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Review Article

Role of Wnt Ligand Secretion Mediator Signaling in Cancer Development

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Abstract

Objective: The Wnt signaling pathway is among the crucial cascades that regulate development and homeostasis of tissue. **Data Sources:** Further, it is closely associated with different types of cancer, which includes glioma, breast, colorectal, lung, and prostate cancer, and hepatocellular carcinoma (HCC). The deviant activation or inhibition of Wnt signaling regulates cancer progression, thereby exerting oncogenic or tumorsuppressive effects that control the invasion, metastasis, and metabolism of cancer cell. **Study Selection:** In the Wnt secretory pathway, lipidmodified Wnt molecules interact with Wnt ligand secretion mediator (WLS), a Wnt cargo receptor. Moreover, they are directed to the plasma membrane and then secreted. **Results:** Loss of WLS function leads to the accumulation of Wnt in the endoplasmic reticulum (ER), leading to retrograde Golgi–ER transport and ER stress associated with the pathogenesis of several conditions, including early embryonic death, and developmental defects related to lymphopoiesis, neurogenesis, and osteogenesis in adults. Although there is substantial evidence, the regulatory mechanisms through which WLS controls cellular functions are not fully elucidated. **Conclusion:** Therefore, the current study aimed to identify the underlying mechanism of the effects of WLS on the development of human diseases.

Keywords: Cancer, Wnt, Wnt ligand secretion

Introduction

Cancer is the leading cause of mortality within worldwide. In accordance with the World Health Organization, cancer occupies nearly 10 million deaths in 2020. Cancer develops via a multistep process that is strongly associated with the progressive accumulation of genetic alterations. Further, alterations in cancer-relevant genes, such as those involving the Wnt signaling pathway, lead to uncontrolled cell proliferation and resistance to cell apoptosis, and they are considered the

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most common driver of cancer. Nevertheless, the molecular mechanism underlying the development, progression, and metastasis of cancer remains unclear. Hence, several studies regarding this topic have been conducted in recent decades.

Role of Wnt Signaling in Cancer Development

From the 1980s to 1990s, the sequence of *Wingless* in *Drosophila* was found to be identical to that of murine proto-oncogene *Int1* (int = integration), and it has important regulatory effects on tumorigenesis.[1,2] The term Wnt stands for Wingless-related integration site, which includes *Int* and *Wingless* to denote where genes belong.[3] Wnt signaling is among the essential cascades regulating tissue development and homeostasis. Moreover, it is closely associated with different types of cancer, which includes glioma, breast, colorectal, lung, as well as prostate cancer, and HCC. The abnormal activation or inhibition of Wnt signaling regulates cancer progression, thereby exerting both oncogenic or tumor-suppressive effects that control cancer cell invasion, metastasis, metabolism, as well as inflammation.[4] However, the thorough mechanism underlying the complexity of the Wnt networks remains unclear.

Wnt proteins comprise a family of secreted, hydrophobic, and Cys-rich glycolipoproteins,[5,6] which bind to highly conserved cell surface receptors and provoke cell-to-cell communications in either a paracrine or autocrine manner.[7] In total, 19 different Wnts are classified as canonical (Wnt1, Wnt2, Wnt3, Wnt3a, Wnt8a, Wnt8b, Wnt10a, and Wnt10b) and noncanonical (Wnt4, Wnt5a, Wnt5b, Wnt7a, Wnt7b, and Wnt11) proteins, initiating the canonical and/or noncanonical pathways by activating Frizzled receptors, in rodents and humans.[8,9] If the canonical pathway (Wnt/β-catenin) is activated, the destruction complex, which comprises axin, adenomatosis polyposis coli, protein phosphatase 2A, glycogen synthase kinase 3, and casein kinase 1 alpha, was disrupted, thereby leading to the phosphorylation of cytoplasm β-catenin translocating into the nucleus that acts as a transcriptional coactivator of T-cell/lymphoid enhancer transcription factors.[9] Noncanonical Wnt signaling pathways mostly made up of Wnt/planar cell polarity signaling and Wnt-cyclic guanosine monophosphate/Ca^{2+} signaling.[10]

Secretion of Wnts

In a study conducted in 2006, vesicle-membrane retromer protein-Wnt ligand secretion mediator (WLS, GPR177) was found to be responsive to the secretion of Wnt proteins.[11,12] In general, the secretion of Wnt is dependent on the retromer complex of WLS in the cellular transport vesicle, and Wnt accumulates in the cytoplasm if the expression of WLS is inhibited. In *Drosophila*, the sequence of WLS is similar to that of Wntless, a novel segment-polarity gene.[12] Further, it is referred to as evenness interrupted[11] or Sprinter.[13] The conserved requirement for the Wntless protein in the secretion of Wnt was first reported by multiple groups working with *Caenorhabditis elegans*, *Drosophila melanogaster*, and human tissue culture cells in 2006.[14] Among the other components of the Wnt signaling pathway, only the WLS gene was found to exist in vertebrate genomes.[15] Moreover, it is required for the secretion of all Wnts in both the canonical and noncanonical Wnt pathways. In addition, WLS (GPR177) is a putative orphan G protein-coupled receptor that plays a key role in embryogenesis in mice.[16]

