D J Med Sci 2021;7(1):66-76
doi: 10.5606/fng.btd.2021.25050

Review

The role of WNT/β-catenin pathway in cancer and autism

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ABSTRACT

The WNT family is a group of signaling molecules that have been shown to control various developmental processes, including cell specification, proliferation, polarity, and cell migration. Dysregulation of WNT signaling plays a role in developmental defects and tumor formation. The importance of WNT signaling in development and clinical pathologies has been emphasized by studies examining various aspects of WNT signaling. There is data suggesting that WNT signaling hyperactivation leads to the pathogenesis of autism spectrum disorder. In this review, the molecular mechanism of WNT/β-catenin signal transduction as well as the relationship of WNT/β-catenin signaling dysregulation with tumor formation and autism are discussed.

Keywords: Autism, cancer, Rett syndrome, tumor, WNT/β-catenin.

WNT signaling is one of the main mechanisms that determine cell proliferation, cell polarity, and cellular outcome during embryonic development and tissue homeostasis.[1] Mutations in the WNT pathway are often linked to developmental defects, cancer, and other diseases. Critical to these processes and the most studied WNT pathway is the canonical WNT signal, which controls gene expression programs that play a key role in development and also regulates the amount of the transcriptional co-activator beta (β)-catenin.[2]

Beta-catenin was first defined as a protein located in the cell membrane that plays a role in cell adhesion. By acting as a bridge between the cytoplasmic portion of E-cadherin and alpha (α)-actin, one of the cytoskeletal elements in the cytosol, it is a molecule which plays a role in cell-cell interactions.[3] Determining of the homology of the β-catenin protein with the Armadillo protein (Arm) found in Drosophila revealed that it also functions as a transcription factor.[4,5] The β-catenin protein contains binding sites in its structure that bind to many molecules such as adenomatous polyposis coli (APC), Axin, and T-cell factor/lymphoid enhancer factor 1 (TCF/LEF-1).[6,7] By identifying the interacting biomolecules, it was revealed that the β-catenin protein plays important roles not only in cell adhesion but also in the WNT/β-catenin signaling pathway.

Another remarkable region of the β-catenin protein structure is the phosphorylation regions located at the N-terminal end which are significant for its stabilization.[8,9] The WNT/β-catenin signaling pathway regulates the level of β-catenin in the cytoplasm and nucleus via the destruction complex in the cytosol. When the signaling pathway is inactive, phosphorylation of regions rich in serine amino acids found in the structure of the β-catenin protein acts as a marker for the degradation of the β-catenin protein. While there is no mutation in the biomolecules involved in

Received: January 18, 2021 ***Accepted:*** February 23, 2021 **Published online:** May 05, 2021

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Cite this article as:
Candar F, Erbaş O. The role of WNT/β-catenin pathway in cancer and autism. D J Med Sci 2021;7(1):66-76.

the signaling pathway, the β-catenin left over from destruction is found in the cell membrane to function in cell-cell adhesions.[10] When the signaling pathway is active, the destructive complex disintegrates, the β-catenin cannot be phosphorylated and the level of β-catenin in the cytoplasm increases. When any mutation occurs in the CTNNB-1 (Catenin Beta 1) gene, which encodes the β-catenin protein, especially those which prevent the protein from being phosphorylated at the N-terminal, various diseases and many types of cancer formation may occur.[11,12]

The WNT signal controls and regulates anteroposterior axis formation and neural differentiation in the brain during early vertebrate development.[13] Therefore, any disruption in WNT signaling can trigger disorders related to the structure and function of the central nervous system.[14,15]

Irregularity of the WNT signal has been reported in various psychiatric disorders such as Autism Spectrum Disorder (ASD), bipolar disorder, schizophrenia, as well as mental disability.[14,16]

WNT SIGNALING PATHWAY MECHANISM

Three types of WNT signaling pathways have been identified: WNT/β-catenin (canonical), WNT/Ca+2 (non-canonical), and WNT/Planar Cell Polarity (PCP) (non-canonical).[17]

