We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,100
Open access books available

149,000
International authors and editors

185M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Insights into the Wnt Signaling Pathway Evolution

Elham Rismani, Nasrin Haghighi-Najafabadi, Babak Elyasi Far, Behzad Shahbazi and Ladan Mafakher

Abstract

Animals' Wnt signaling pathways are highly preserved signal transduction pathways, which play a crucial role in embryogenesis and adult tissue homeostasis. This chapter reviews the three major Wnt pathways, focusing on some critical proteins in the Wnt/β-catenin path in terms of their evolution and role in homeostasis. Wnt proteins act as a gateway between extracellular, cytoplasmic, and nuclear components to transmit signaling pathways. The Frizzled (FZD) family as G-protein-coupled receptors activates the signaling pathways by binding to Wnt ligands. LRP5/6, members of the family of low-density lipoprotein receptors (LDLR), associate with FZD receptor and Wnt ligands as co-receptors to initiate the Wnt/β-catenin pathway. The Wnt/β-catenin pathway is regulated by antagonists such as the Dickkopf and secreted Frizzled-related protein (SFRP) families.

Keywords: Wnt signaling pathway, frizzled, Wnt, LRP, Dickkopf, SFRP

1. Introduction

The Wnt signaling pathway activates numerous proteins and transmits biological signals into a cell through cell surface receptors, a constant mechanism in all animal kingdom phyla [1]. In the 1980s, studies on oncogenic retroviruses in colon and breast tumors and identifying the Wingless (Wg) gene in *Drosophila* led to the discovery of Wnt signaling [2]. The Wnt signaling route consists primarily of three signal transduction pathways: the canonical Wnt pathway, the noncanonical planar cell polarity (PCP) pathway, and the noncanonical Wnt/calcium pathway. The categories are based on the presence of the protein beta-catenin (β-catenin) in the canonical pathway, whereas the noncanonical pathway operates independently [3]. The central function of WNT signaling in controlling embryonic progression and adult tissue homeostasis is well understood. From primitive metazoans to humans, WNT signaling pathway elements comprising ligands and receptor genes have duplicated and diversified to establish a robust regulatory response [4]. This chapter comprehensively introduces the Wnt signaling pathway and looks at the critical proteins in terms of their evolution and role in homeostasis.

1.1 The Wnt/β-catenin signaling pathway

The Wnt/β-catenin pathway comprises four protein groups: the extracellular signal ligands, membrane proteins, cytoplasmic members, and nuclear proteins (**Figure 1**). For example, secreted Frizzled-related protein (SFRP) and Dickkopf (DKK) are the family of regulatory proteins in the Wnt signaling pathway that make up the majority of extracellular signals. The cell membrane proteins include the Wnt receptors Frizzled (specific sevenfold transmembrane receptor Frizzled protein) and lipoprotein receptor-related protein (LRP5/6). The cytoplasmic members consist of β-catenin, the Disheveled family (DVL), glycogen synthase kinase-3β (GSK-3β), adenomatous polyposis coli (APC), axis inhibition protein (AXIN), and casein kinase I (CK1). The translocated β-catenin to the nucleus, T cell-specific transcription factor (TCF)/ lymphoid enhancer-binding factor (LEF)