Characteristics of the Wnt Ligand Secretion Protein

The WLS (Wls, Wntless) is a conserved multipass (seven or eight) transmembrane (TM) protein that recycles between the endoplasmic reticulum (ER) trans-Golgi network and the cell surface [Figure 1].[12,17] Although previous studies have been investigating the rough contour of WLS since 2006, its exact structure has not been identified until recent years. Based on an amino acid sequence analysis, WLS is considered an integral membrane protein with at least four major domains (signal peptide [SP], a.a. 13–35; predicted lipocalin fold [PLF], a.a. 46–213; TM, 233–493; and C-terminal [CT], a.a. 494–541) defined according to functional binding characteristics.[11] Different interaction signals have various functions. Recently, Nygaard *et al*. and Zhong *et al*. showed the potential interaction between Wnt and WLS protein.[18] The PLF domain had a hydrophobic cavity. A highly charged luminal domain provides multiple binding sites for Wnts.[18,19] Further, the lipid bilayer of the G protein coupled receptor-like TM domain binds to the O-linked palmitoleate of Wnt hairpin 2, which contributed to the tight binding contacts between Wnt and WLS.[18,20] The luminal domain is arranged into a β-sandwich fold together with two disulfide bonds to stabilize the structure. It is similar to the structure of the monomeric form of the lipid-binding protein Seipin, which is involved in lipid droplet biogenesis.[19,21] Aside from the PLF and TM domains, Wang *et al*. showed that IRE1alpha interacted with the SP domain to regulate relevant ER signals. Meanwhile, the CT domain interacted with the E2lf, ATF6, and CANX/CALR complex regulating glycosylation activity [Figure 1].

Role of Wnt Ligand Secretion in Wnt Signaling

WLS plays an essential role in Wnt signaling. An understanding of the localization of WLS can identify its function in the pathway. WLS was mostly detected in the Golgi, vesicular membrane, and ER.[11,20,22] Belenkaya *et al*. have revealed that WLS is trafficked from the trans-Golgi network into the cell membrane and is then endocytosed from the cell surface.[22] Then, WLS is recycled in the Wnt-producing cell through the retromer complex, which includes five proteins (specifically, SNX1/2, SNX5/6, Vps26, Vps29, and Vps35) in mammalian cells, that redirects WLS away from the lysosomal degradative pathway and back to the ER.[23-25]

By SPs, Wnts are targeted to the lumen of the ER, and the membrane bound O-acyl transferase PORCN (porcupine) add a single monounsaturated palmitoleate to Wnts. The palmitoylation of Wnts allow them to bind to WLS in the ER.[26]

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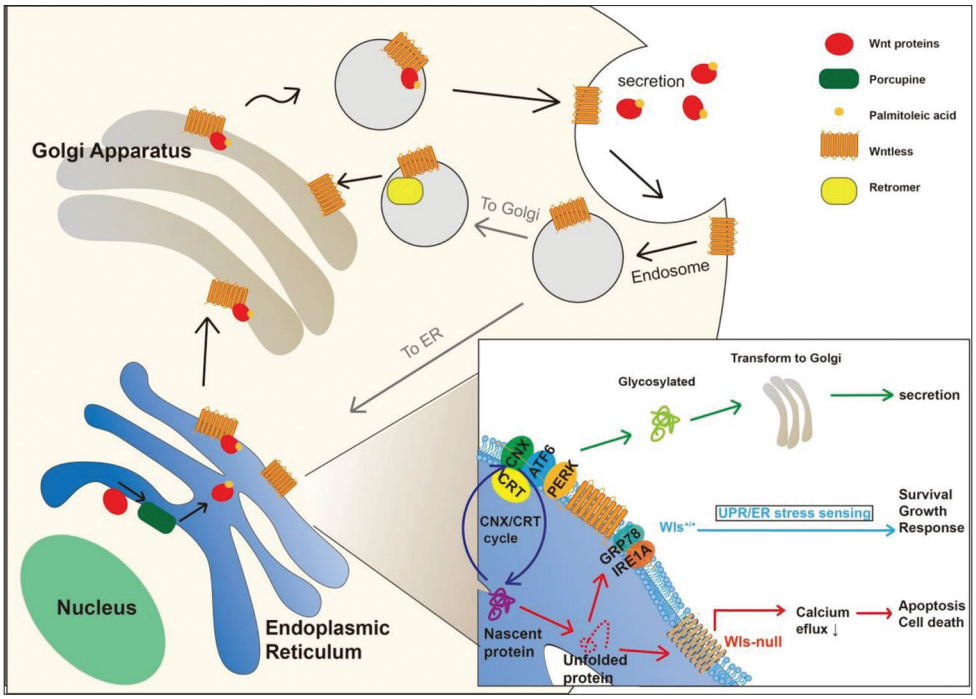