In the WNT/β-catenin signaling pathway, intracellular signalization starts the Wnt ligand (WNT1, WNT2, WNT3, WNT3a, WNT7a, WNT7b, WNT8a, WNT8b, WNT10b, or WNT16) binding to the Frizzled (Fz) receptor and the Fz coreceptor defined as low-density lipoprotein receptor-related protein 5/6 (LRP5/6) protein. Thus, the ternary structure (Fz-WNT-LRP5/6) required to initiate the WNT signaling mechanism is formed.[18] After this binding, the signal first passes to the cytoplasm and DVL (disheveled) phosphorylation is activated. Then, this phosphorylation disintegrates the destruction complex, which is comprised of Axin, APC, CK1 (casein kinase 1), GSK-3 (glycogensynthase kinase 3), which provides stabilization and nuclear translocation of β-catenin. β-catenin enters the nucleus and binds to TCF/LEF which are members of the transcription factor family, and with the help of coactivators p300 and CBP (CREB-binding protein), allowing the transcription of several WNT target genes that enable cell proliferation.[19] In the absence of the WNT ligand, the cytoplasmic β-catenin is marked for degradation by the destruction complex, which occurs by phosphorylation of serine and threonine residues in the N-terminal of β-catenin by CK1 and GSK3 components.[20]

The other two non-canonical pathways are associated with differentiation, cell polarity, and migration. In the non-canonical WNT/PCP pathway, when WNT ligands bind to the Fz receptors, GTPases such as RhoA (Ras homolog family member A), RAC (Ras-related C3 botulinum toxin substrate), and Cdc42 (cell division control protein 42) are activated.[21] The PCP pathway affects the cytoskeleton and stimulates transcriptional activation of target genes responsible for cell adhesion.[22] In the non-canonical WNT/Ca+2 pathway, when WNT ligands bind to Fz receptors or alternative receptors (Ryk or ROR), cell migration and inhibition of the WNT/β-catenin pathway occurs through intracellular Ca+2 influx and activation of calmodulin kinase II (CaMK2), Jun kinase (JNK), and PKC.[23]

Aside from secreted WNT, R-spondin ligand family members have been discovered as positive effectors of WNT signaling.[24-26] R-spondins bind to the leucine-rich repeat-containing G-protein coupled receptor (LGR) 4-6.[27] When R-spondin does not bind, the two homologous E3 ubiquitin ligases ZNRF3/RNF43 targets the Frizzled (Fzd) receptor for lysosomal degradation.[28] The binding of R-spondins to the G-protein coupled receptor 4-6 inhibits the activity of ZNRF3/RNF43 and leads to the accumulation of Fzd receptors on the cell surface.[26,29] The interaction of ZNRF3 and RNF43 with the Fzd receptor has been found to be promoted by DVL.[30]

RELATIONSHIP OF CANCER TYPES TO WNT/β–CATENIN SIGNALING PATHWAY

Abnormal WNT signaling often leads to high β-catenin levels in the nucleus, and this consequence is associated with various types of cancer. One of the malignancies most strongly

associated with abnormal WNT signaling is colorectal cancer.[31]

The WNT pathway is up-regulated in colorectal cancers. WNT, which is normally activated in the crypts of intestinal glands, plays a critical role in cell repair and maintenance of stem cell functions. The primary mechanism of WNT pathway activation is the deactivation of APC, which acts as a negative regulator. Deactivation of the APC protein eliminates destruction complex-mediated ubiquitination of β-catenin and activates WNT/β-catenin signaling.[32]

Aside from APC, mutations of the R-spondin/Lgr5/Rnf43 module have been identified as driving forces of WNT-dependent tumor growth, and deleterious RNF43 mutations have been identified in approximately 19% of colorectal cancer cases. These mentioned mutations are not independent of APC mutations. In addition, excessive expression of R-spondin-3 mutations and fusion proteins have been demonstrated in 10% of colorectal cancer cases.[33]

Chromosomal instability is common in colorectal cancer and is associated with poor prognosis. Dysfunction of WNT pathway components, particularly APC, has been linked to chromosomal instability through multiple mechanisms. The direct interactions of APC with the cytoskeleton and the transcriptional WNT response via the β-catenin pathway are among the routes to chromosomal instability.[34,35]