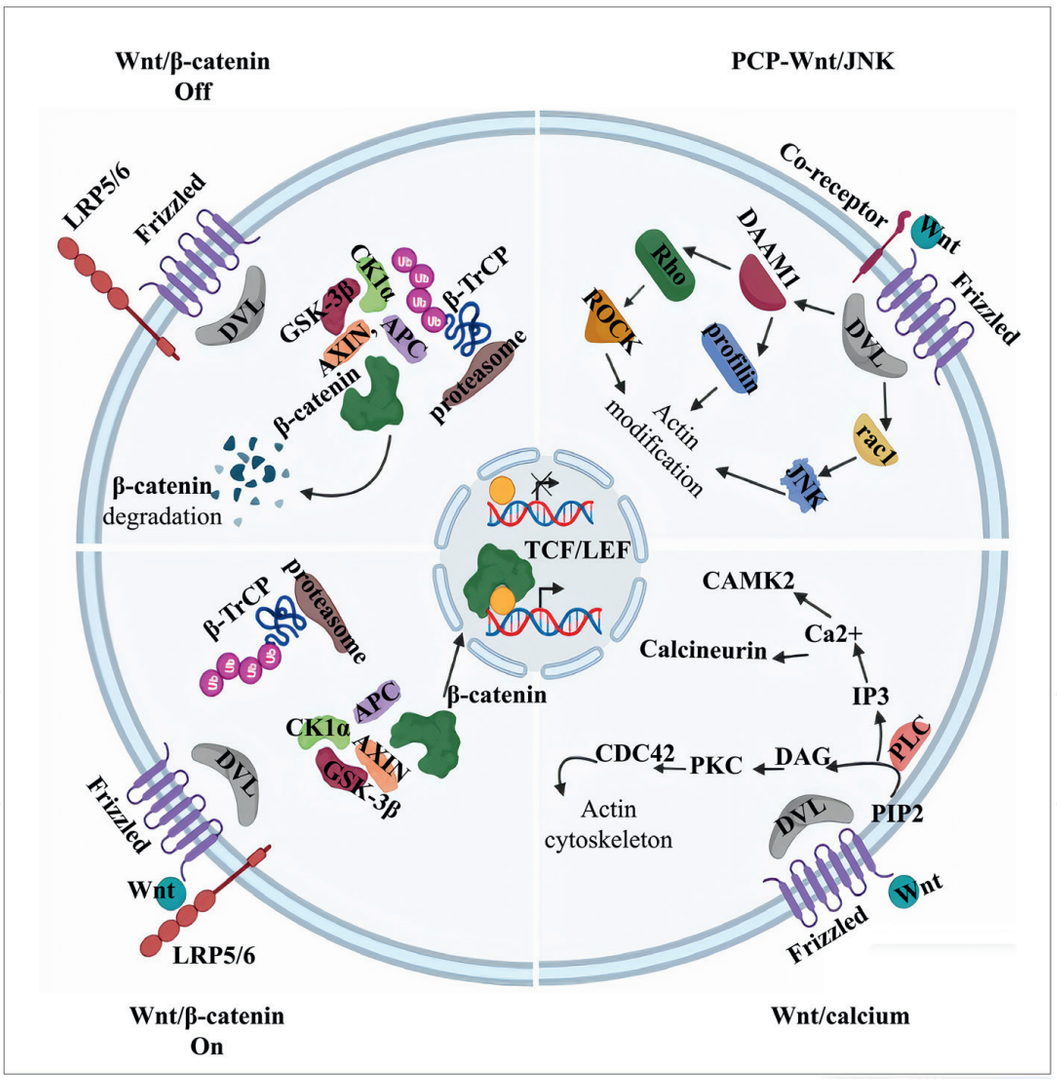

Figure 1.
A schematic representation of the Wnt signaling pathway. In the absence of Wnt ligand (off), β-catenin is targeted for degradation by the Axin, APC, and GSK-3 degradation complex. If Wnt attaches to the Frizzled and LRP receptors (on), the degradation complex is inactivated, causing β-catenin to be stabilized and translocated to the nucleus. The other two pathways function independently of β-catenin. The PCP pathway begins with Wnt molecules interacting with Frizzled and its co-receptor. Attaching Wnt molecules to the Frizzled protein turns on the Wnt/calcium pathway, which can cause calcium to be released from inside the cell.

family members, and downstream target genes, such as MMPs and c-Myc, are members of nuclear proteins [5, 6].

The cytoplasmic level of β-catenin is regulated by the AXIN, APC, CK1, and GSK-3 destruction complex, which causes β-catenin phosphorylation and ubiquitination by beta-transducin repeat-containing E3 ubiquitin-protein ligase (b-TrCP), followed by proteasomal degradation of β-catenin [7, 8]. The binding of Wnt ligands to membrane receptors triggers the binding of AXIN to phosphorylated LRPs, which in turn activates the Wnt/β-catenin pathway. This event makes the destructive complex break apart, stabilizing the cytoplasm's B-catenin and turning on DVL. The primary function of the Wnt/β-catenin pathway comprises the nuclear translocation of the critical protein β-catenin and engagement of target genes via T cell-specific transcription factor and lymphoid enhancer-binding factor (TCF/LEF) [9].