Figure 1: A schematic diagram illustrates the role of WLS in vesicle transport, ER signaling and UPR stress. In ER, porcupine adds a single mono-unsaturated palmitoleate to Wnts, allowing it to bind to WLS, which is an essential cargo for Wnts to commute to Golgi and complete the additional posttranslational modifications. After Wnts is secreted, WLS is re-endocytosed and transformed back to the ER or Golgi through the retromer complex. Aside from its role in Wnts transport, WLS is also a critical chaperone docking ER stress sensors and CANX-CALR complex and controlling ER homeostasis as well as UPR stress (enlarged picture). WLS integrates the CANX-CALR cycle, ER stress regulators (IRE1A, PERK, ATF6, and GRP78) and calcium regulators (not depicted in the scheme). WLS deficiency leads to UPR dysregulation and decreases ER calcium efflux in bone marrow-derived dendritic cells, resulting in abnormal protein glycosylation and UPR signaling activation, leading to apoptosis and cell death. UPR: Unfolded protein response, WLS: Wnt ligand secretion, ER: Endoplasmic reticulum

WLS is an essential cargo when Wnts leave the ER. The Wnts–WLS complex afterwards commutes to the Golgi from the ER to complete the additional posttranslational modifications of Wnts. Next, it shuttles to the plasma membrane and then moves to a Wnt receiving cell through interaction with glypicans and/or lipid-binding proteins where the Wnt–WLS complex dissociates and Wnts are secreted. Then, WLS is re-endocytosed and transformed back to the ER or Golgi.[23,27]

Role of Wnt Ligand Secretion in Endoplasmic Reticulum Signaling and Unfolded Protein Response Stress

In addition to its critical role in the Wnt signaling pathway, WLS is commonly detected in the ER to modulate ER homeostasis and protein glycosylation without dependence on Wnt signaling [Figure 2a and b]. ER is a critical organelle involved in the synthesis, folding, and modification of proteins. Damaged protein glycosylation can result in ER stress and, ultimately, a series of responses including the UPR and cell autophagy or apoptosis.[28] Wang *et al*. showed that WLS is a major molecular chaperone to organize the UPRosome consistent with multiple ER stress regulators, including IRE1alpha, PERK, ATF6, and GRP78 (BiP), and calcium influx regulators InsP3R1 and RYR1/RyanR1. WLS deficiency leads to UPR dysregulation, decreased ER calcium efflux, and protein hypoglycosylation in bone marrow-derived dendritic cells [Figure 1, enlarged]. The loss of WLS in DCs leads to abnormal protein glycosylation, which then removes cellular signaling molecules, including Toll-like receptors, chemokines, and cytokines. Further, it eventually results in the inability to develop Th1-, Th2-, and Th17-associated immune responses.[28]

WLS assists glycosylation modification that facilitates Wnts transportation from the ER via the Golgi to the plasma membrane, and it is re-endocytosed and recycled to continue the pathway. Wnts are shown to be hypo-glycosylated in WLS-deficient cells [Figure 2c and d]. Hence, it is an important factor in the Wnt signaling pathway. Moreover, independent of the Wnt pathway, WLS is important in modulating ER homeostasis, and the loss of WLS causes protein glycosylation and immune defect in DCs.

Role of Wnt Ligand Secretion in Embryonic Development

Wnt signaling is an essential pathway that modulates embryogenesis, stem cell maintenance, and also the neural connection in several aspects.[5] The regulation of WLS to the Wnt signaling pathway is required to establish the body axis during early embryogenesis.[16] Homozygous mutations in *WLS* have been shown to affect Wnt stability and signaling,

