechanistic elucidation of intermolecular signal transmission has only begun. Numerous questions remain unresolved, including the precise molecular mechanisms by which Wnt prevents GSK-3β/APC/Axin-mediated β-catenin phosphorylation in the activated pathway, how information is exchanged among various factors, and how β-catenin produces differential target gene expression outcomes after nuclear entry. Current understanding of Wnt/β-catenin signaling remains largely derived from overexpression studies, necessitating loss-of-function approaches to ensure physiological relevance. Future research should focus on selecting appropriate methodologies for investigating signaling mechanisms at different stages and pathways. Furthermore, given that Wnt/β-catenin signaling is involved in multiple developmental stages and its dysregulation causes various reproductive diseases, studies of this pathway and its antagonists hold promising therapeutic potential.

Note: Figure translations are in progress. See original paper for figures.

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