1.2 The planar cell polarity (PCP) signaling pathway

Mutagenesis investigations of Wnt signaling components in Drosophila, particularly Frizzled and Disheveled, revealed that the noncanonical PCP route is fundamental in regulating the posture of epithelial structures such as cuticle hairs and sensory bristles (**Figure 1**). This pathway feature is due to its role in regulating the actin cytoskeleton [10]. The PCP pathway begins with Wnt ligands interacting with Frizzled and its co-receptor (NRH1, Ryk, PTK7, or ROR2). After DVL is recruited, it makes a structure with a Disheveled-associated activator of morphogenesis 1 (DAAM1), which triggers the G-protein Rho and then the Rho-associated kinase. The DVL-Rac1 complex stimulates c-Jun N-terminal kinase (JNK) and facilitates profilin adhesion to actin, which ultimately leads to cytoskeleton remodeling and gastrulation [11, 12].

1.3 The Wnt/calcium signaling pathway

Wnt ligand binding to the Frizzled binding site activates the noncanonical Wnt/calcium cascade. (**Figure 1**). The PDZ, DEP domains of DVL, and a G-protein interact directly with Frizzled receptors, stimulating either PLC or cGMP-specific PDE. Activating PLC cleavage of PIP2 as the plasma membrane component into DAG and IP3 triggers calcium release by binding IP3 to its receptor on the ER. Calcium and DAG, along with PKC, can activate CDC42. Calcineurin and CaMKII are also activated by calcium; the latter activates the transcription factor NFAT, which regulates cell attachment, migration, and tissue dissociation. Calcium release is inhibited by activation of PDE and mediates the inhibition of PKG [12, 13].

2. The Wnt signaling pathway's function and role

In the world of multicellular creatures, all three pathways have multiple functions. One of the crucial functions of Wnt signaling is in the orientation of the body axis of the developing embryos in all metazoans [14]. Wnt signaling is critical in embryonic development and controls processes like cell fate decisions, body orientation architecture, cell growth, cell migration, cellular differentiation, and tissue patterning. Wnt signaling appears to regulate the differentiation of embryonic stem cells (ESCs) into different germ layers, consistent with its role in embryonic development. Wnt signaling is an essential regulatory pathway during adult tissue homeostasis in multicellular animals. The involvement of the components of this pathway has been recognized in

homeostatic tissue regeneration in the developing and adult brain, metabolic homeostasis, skin homeostasis, and bone homeostasis. Examples come from various studies summarized in the following, considering their references to indicate more details.

One of the most studied cases of the Wnt pathway in the homeostasis of mature tissue is related to the preservation of ESCs niches, whose proliferation and self-renewal are driven by exogenous Wnt signals. Disrupting some components, such as Tcf4, β-catenin, or APC, results in a loss of intestinal epithelial compartment proliferation and differentiation, confirming the importance of Wnt/β-catenin regulation [15]. The maintenance of a balance between the systemic calcium homeostasis and the biomechanical properties of bone tissue is another example of the crucial function performed by Wnt signaling. Loss-of-function mutations in Wnt/β-catenin signaling components such as LRP5 and SOST have led to severely decreased or increased bone mass and osteoporosis [16]. Interestingly, an increase in bone mass is associated with decreased activity of Wnt inhibitors such as Dickkopf-1 or Sclerostin. This data showed that Wnt signaling has a substantial role in bone growth [17].

In Gurley et al.'s study to understand the mechanisms of anterior-posterior differentiation, β-catenin was suggested as a molecular switch during regeneration and homeostasis in planarians [18]. Regarding metabolic homeostasis, attention to the association of TCF7L2 polymorphisms with type 2 diabetes has emphasized the function of the canonical Wnt pathway in glucose homeostasis [19]. Furthermore, new research indicates that skin homeostasis requires an appropriate level of Wnt/β-catenin signaling. The high grade of Wnt signaling directs keratinocytes to form hair, whereas low or intermediate levels of Wnt signaling determine interfollicular epidermis and the sebocyte lineages. Disturbing the balance of Wnt/β-catenin signaling can lead to the excessive formation of particular skin cell lineages and cancer [20].

The equilibrium between biomaterial synthesis and degradation is essential to life. One of the most catabolic in eukaryotic cells is autophagy which plays a critical function in preserving stability by eliminating destroyed or aged organelles and toxic protein aggregates. So, autophagy is essential in cellular homeostasis by maintaining bioenergetics and establishing a critical response to microenvironmental stress. Current literature illustrates that autophagy and Wnt/β-catenin signaling are related and have cross-talk at different stages of cellular development and differentiation to maintain cellular homeostasis. During nutritional deficiencies, β-catenin and Disheveled become targets for degradation by microtubule-associated protein light chain 3 (LC3) as an autophagic degradation protein. The Wnt signaling pathway by β-catenin, a corepressor of autophagy proteins of p62 and GSK3β, could regulate Wnt and control autophagy adversely [21].