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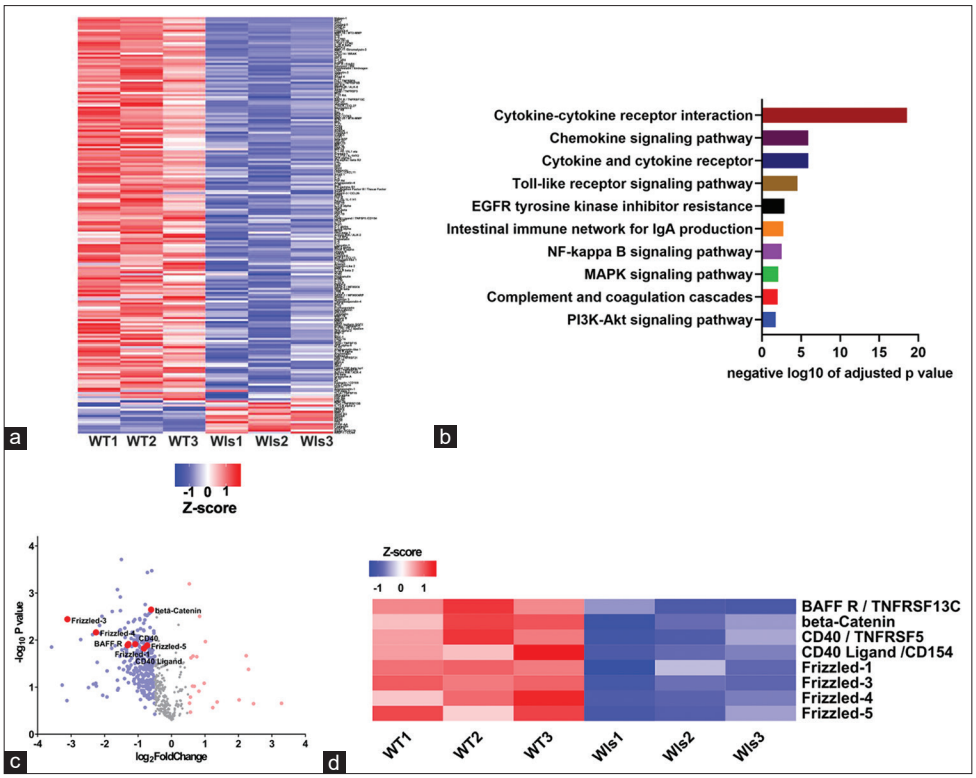

Figure 2: WLS-mediated molecular chaperone super-complex modulates protein glycosylation, cytokine secretion, and cell fate in bone marrow-derived dendritic cells (BMDCs). (a) Heatmap presentation of the major differentially glycosylated membrane and secretory protein clusters in control and *Wls* Knockout mice, (b) Major functional pathways of differentially glycosylated proteins in *Wls* Knockout mice as analyzed by KEGG analysis, (c) Volcano plot showing in red DEGs significantly hypo-glycosylated Wnt signals and in blue DEGs significantly hypo-glycosylated proteins. Proteins of interest are labeled, (d) Heatmap presentation of the glycosylation level of Wnt-relavant signaling proteins in control and *Wls* Knockout mice. WLS: Wnt ligand secretion

causing syndromic structural congenital abnormality, including microcephaly and facial dysmorphism and foot syndactyly, renal agenesis, alopecia, iris coloboma, and heart defects.[29] The role of WLS in tissue development is important, and this has been discussed in several studies.[30,31]

Neuron

WLS is involved in the development of the nervous system, and the mutation or deletion of WLS causes cerebellar, spinal cord, and neuromuscular junction (NMJ) defects.[16] The Wnt proteins, such as Wnt1 and Wnt3a, affect the establishment of the body axis. The deletion of WLS induces brain and craniofacial abnormalities. Fu *et al*. showed that WLS is important for inducing isthmic organizer activity mediated by the Wnt signaling pathway in the midbrain or cerebellar development.[32] Yeung *et al*. investigated the WLS function in the rhombic lip, which plays an essential role in neural system development, by which granular cell and mossy and climbing fiber neurons are derived from.[33,34] Yeung *et al*. found that WLS regulates canonical and noncanonical Wnt signaling in the rhombic lip- and ventricular zone lineages, thereby organizing cells at the region of anchoring centers and promoting cell differentiation during cerebellar development.[35,36] Kuan *et al*. revealed that the characterization of the *WLS* mutant phenotype supports unique requirements for Wnt signaling in habenular development.[37] Ependymal cell is a type of glia cell that plays a crucial role in the production and regulation of cerebrospinal fluid. Wnt/β-catenin signaling contributes to the formation and regulation of ependymal cells.[38] In addition, WLS is involved in the development of roof plate, which is the most dorsal region of the spinal cord and is an organizing center during the early development of the spinal cord.[39] Shinozuka *et al*. revealed that the stretched roof-plate cells secrete Wnt1 and Wnt3a. The conditional knockout of WLS resulted in the downregulation of Wnt proteins, stretched roof-plate cells during development, and induced spinal cord injury in adult mice.[40] The NMJ is a synapse between motoneurons and skeletal muscles that controls motor behavior. The lack of WLS caused significant enhancement of the axon outgrowth defects, indicating that the Wnts function in motor neuron axon outgrowth.[41] Wnt7A and Wnt7B were major Wnts for nerve terminal differentiation. Motoneuron-specific WLS mutant mice formed NMJs but presented with axonal deficits in adulthood, thereby indicating the age-dependent requirement of WLS in NMJ maintenance.[42]