Unlike colorectal cancer, mutations of key WNT pathway components are rare in pancreatic ductal adenocarcinoma (PDAC). However, aberrant nuclear localization of β-catenin is frequently observed.[36] Results from mouse models indicate that WNT signaling promotes tumor formation when activated at different tumor stages.[37]

Recent studies have shown that PDAC cell lines that carry an RNF43 mutation are particularly susceptible to treatment with porcupine (PORCN) inhibitor LGK974.[38] PORCN is a member of the MBOAT (membrane associated O-acyl transferase) family and is responsible for the lipid modification and secretion of WNT.[39] Response to treatment with LGK974 indicates that PDAC is based on WNT ligand stimulation.[38] Furthermore, in addition to induction of the WNT antagonist DKK1, treatment with anti-Fzd antibody OMP18R5 delays PDAC formation.[40,41]

WNT signaling is also activated in cholangiocarcinoma, however, genomic changes of major WNT pathway components, with the exception of RNF43, are rare.[41,42] Pharmacological inhibition of the WNT signal at both β-catenin and WNT secretion levels decreased the proliferation of cholangiocarcinoma cells in a studied mouse model.[43] Moreover, secreted WNT signal inhibitors such as SFRP2 are often silenced by hypermethylation in cholangiocarcinoma.[44,45]

In recent years, knowledge of the role of the WNT signaling in hematopoiesis and leukemia has increased.[46] Normal hematopoietic stem cells (HSC) depend on a sensitive WNT signal level for long-term maintenance, whereas WNT activity is significantly increased in most leukemias.[47]

Beta-catenin is considered necessary for the progression of leukemia-initiating cells (LIC) from pre-LIC to LIC state and for the self-renewal of the LIC.[48,49]

The most common leukemia in childhood is acute lymphoblastic leukemia. It has been observed that canonical WNT signaling is a driving force during tumorigenesis of the specific T-cell acute lymphoblastic (T-ALL).[50]

Chronic lymphocytic leukemia (CLL) is the most common form of adult leukemia in Western countries. Canonical WNT signaling is active in CLL cells and its inhibition increases *in vitro* apoptosis. In a portion of studied cases, alongside frequent silencing of WNT inhibiting factors such as DKK1/2, somatic mutations in genes related to the WNT pathway were found in a fraction of studied patients. While knockdown of mutated WNT pathway members reduced cell viability in CLL cells carrying the targeted WNT pathway alteration, those without WNT pathway mutations remained unaffected. These findings demonstrate that a subset of CLL is dependent on active WNT signaling for survival.[51]

WNT signaling is activated in over half of breast cancer cases and is associated with reduced overall survival.[52] The role of canonical WNT signaling in the development and progression of triple negative breast cancer has been studied.[53] Furthermore, high levels of nuclear β-catenin have been found in other breast cancer subtypes.[54]

Although only a small fraction of tumors harbors somatic mutations of key regulators of this pathway, such as β-catenin, canonical WNT ligands and receptors are often overexpressed in breast cancers.[55,56] The secreted antagonists are silenced.[57] In addition, overexpression of R-spondin-2 has also been shown to induce breast tumors in mouse models.[58]

In a significant portion of prostate tumors, increased β-catenin levels are seen in the cytoplasm or nucleus either as a result of gene mutations or as a result of non-genomic changes in the expression of inhibitors and activators of the WNT signal.[59]

WTX, a tumor suppressor protein involved in destruction complex function, is mutated in some cases of Wilms' tumor, a form of pediatric kidney cancer.[60] WTX occurs in the destruction complex in which β-catenin promotes degradation, making its tumor suppressor properties equivalent to those of APC and Axin.[61]

WNT signaling has an important place in neural differentiation during early vertebrate development.[13] In contrast, the abnormal WNT signal in neural stem cells (NSCs) stimulates malignant transformation and initiates the formation of brain tumors.[62]

The DNA repair gene platelet activating factor (PAF) is specifically overexpressed in colon cancer and intestinal stem cells. PAF mechanically strengthens WNT signaling by incorporating histone methyltransferase EZH2 into the TCF transcriptional complex.[63]