3. Key proteins of the pathway

The original Wnt signaling pathway in simple metazoans, including *Porifera* (sponges), *Placozoa*, and *Ctenophora* (comb jellies), is made up of a basal number of transducers, including Wnt molecules, receptors, and cytoplasmic [22]. Despite variations in details of Wnt ligands, receptors, and cytoplasmic proteins involved in signaling pathways, they shared the main components, such as extracellular ligands and membrane receptors. Since The Wnt/−βcatenin pathway, also known as the canonical pathway, is one of the main pathways in the renewal, proliferation, and differentiation of stem cells and adult tissue homeostasis, in the following, we will introduce the essential proteins of this pathway.

3.1 The Wnt family

Wnts (Wingless-type MMTV integration site family) are a group of genes that encode Wnt-secreted glycoproteins. Beyond the metazoans, no genes encoding for Wnt ligands have been discovered. The number of Wnt gene subfamilies has increased from the first metazoans to cnidarians, which have a complete set of Wnt genes. However, the loss and gain of Wnt gene subfamilies have been traced in metazoan evolution [22].

3.1.1 Structure

The Wnt family, as the initiators of the Wnt signaling pathway, is comprised of 19 cysteine-rich proteins in mammals [21, 23], which share similarities in size (350–400 amino acids in length). Each includes an amino-terminal signal sequence with 22–24 cysteine residues, highly preserved during evolution, which are critical in Wnts for their function [24, 25]. Despite about 35% sequence identity among the majority of Wnt proteins, members of a subgroup (such as WNT3 and WNT3a) show more identity in sequence (58–83%) and have overlapping expression sites [26]. Among human Wnt proteins, only crystal structure of human Wnt3 (PDB ID:6AHY) (**Figure 2A**) has been characterized. The Wnt conservation analysis discovered that binding site regions of the Wnt to Frizzled receptor, named thumb and finger index, were highly conserved during evolution (**Figure 2B**). The result represents the critical role of the interacting Wnt protein with Frizzled in regulating the Wnt signaling pathway. In addition, comparing the