Bone

Wnt signaling promotes bone formation through different aspects, which include stimulating preosteoblast proliferation, promoting osteoblast differentiation, and preventing apoptosis

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of osteoblast and osteocyte.[43] Although several papers have reported the roles of the Wnt signaling pathway in bone embryogenesis, the function of WLS in bone tissue development has not been a significant cause of concern until the last 10 years. WLS is required during intramembranous and endochondral ossification and tooth development. *WLS* deletion or mutation in different sites, such as the mesenchyme, ectoderm, endoderm, osteoblast progenitors, immature/mature osteoblast, and chondrocyte, causes different skeletal or dental defects.[44] WLS is expressed in the mesenchyme of the ventral body wall and is essential for mesenchymal cells' migration to the midline by modulating Wnt/β-catenin signaling activity, which is required for the fusion of the sternum.[45] In addition, the deletion of WLS impaired the maintenance of bone marrow mesenchymal stem cells in postnatal bone remodeling in mice.[43] The knockout of *WLS* in mesenchymal cells resulted in shortened anteroposterior axis, hypoplastic skeletons with truncated auto, and severe abnormalities in the craniofacial skeleton, including clefts involving the secondary palate.[44,46,47] The deletion of WLS in the ectoderm leads to distal limb agenesis, upper facial structure defect, abnormal digital bone regression, impaired intramembranous ossification, and skull suture fusion.[48,49] In addition, WLS deletion in the endoderm caused abnormal tracheal formation with impaired dorsal–ventral modeling of the tracheal mesenchyme, cartilage, and smooth muscle.[50] In chondrogenesis, WLS is required for Wnts secretion, regulating the proliferation of chondrogenic cells.[51] WLS ablation causes defects in endochondral ossification and delayed suture fusion in the frontal bones in mice.[46,52]

Lung and Ovary

Epithelial–mesenchymal interactions play an essential role in the induction and coordination of vascular development in the lung. Signals from developing pulmonary mesenchyme are important in determining the stereotypical branching pattern of the mammalian lung while epithelium cells secrete growth factors that promote the differentiation of pulmonary mesenchymal cells.[53,54] Epithelial Wnts modulate normal lung development and maintain pulmonary vasculature via Klf2, KDR, Vegfa, TEK, and Ang-1 through the Wnt/β-catenin signaling pathway.[53,54] The loss of WLS from the embryonic pulmonary epithelium was proven to inhibit peripheral lung growing and differentiating, and disrupt branching morphogenesis as well as the formation of the pulmonary vasculature.[53,55] Further, Wnts signaling pathway is essential for alveoli formation in the neonatal stage. The ablation of WLS results in the mesenchymal cells' abnormal differentiation, leading to the persevering presence of myofibroblasts and ectopic expression of α-SMA$^+$ in the endothelial cells lining the enlarged air-sac.[54]

The Wnts signaling pathway is significantly affecting ovarian cell function.[56,57] WLS is greatly expressed in follicles, together with oocytes, granulosa cells (GCs), and primordial cells.[58] The deletion of WLS in GC resulted in the smaller corpus luteum in mice ovaries with a notable reduction in luteal cell marker gene expression and a remarkable increase in apoptotic gene expression, causing to female infertility.[58,59] This finding might be attributed *WLS* to multiple gene changes involved in follicular processes, including steroidogenesis, tissue remodeling, and GC proliferation and differentiation.[60]

Hair Follicle

The growth of hair follicle (HF) occurs through cycles of three phases (growth [anagen], regression [catagen], and rest [telogen]), and the Wnt/β-catenin pathway is indispensable for HF induction by directing anagen onset of hair follicle stem cells.[61] The deletion of WLS in the follicular epithelium resulted in hair cycle arrest in the telogen or early anagen phase, causing premature regression of hair follicles during postnatal HF morphogenesis.[62-64] The dermal papilla (DP) comprises mesenchymal cells in the HF. CD133 + DP cells are a family in the DP that has trichogenic ability and can regulate DP size. The loss of WLS not only impact on the proliferation and differentiation of matrix keratinocytes but also modulates the biological properties of DP cells, causing delayed hair growth and premature HF regression.[65] TGF-β/JNK pathway, an abnormal catagen-inducing signal causing premature HF regression, has been reported to be activated in the *WLS*-deficient hair follicles.[66] In addition, *WLS* is essential for promoting regeneration of adult wound-induced *de novo* hair follicle, in which hair follicles regenerate *de novo* within the center of re-epithelialized wounds.[63] In conclusion, WLS is required in hair follicle induction and hair cycling by regulating the Wnt signaling pathway, which provides insights regarding strategies for treating hair disorders such as alopecia or hirsutism in the future.

Wnt Ligand Secretion across Different Types of Cancer

Wnt signaling is correlated to cancer carcinogenesis and the overexpression of WLS is detected in different types of cancer. The overexpression of WLS can result in aberrant Wnt signaling, which regulates oncogenesis, and can be associated with poor outcome or chemoresistance. In this section, several cancers related to WLS are evaluated [Table 1].