There is evidence indicating that WNT signaling contributes to the growth of cancer cells or cancer stem cells in a paracrine mode, and that metastatic tumor cells carry the activating properties of WNT signaling.[64,65]

WNT SIGNALING PATHWAY MUTATIONS IN AUTISM

Studies have shown that mutations of genes related to the WNT pathway contribute to autism spectrum disorder (ASD).[14]

WNT1 mutation, found in some ASD patients, has a higher capacity to activate WNT/β-catenin signaling than wild-type WNT1.[66] In particular, the increase in WNT3 expression, which normally plays a role in gastrulation and hippocampal neurogenesis in the prefrontal cortex of ASD patients,[67] suggests that hyperactivation of the WNT signal leads to ASD pathogenesis.[68,69] WNT2 has been shown to be essential for cortical dendrite growth and dendritic arborization. Its expression is regulated by brain-derived neurotrophic factor (BDNF), while its overexpression leads to dendritic spines resulting in neurodevelopmental and neurodegenerative disorders.[70] WNT2 has been associated with susceptibility to autism, WNT2 gene polymorphisms have been reported to cause speech delay inherent to autism.[71,72]

Human APC inactivating gene mutations have been associated with ASD.[73] Compared to wild-type offspring, conditional knockout (cKO) APC mice exhibit learning and memory impairments, and autistic-like behaviors. β-catenin and canonical WNT target gene expressions (Dkk1, Sp5, Neurog1, Syn2) are increased in APC-cKO forebrain neurons.[1] Furthermore, the lysates from the hippocampal, cortical, and striatal regions of Apc-cKO mice showed higher β-catenin levels compared to those of control mice.[74] These results also indicate that hyperactivation of WNT/β-catenin signaling may be a cause of ASD.

Loss of function mutations in *de novo* β-catenin (CTNNB1) have been reported in people with ASD mental disability, microcephaly, motor delay, and speech impairment. Conditional ablation of β-catenin in parvalbumin interneurons in mice leads to impaired object recognition and social interactions, as well as elevated repetitive behaviors, which are core symptoms of ASD patients, and surprisingly, they showed enhanced spatial memory.[66] To determine the effect of CTNNB1 conditional knockout in overall neuronal activity, it was determined that c-Fos was significantly reduced in the cortex, but not in the dentate gyrus and the amygdala. The findings revealed a cell type-specific role of CTNNB1 gene in regulation of cognitive and autistic-like behaviors.[75]

TCF7L2 is one of the TCF/LEF1 transcription factors in the canonical WNT/β-catenin signaling pathway and is associated with type II diabetes in humans.[76] *De novo* loss of function mutations of TCF7L2 have been found in ASD patients.[77,78]

Whole-genome and whole-exome sequencing studies have identified mutations of the ANK3 gene in ASD patients.[79] The ankyrin G protein encoded by ANK3 functions as a scaffold protein.[80] Ankyrin-G facilitates cell-cell contact by binding E-cadherin in a protected region different from β-catenin and localizes β-catenin to the cell adhesion site in early embryos and cultured epithelial cells. Ankyrin-G facilitates cell-cell contact by binding E-cadherin in a conserved site, distinct from β-catenin, and localizes β-catenin to the cell adhesion site in early embryos and epithelial cell cultures.[81] Ankyrin-G is enriched at the ventricular zone of the embryonic brain, where it regulates the proliferation of neural progenitor cells. Ankyrin-G loss-of-function increases the proliferation of neural progenitor cells and nuclear β-catenin, probably by disruption of the β-catenin/cadherin interaction.[82]

Classical cadherins form a complex with β-catenin and play a role in cell-cell adhesion.[82,83] Loss of function mutations in classical cadherins leads to decreased cell adhesion, increased cell motility, and an increase in β-catenin release and level of canonical WNT signaling.[84] One study demonstrated a rare microdeletion of classical cadherin CDH8 in a group of individuals suffering from autism and learning disabilities.[85] Classical cadherin CDH9, CDH10, CDH13, and CDH15 mutations have also been encountered in some autism patients. The predicted functional consequence of haploinsufficiencies of these cadherins would be enhanced β-catenin release and activation of the WNT pathway.[86,87]