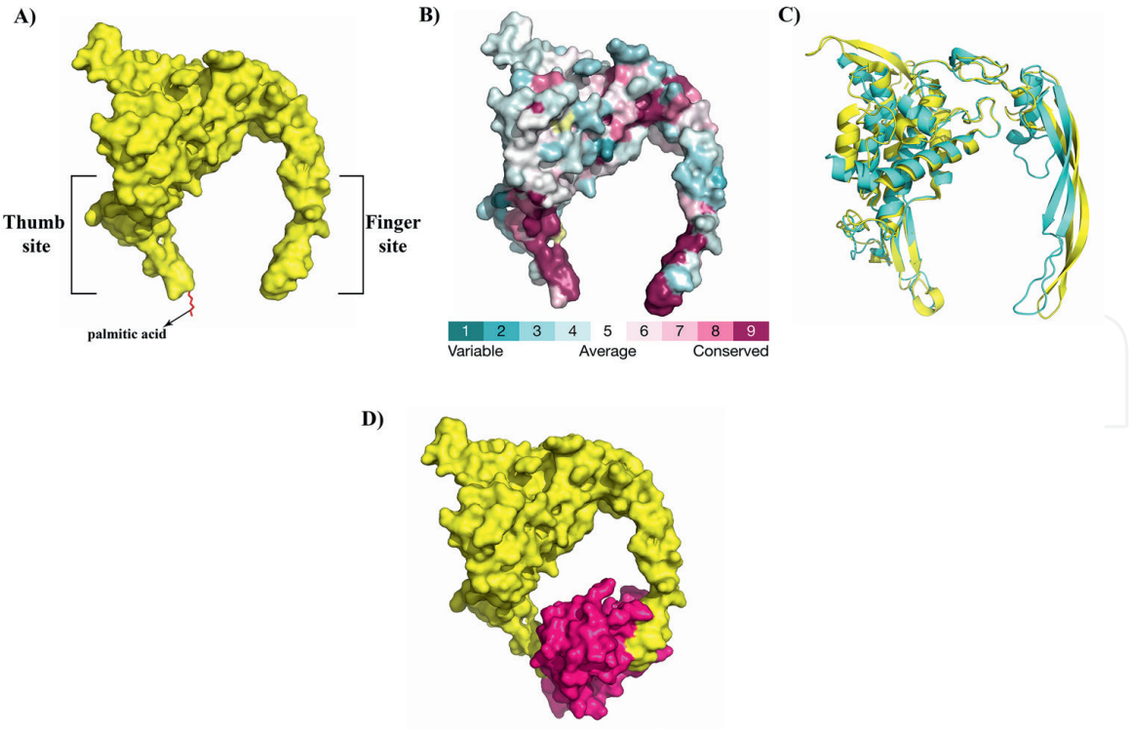

Figure 2.
The tertiary structure of human Wnt3. (A) Surface representation, (B) ConSurf webserver conservation analysis of WNT3. Data showed that binding site regions of WNT are conserved during evolution, (C) structure alignment of human Wnt3 (colored yellow) to Wnt8 Xenopus (colored cyan) shows the structure similarity of Wnts during evolution, and (D) complex of human Wnt3 (colored yellow) with the CRD domain (colored magenta) indicates two binding regions of Wnt to frizzled.

structure of human Wnt3 with that of Wnt8 *Xenopus* revealed the tertiary structure of Wnts conserved throughout evolution (**Figure 2C**).

Because of the existence of O-lipidation at a conserved serine, Wnt proteins are highly hydrophobic. The first Wnt protein structure (complex of *Xenopus* Wnt8 with mouse Frizzled 8) revealed that Wnts attach to Frizzled at two different sites on opposite faces of the cysteine-rich domain (**Figure 2D**) (CRD) [27]. This conserved extracellular domain in all Frizzled proteins contains 10 cysteine residues that bind to multiple Wnts with high affinity [28].

The Wnt family can be categorized according to the features and functions of Wnt1 and Wnt5a. The canonical signaling pathway comprises the Wnt1 group, including Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt7a, Wnt8, Wnt8b, and Wnt10a components. While Wnt4, Wnt5a, and Wnt11 are all members of the Wnt5a family, they have the potential to stimulate a noncanonical signaling pathway [29].

3.1.2 Role and function

In all Wnt signaling pathways, Wnt molecules interact with their Frizzled or co-receptors to initiate a signaling cascade. The canonical Wnt pathway begins by binding Wnt molecules to the Frizzled receptor and LRP5/6. Also, Wnt ligands in the category of Wnt5a could stimulate the PCP and Wnt/Ca2+ pathways, which are characterized as noncanonical pathways. Signaling is transferred via Frizzled binding and DVL activation without LRP as a co-receptor [12].

3.1.3 Evolution of Wnts

Wnt ligands are an ancient and diverse protein family that induces target cells by binding to Frizzled receptors [4]. Wnt genes are identified from sponges to humans that become 13 subfamilies (Wnt1 to Wnt11, Wnt16, and WntA) by duplication and diversification before splitting the bilaterian and cnidarian. Phylogenetic and genomic investigations on a large scale have discovered that some Wnt genes are lost and retained during evolution in animals. This data means that each lineage of animals constructs its reservoir of Wnt genes [30]. Also, phylogenetic analysis of the orthologues of Wnts during evolution demonstrates high sequence similarity; for example, the human Wnt1 has 98% identity to the mouse Wnt1, which means evolutionary relationships between these proteins during development [31]. According to the structural analysis of Wnt proteins during evolution, the thumb site of Wnt proteins is closely related to saposin-like proteins, a primitive category of multifunctional lipid-interacting and helical carrier folds. In contrast, the finger site of Wnt proteins resembles a cytokine-like domain. During evolution, these two domains of Wnt came together to make more specific and robust interactions with Frizzled receptors to control animal development and homeostasis [32].

3.2 The SFRP family

Numerous antagonists regulate Wnt signaling, such as Wnt inhibitory factor 1 (WIF1), Cerberus, Sclerostin, members of the Dickkopf, and secreted Frizzled-related protein (SFRP) families. Sclerostin and Dickkopf proteins bind to LRP5 or LRP6 and block canonical signaling, whereas WIF1, Cerberus, and SFRPs can interact directly with Wnt proteins and inhibit their activation [33, 34].