Breast cancer

The oncogenic potential of Wnt proteins and WLS in the mammary gland has been observed in humans and mice with breast cancer (BC).[60,61] The WLS levels are negatively correlated with estrogen and progesterone receptors in patients with breast cancer as well as in BC cell lines.[62] The tumor epithelial–mesenchymal transition (EMT) signals were upregulated in the *WLS* high-expression group and correlated with BC progression.[84] WLS regulates cell proliferation by adjusting Wnt signaling and is highly detected in clinical breast tumors, but not in noncancerous tissues.[84] The loss of WLS can significantly suppress the proliferation of BC cells.[64] Further, the overexpression of human epidermal growth factor receptor 2 (HER2) have been recognized and

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Table 1: Wnt ligand secretion in cancer development

Cancer type	Wntless's function	Reference
Breast cancer	Proliferation - WLS is essential in WNT4 signaling, which is required for survival and proliferation of invasive lobular carcinoma cells, a subtype of breast cancer. Knockdown of WLS suppressed the proliferation of breast cancer cells	[67,68]
	Cell growth - Downregulation of WLS inhibits the secretion of Wnt and its downstream signaling and hence reduced colony formation and tumor cell growth	[67]
	EMT/tumor metastasis - Gene Set Enrichment Analyze showed that EMT pathways were upregulated in the WLS high-expression group	[69]
Gastric cancer	Proliferation - Anti-WLS antibody is observed to inhibit the proliferation of gastric cancer cell, indicated that WLS is crucial for cell proliferation in gastric cancer	[70]
	Cell growth - Anti-WLS antibodies greatly reduced tumor growth in gastric cancer. Progression-free survival was significantly shorter in intestinal gastric tumor with positive WLS expression	[70,71]
	EMT/tumor metastasis - The expression level of WLS is greatly expressed in metastatic cancer cell line and is related to advanced TNM stage and lymph node metastasis	[72]
Ovarian cancer	Proliferation	
	Cell growth - High-grade serous carcinomas expressed significantly higher levels of WLS expression than lower grade. WLS forms a complex with GOLPH 3 and increases tumor cell viability	[71,73]
	EMT/tumor metastasis - Metastatic cases showed significantly higher expression of WLS than borderline or low-grade carcinoma	[71]
Liver cancer	Proliferation - Blocking the expression of WLS reduces the proliferation of HCC tumor cells and the percentage of M2-like TAMs, and TAMs are major elements of tumor microenvironment and are essential in the progression of HCC	[74]
	Cell growth - HCC cell is found to depend on Wnt/β-catenin signaling for maintaining optimal growth and WLS is a crucial factor of maintaining the pathway	[75]
	EMT/tumor metastasis - WLS expression was positively associated with tumor size and TNM stage and was correlated with poor differentiation of HCC	[76]
Prostate cancer	Proliferation - Ablating WLS in prostate stromal cells bring on decreasing epithelial proliferation during early prostate development in mouse model. The inhibition of WLS resulted in the decrease of the tumor proliferation	[77]
	Cell growth - WLS is required for NEPC cell growth. The expression of WLS is associated with high Gleason score prostate cancer	[78,79]
	EMT/tumor metastasis	
Glioma	Proliferation - WLS is overexpressed in astrocytic gliomas and the overexpression of WLS promotes downstream Wnt signaling pathway which plays an essential role in glioma tumorigenesis	[80]
	Cell growth - By silencing the expression of WLS, it significantly decreased number of cells in S-phase, and also decreased the viability of glioblastoma cells	[80]
	EMT/tumor metastasis - GOLPH 3 is involved in glioma cell migration and invasion by regulating AKT-mTOR-YB1 pathway, and the expression of GOLPH 3 in tumor cell is positively correlated with WLS and Vps35. Additionally, though the mechanism is unclear, GOLPH 3 is proved to form a complex with WLS	[81]
Bone tumor	Proliferation - The inactivation of WLS is observed to reduce the Loxl2 expression and inhibit the OS proliferation *in vitro*	[82,83]
	Cell growth - Loxl2 modulates collagen packing and influences osteoblast differentiation as well as tumor pathology	[83]
	EMT/tumor metastasis	

EMT: Epithelial-mesenchymal transition, WLS: Wnt ligand secretion, HCC: Hepatocellular carcinoma, NEPC: Neuroendocrine prostate cancer, TNM: Tumor-node-metastasis, TAMs: Tumor-associated macrophages, GOLPH 3: Golgi phosphoprotein 3, OS: Osteosarcoma

related to poor prognosis in BC.[65] The HER2-mediated nuclear factor-κB (NFκB) signaling plays a key role on tumor resistance to radiotherapy and contributes to BC progression.[66] WLS was found to activate HER2-mediated NFκB signaling in several cancer cells although the detailed mechanism underlying the activation between WLS and the HER-nuclear factor-κB signaling pathway has not been validated yet.[84] Overall, the overexpression of WLS was positively associated with tumor cell proliferation, upregulated EMT pathway,[84] and poor overall survival and disease-free survival in patients with BC.[63] WLS might not only act as a predictive biomarker of therapeutic response but also serves as a possible strategy for addressing tumor resistance in BC.