Dysfunction of the UBE3A gene has been linked to autism, Angelman syndrome, and cancer. UBE3AT485A, a *de novo* autism-dependent UBE3A mutant that disrupts phosphorylated control of UBE3A activity, converts multiple proteasome subunits to ubiquitin, reduces proteasome subunit abundance and activity, stabilizes nuclear β-catenin, and stimulates canonical WNT type UBE3A signal more effectively than wild type UBE3A.[88]

Rare missense variants have been identified in ASD patients. These variants inhibit DIXDC1 isoform 1 phosphorylation, causing impairment to dendrite and spine growth. These data reveal that DIXDC1 is a regulator of cortical dendrite and synaptic development and provide mechanistic insight into morphological defects associated with neurodevelopmental disorders. DIXDC1 is a positive modulator for WNT signaling and regulates excitatory neuron dendrite development and synapse function in the mouse cortex. MARK1, which is also linked to ASD, phosphorylates DIXDC1 to regulate dendrite and spine development through modulation of the cytoskeletal network in an isoform-specific manner. Mice deficient in DIXDC1 exhibit behavioral disturbances including reduced social interaction that can be mitigated by pharmacological inhibition of GSK3 to up-regulate WNT/β-catenin signaling.[89]

Prostaglandin E2 (PGE2), an endogenous lipid molecule, is linked to ASD. The association between prostaglandin and autism derives from the Möbius sequence with autism and history of misoprostol use during pregnancy.[90] The prostaglandin analogue misoprostol is used for termination of pregnancy as well as for the prevention of stomach ulcers. Four out of seven children (57.1%) with ASD were exposed to misoprostol prenatally.[90,91] Studies have shown that PGE2 interacts with canonical WNT signaling in neuroectodermal (NE-4C) stem cells and that it increased the average speed of migration in WNT-activated neuroectodermal stem NE-4C cells. PGE2 alters distinct cellular phenotypes that are characteristic of WNT-induced NE-4C cells, corresponding to the modified splitting behavior of the cells. Furthermore, expression levels of WNT-target genes (CTNNB1, PTGS2, CCND1, MMP9) were found to increase significantly in response to PGE2 treatment.[92]

Mutations in the neuroligins NLGN3 and NLGN4 have been reported in patients with autism.[93] Such type I transmembrane proteins are neural cell adhesion molecules and are required for synapse formation and development.[94] Studies have demonstrated that WNT/β-catenin signaling directly regulates NLGN3 expression.[95]

SHANK3 is a synaptic scaffold protein enriched in the postsynaptic region of excitatory synapses and plays important roles in the formation, maturation and maintenance of synapses.[96] Findings from genetic studies in patients with ASD suggest a strong relationship between SHANK3 and ASD.[97] Various mutations in the SHANK3 gene have implications for dendritic branching morphology and synaptic transmission.[98]

Haploinsufficiency of the SHANK3 gene causes 22q13.3 deletion syndrome (Phelan-McDermid syndrome), a developmental disorder characterized by speech delay, hypotonia, developmental delay and autistic behavior.[99]

Studies have shown that Shank proteins, in addition to scaffold functions, also play a role in signaling pathways, therefore, thus, Shank3 has been proposed to regulate the WNT signaling pathway by sequestering β-catenin at post-synaptic sites. As a result, heterozygous loss of Shank3, as observed in autistic patients, allows for increased translocation of β-catenin to the nucleus where it may induce transcription of β-catenin-responsive genes.[100]

Taken in its entirety, the available genetic information indicates that not only canonical WNT pathway activation, but also inhibition seems to increase autism risk.