The most prominent family of Wnt inhibitors is the SFRPs. This family is identified in all vertebrates and comprises five secreted glycoproteins: SFRP1, SFRP2, SFRP3 (Frzb), SFRP4, and SFRP5 [35]. Sequence and phylogenetic analysis illustrate that the SFRPs family consists of two subgroups: SFRP1, SFRP2, and SFRP5, which are strongly linked, and SFRP3 and SFRP4, which cluster together. This classification reveals a distinct organization of SFRPs' genomes. Surprisingly, the third subgroup of SFRPs, which appears to be absent in mammals, has been discovered in *Xenopus*, zebrafish, and chicks named "Sizzled," "Crescent," and "TLC," respectively. This group shows sequence similarity with the SFRP1-SFRP2-SFRP5 subgroup with an additional inter-domain disulfide bond in the Sizzled and Crescent subgroups [17].

3.2.1 Structure

The SFRP family consists of a CRD with 10 cysteine residues at the N-terminus that shares 30–50% sequence homology with the CRD of the Frizzled protein and a netrin-related motif (NTR) at the C-terminus that folds into two independent domains (**Figure 3A**) [35]. It is presumed that the CRD domain is composed mainly of the alpha helix [37] and interacts with the Wnt in a similar interface mode to the Frizzled CRD domain [38–40]. The NTR domain in the SFRP family has approximately 120 amino acids with six cysteine residues. Compared to the CRD domain, the cysteines in this domain are not conserved during evolution [41]. The NTR domain displays homology with netrins, complement C3, C4, C5, and procollagen C-endopeptidase enhancers [42]. The tertiary structure of this domain comprises five beta barrels [43–45]. The role of the NTR domain in SFRPs is not yet fully understood [46]. However, Bhat et al. revealed that both parts of SFRPs are critical for their particular inhibitory activity [47]. Some studies have illustrated that the NTR domain regulates the Wnt signaling

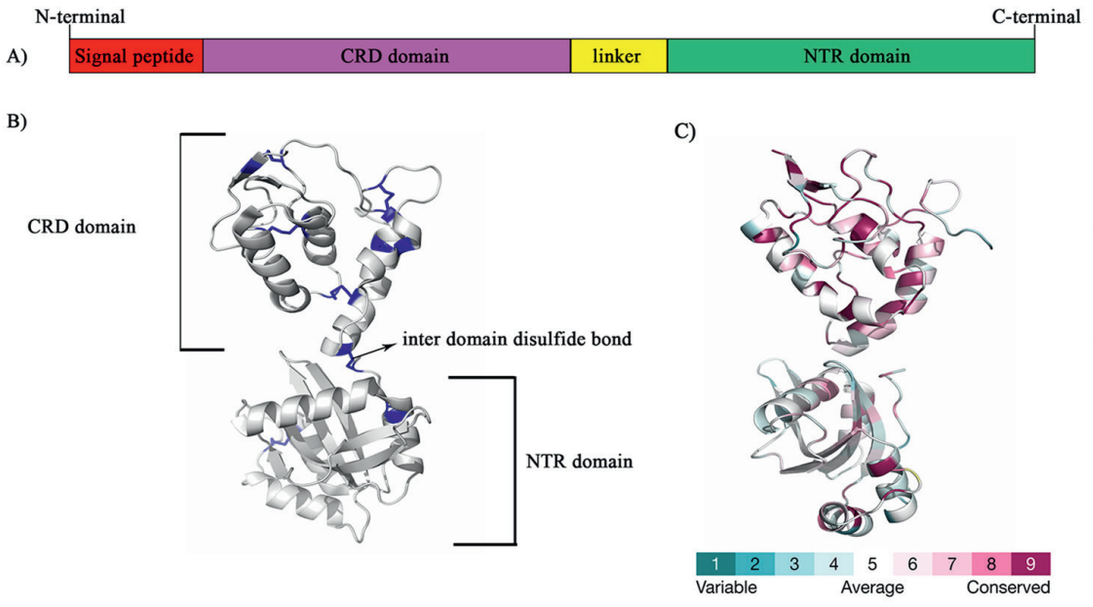

Figure 3.
Schematic view of sequence and structure analysis of sizzled protein. (A) Sequence analysis of sizzled protein with its different regions, (B) tertiary structure of the sizzled protein (PDBID:5XGP) [36]. Disulfide bonds in a structure characterized by the blue stick. Inter-domain disulfide bond has just existed in sizzled protein and not in SFRPs, and (C) conservation analysis of sizzled during evolution by ConSurf webserver. Data show the CRD domain is more conserved than the NTR domain during evolution.