Gastric cancer

The role of the WLS–Wnts signaling pathway in gastric cancer is well documented in the carcinogenesis of different types of gastric cancer. Evidence showed that high WLS levels are more common in intestinal gastric tumors than in diffused and mixed gastric tumors and positively associated with a significantly short progression-free survival.[71] In addition, WLS levels in intestinal gastric tumors are positively correlated with the expression of HER2 involved in the HER-nuclear factor-κB signaling pathway and promote tumor progression.[71] From a pathological aspect, WLS is significantly expressed in the metastatic cancer cell line but not in the poorly differentiated cell line from primary gastric cancer tissue, correlating with advanced tumor-node-metastasis (TNM) stage and lymph node metastasis.[85] The inhibition of WLS resulted in the inhibition of the Wnt/β-catenin signaling pathway and led to the suppression of gastric tumorigenesis *in vitro* and *in vivo*.[71] The antibody-targeting WLS exhibits anticancer properties not only in mice but also in patient-derived xenograft models,

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providing a promising approach of targeting WLS to prevent gastric cancer progression.[86] From a molecular aspect, high levels of WLS are correlated with ER stress insensitivity and decreased ER stress-induced apoptosis of tumor cells, promoting cancer proliferation in gastric cancer.[87] The role of WLS provides potential mechanisms for gastric cancer development independent of the Wnts signaling pathway by which decreased ER stress-induced apoptosis or interaction with the molecules in the HER2 pathway, thereby resulting in advanced-stage cancer and poor prognosis.

Ovarian cancer

In developed countries, ovarian cancer ranked the second most common gynecologic malignancy, and the condition is diagnosed in latent stages in most cases.[88] The role of the WLS and Wnt signaling pathway in ovarian folliculogenesis and female fertility is essential, and the overexpression of *WLS* may result in tumor proliferation and chemoresistance in ovarian cancer.[58] In addition, *WLS* levels are significantly correlated with HER2-positive serous ovarian carcinomas as well as gastric and BC.[71] Mechanistically, WLS directly binds to Golgi phosphoprotein 3 (GOLPH 3) to upregulate miR-509-3p, a microRNA upregulated in cisplatin-resistant ovarian cancer, decreasing tumor cell response to chemotherapy in poor-prognosis ovarian cancer.[73,79] This result indicated that targeting miR-509-3p might be a potential therapy for cisplatin-resistant ovarian cancer.[73] Further, the oncogenic role of the Wnt/β-catenin signaling pathway regulated by WLS has been observed in ovarian cancer. Therefore, therapeutic strategy targeting WLS can address chemoresistance and metastasis in ovarian cancer.

Liver cancer

HCC is the third-ranked cause of universal cancer-related mortality annually.[89] HCC cells are dependent on the activation of canonical Wnt/β-catenin signaling pathway for maintaining optimal tumor growth. Wnts secreted by HCC tumor cells leads to their polarization to M2-like tumor-associated macrophages (TAMs), which are major elements of the tumor microenvironment and are essential in HCC progression.[90] Inhibiting the expression of WLS reduces the proliferation of tumor cells and the percentage of TAMs.[91] WLS level was positively associated with tumor size, TNM stage, and poor differentiation in HCC.[92] Therefore, the expression of WLS might promote tumor progression, which is correlated with poor outcome in patients with HCC.[92] DEN/CCl_4 can lead to HCC after chronic injury and fibrosis, and *WLS*-knockout hepatocyte abolished the overall pretumor environment and comparable tumor burden histologically in response to DEN/CCl_4.[93]

WLS is also a potential biomarker in diagnosing other types of liver cancer such as intrahepatic cholangiocarcinoma (ICC), which is the second most common primary liver cancer and is a threatening malignancy of the bile duct. WLS is upregulated in ICC tissues, and its expression is positively associated with ICC tumor stage, TNM stage, and lymphatic invasion. Hence, WLS can be considered to be a molecular marker for predicting advanced-stage tumor and metastasis in ICC.[94] WLS is correlated with poor prognosis in liver cancers such as HCC and ICC. Thus, WLS can be a predictive biomarker of outcome. Nevertheless, the effect of hepatocyte-specific Wnts on the tumor microenvironment is controversial, and further studies must be conducted to validate this effect.