RETT SYNDROME AND WNT6

The MECP2 protein plays an important role in certain neurodevelopmental disorders, one of which is Rett syndrome (RTT).[101] Rett syndrome is a rare neurological and developmental disorder that shares some common features with ASD.[102] Patients with RTT generally exhibit normal development during infancy, followed by a period of regression. Abnormal behaviors often include motor function deficits, cognitive impairments, and other symptoms associated with mental retardation.[102,103]

Mutations in genes producing the MECP2 protein are observed in almost all cases of RTT.[104] MECP2 regulates the activity of other genes and plays a role in the growth and communication of nerve cells.[105]

One study showed that restoration of Wnt6, a signaling molecule involved in brain function, eased motor and social behavioral deficits in a mouse model of Rett syndrome. Mice carrying MEPC2 T158A mutation showed a nearly 12-fold reduction in the levels of Wnt6, which plays a critical role in development and adult brain functions. These findings suggested that deficient WNT6 signaling may play a role in RTT development, and restoring its activity may alleviate disease symptoms in the mouse model. To test this hypothesis, it was evaluated whether restoring the WNT6 signal could rescue behavioral difficulties in mice with the RTT-associated MECP2 mutation. To this end, an increase in WNT6 levels was achieved, particularly in the amygdala, a region of the brain involving emotions and behavior. The results indicated that the restoration of the WNT6 signal partially, but significantly, rescued not only social behavioral deficiencies, but also motor difficulties compared to untreated mice. The authors noted that while the amygdala itself is not mainly involved in motor control, it is connected with brain regions that play such a role, and as a result, Wnt6 signaling in the amygdala may indirectly influence motor function. Further analysis revealed that the benefits associated with WNT6 were linked to the restoration of MeCP2 SUMOylation (small ubiquitin-like modifier [SUMO]) and normalized or increased BDNF and IGF-1 protein levels in the amygdala. BDNF and IGF-1 play a role in brain development and nerve cell function. Previous studies have shown that BDNF is impaired in both mouse models and patients with Rett syndrome. BDNF and IGF-1 play a role in brain development and nerve cell function. Prior research showed that BDNF is impaired both in mouse models and people with Rett syndrome. Furthermore, a different study found that that IGF-1 treatment eased disease symptoms in a person with Rett syndrome. Taken in its entirety, these findings suggested that WNT6 may promote MeCP2 SUMOylation through an increase in BDNF and IGF-1 levels.[106]

THE ROLE OF WNT NEURAL PATHWAY IN ABNORMAL DENDRITIC BRANCHING IN ASD

Although increased spine densities are seen in autism spectrum disorder, spine pruning and maturation defects are seen as well.[107,108] Neural activity is a driving force in development, and sensory experience influences dendritic branching density and maturation of dentritic branching. Experience-dependent spine pruning and maturation has been observed in the mouse primary somatosensory cortex. Using live imaging, it was demonstrated that locally elevating neural activity or cadherin/catenin-dependent cell adhesion led to enlargement of the stimulated spine and concurrent pruning of

its neighbor, an effect dependent on inter-spine distance and N-cadherin motility. Furthermore, selective enrichment of β-catenin in a small proportion of spines *in vivo* through pre-synaptic manipulations promoted the survival and maturation of β-catenin-enriched spines, at the expense of neighboring spines with lower β-catenin levels. In addition, it was shown that acceleration of spine pruning induced by environmental enrichment was abolished in the absence of endogenous β-catenin. Together, these results demonstrate a critical role of the cadherin/catenin complex in mediating coordinated spine pruning and maturation during neural circuit refinement.[109]

Some of the aforementioned mutations that result in increased WNT signaling lead to increased nuclear translocation of β-catenin. In this case, β-catenin may be unable to adequately form a complex with N-cadherin.[110] Therefore, errors may occur during the critical role of this complex in mediating coordinated spine pruning and maturation.

Conclusion

Some of the mutations associated with WNT signaling and common risk genes demonstrated in different studies and mentioned throughout this study point to an increased risk of cancer in individuals with autism. However, there are studies that report low cancer rates in individuals with autism. As previously mentioned, abnormal activation of the canonical WNT signal plays a role in several forms of cancer. These inconsistent results regarding cancer rates can be attributed to the contribution of mutations that abnormally increase or decrease the WNT/β-catenin signaling in autism spectrum disorders. Further studies to be conducted in the future will shed light upon this subject.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

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