pathway by binding to heparin [48, 49] via its positive charge of lysine and arginine amino acids located at the bottom of the domain, contrary to the NTR-CRD interface. [36]. Also, Bhat et al. revealed that any alteration of the lysine residues to alanine in the NTR domain of SFRP1 caused a reduction of antagonist activity in the Wnt signaling pathway [47]. The only crystal structure of full-length SFRPs belongs to the Sizzled protein of *Xenopus laevis* (**Figure 3B**) as a homolog protein of SFRPs [36]. Conservation analysis showed that the CRD domain of Sizzled was more conserved than the NTR domain due to its critical role in interaction with Wnts (**Figure 3C**). Moreover, SFRPs represent posttranslational modifications in their structure. This alteration seems to confer additional differences which may cause further diversification of various SFRP family functions, like N-glycosylation of SFRP1 and sulfated at two tyrosine residues in SFRP5, which inhibit its binding to heparin and reduce the stability of SFRP5 [17].

3.2.2 Role and function

The SFRP gene family is regarded as a tumor suppressor. However, new studies indicate that SFRPs could play multiple roles in regulating the Wnt signaling pathway and cause various effects in various kinds of cancer due to changes in their expression [35]. Although SFRP has been identified as a critical Wnt signaling regulator, the precise mechanism by which Wnt signaling is controlled remains unknown [17]. Moreover, SFRPs have been linked to disorders such as skeletal diseases, ocular degradation, and hypophosphatemic illnesses, implying that their activity is required for tissue homeostasis. [17]. Phosphorus is essential in a variety of biological processes. Phosphorus homeostasis is linked to various cellular mechanisms involved in energy homeostasis, signal transduction, synthesis of nucleic acids, cellular membrane function, bone wellness, and integrity. Therefore, maintaining phosphorus balance and homeostasis is essential to the organism's health. The SFRP4, characterized as phosphatonin molecules, a new class of hormones that regulate renal phosphorus wasting, plays a crucial role in phosphorus homeostasis by antagonizing the Wnt pathway in the kidney. SFRP4 overexpression has been found in cancers associated with wasting phosphate and osteomalacia in the kidney. In both events, the abundance of Na + -Pi symporters decreases in the proximal tubule of the brush border membrane. This reduction caused a decrease in the expression of the Na + -Pi IIa symporter at the surface of the proximal tubules and opossum cells in the renal. Also, subsequent studies illustrate that this protein participates in other pathophysiological circumstances related to the wasting of phosphate, which could contribute to physiological regulation [50, 51]. Moreover, SFRP1 could also affect this pathway by interaction with RankL. Likewise, SFRP3 is related to the progress of osteolysis or heterotopic ossification [17].

Alternately, SFRPs have an impact on promoting and generating photoreceptors during the development of embryos. Elevated SFRPs expression, especially SFRP1, caused retinal damage in patients with retinitis pigmentosa, a congenital disorder characterized by the promotion of photoreceptor loss [17].

3.2.3 Evolution of SFRPs

SFRP homologs have not been discovered in the *Drosophila* genome. At the same time, this family exists in other invertebrates, such as the purple sea urchin, the nematode *Caenorhabditis elegans*, and even the sponge *Lubomirskia baikalensis*,

demonstrating the SFRPs' historical basis (4). This organism only has one SFRP protein, whereas most invertebrates (except *lamprey*) have two SFRP proteins and all vertebrates have five SFRP proteins. This data demonstrated how SFRP proteins duplicated and diverged during the evolution of vertebrates to more precisely and accurately control signaling pathways [52]. Moreover, sequence analysis of SFRP protein in humans found that disulfide bonds in the CRD domain are completely conserved while not in the NTR domain. This result could be interpreted since the origin of the CRD domain of human SFRPs came about by the duplication of CRD domains of Frizzled receptors during evolution. Still, the NTR domain has been independently created [53].

3.3 The DKK family

The DKK (Dickkopf) protein family is a group of four Wnt modulators in vertebrates, with sizes ranging from 255 to 350 amino acids. The distinct feature of these proteins is two conserved CRDs that differ from the CRDs found in SFRPs and Frizzleds [54].