Prostate cancer

In a pathological context, prostate stromal cells are a major source of several Wnts. Altered Wnt signaling may be responsible for the progression of antiandrogen resistance in prostate cancer.[95] Ablating WLS in prostate stromal cells can decrease epithelial proliferation during early prostate development in a mouse model.[77] Further, WLS was highly expressed in castration-resistant prostate cancer and neuroendocrine prostate cancer (NEPC) tumors *in vitro* and *in vivo*. Studies showed that *WLS* induces NEPC via the receptor tyrosine kinase-like orphan receptor (ROR) 2-dependent PKCδ/ERK pathway.[78] Notably, WLS is overexpressed in enzalutamide-resistant prostate cancer cells and associated with a high Gleason score in prostate cancer. (?) The inhibition of WLS decreased tumor proliferation and increased the sensitivity of enzalutamide treatment to resistant cells. Therefore, WLS plays a vital role in progression and antiandrogen therapy resistance of prostate cancer.[79]

Glioblastoma

Gliomas are prevailing primary brain tumors classified histologically into grades I–IV. Grade IV gliomas referred to as glioblastomas, are malignant and aggressive with extremely proliferative and invasive characteristics.[96] WLS is highly detected in astrocytic gliomas, and promotes glioma tumorigenesis by upregulating Wnt signaling pathway.[80] By silencing the expression of *WLS*, it significantly decreased the viability and number of glioblastoma cells in the S phase.[80] Further, studies have manifested that the inhibition of retromer function by depleting Vps35 disrupts the recycle of WLS after internalization, which resulted in the inhibition of Wnt secretion.[97] GOLPH 3, a component of WLS-Vps35 complex, is involved in cell proliferation, cell migration and invasion by regulating the consequent AKT-mTOR-YB1 pathway in glioma cells, leading to poor prognosis in glioma patient.[96] Thus, the GOLPH 3-WLS-Wnt axis can be a therapeutic target in glioblastoma.[96]

Osteosarcoma

The mutation or deletion of WLS is correlated with severe skeletal diseases in humans, such as osteosarcoma (OS), which is the most common primary malignant bone tumor.[44] Loxl2, a modulator of collagen packing, influences osteoblast differentiation and tumor pathology regulated by Wnt signaling, promotes the development of aggressive OS. In addition, proto-oncogene-c-Fos regulates the expression of the Wnt proteins-Wnt7b and Wnt9a in OS cells, upregulating Loxl2 expression and consequent tumorigenesis in OS cells. *WLS* is referred as a driver gene in OS. The overexpression

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of *WLS* results in upregulating Wnt signaling, and the inactivation of WLS reduces the expression of Loxl2 and inhibits the proliferation of OS *in vitro*.[82,83] In addition, an immunohistochemical marker called YJ5, a monoclonal antibody-targeting WLS that is able to highlight the overexpression of WLS, can be applied as a useful marker for difficultly diagnose bone tumor.[98] In OS cells, the Wnt signaling pathway participates in cell proliferation, induction of an EMT-like process, and tumor metastasis.[99] Although the role of WLS in the Wnt signaling pathway has been identified, more clinical and pathological studies on WLS targeting must be perform to validate its oncogenic role in OS.

Others

WLS has other interesting functions beyond regulating embryogenesis and cancer development through the Wnt signaling pathway. For example, there is an interplay between WLS and the mu-opioid receptor, the main cellular target of opioid drugs. Hence, WLS may play a role in the reward or addiction of heroin, opioids, or cocaine.[81,100-102] Further, according to recent studies, WLS is an important regulator in modulating ER stress in dendritic cells, and it affects immunity and protein glycosylation control independent of the Wnt signaling pathway.[28] WLS is involved not only in the Wnt pathway but also in the pathways that crosstalk the Wnt signaling pathway (e.g., TGF-β/JNK pathway,[66] HER-NFκB pathway,[71] ROR2-dependent PKCδ/ERK pathway,[78,103] and AKT pathway[104]).

Conclusion

WLS is not only a biomarker for predicting clinical outcomes but is also a potential therapeutic target of several cancer types. WLS-targeted therapy against cancer is gaining increasing attention. Although targeting molecules that are involved in the Wnt-signaling pathway for treating cancer has been developed vigorously in recent years; there is relatively less clinical trials that target WLS; instead, there are several phase I and phase II trials for treating colon, gastric and endometrial cancer that target porcupine, an essential factor for Wnts to bind to WLS.[105-107] The current review discussed the basic structure of WLS and its role in Wnt signaling, embryogenesis, and tumorigenesis. WLS is a multipass ER TM protein that is essential in secreting almost all Wnts although a large amount of character of WLS is still under revealed. This study could provide more information about the importance of WLS, which can be used to develop novel therapies against cancers or other diseases in the future.

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Conflicts of interest

There are no conflicts of interest.

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