3.3.1 Structure

The Dickkopf protein in vertebrates is composed of two conserved CRD regions(CRD1 and CRD2), which are separated by a spacer region of different lengths in DKK members (**Figure 4A**). CRD2 competes with Wnt ligands for LRP5/LRP6 interaction, whereas CRD1 modulates CRD2 [55]. In their CRD1 and CRD2 domains, DKK1 and DKK2 share 50 and 70% of their identity, respectively. The CRD2 domains of DKK1 and DKK2 have a high affinity for direct interaction with LRP 5/6. The DKK2 (CRD2) NMR structure revealed a relatively flat molecule with six-sheet regions composed of five disulfide bonds, stabilizing the CRD2 structure [56]. Furthermore, DKK3 is unique within the Dickkopf family in that it contains an N-terminal Soggy

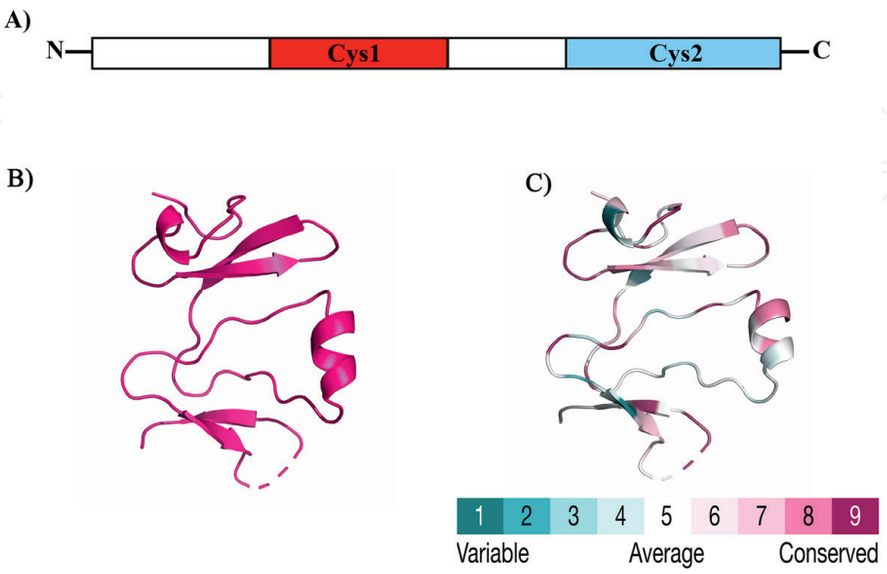

Figure 4.
Schematic view of the human DKK1 domain and the 3D structure of the human DKK1 CRD2 domain. (A) DKK domains, (B) tertiary structure of DKK1's CRD2 domain (PDB ID: 3SOQ), which interacts with the LRP receptor, and (C) conservation analysis of the CRD2 domain throughout evolution.

domain as well as two CRD domains [57]. The tertiary structure of the CRD2 domain of human DKK1 is represented in **Figure 4B**. Conservation analysis of this domain illustrated that this domain is approximately highly conserved during evolution (**Figure 4C**).

3.3.2 Role and function

Generally, Dickkopf proteins are critical for embryogenesis and osteogenesis. So, any abnormalities in DKK protein have been linked to a range of cancers, bone diseases, and neurodegenerative disorders [56]. DKK1 binds to LRP5/LRP6 and its co-receptor Kremen to block the Wnt pathway, which is needed for the development of the head and limb formation in vertebrate embryos. Dickkopf proteins were involved in one of the negative-feedback loops that terminated or repressed activation of Wnt at the cell surface receptor. Indeed, DKK1 is a promising target gene of the Wnt/β-catenin pathway that inhibits the initiation of signaling by competing with Wnts to interact with LRP5/6 [58, 59].

Wu et al. discovered that DKK2 and Wnt collaborate synergistically to stimulate the Wnt/β-catenin pathway in embryos of Xenopus. It seems that the function of DKK2, disparate from other members of the DKK family, appears to be a stimulated co-factor for Wnt signaling [55, 60]. DKK3, on the other hand, unlike the other DKKs, is not a Wnt signaling antagonist [57].

3.3.3 Evolution of DKKs

Although the homologs of the receptor proteins Frizzled and LRP5/LRP6 have been identified in vertebrates and insects, no Dickkopf protein was discovered in the genomes of insects and nematodes. By finding a DKK3-related protein in *Hydra*, DKK3 was proposed as the ancestral Dickkopf type. Structural and phylogenetic analysis indicated that the vertebrate DKK1/2/4 subfamily was created by subsequent gene duplication. Furthermore, functional analyses of HyDkk1/2/4 and canonical Wnts in *Xenopus* and *Hydra* proposed that the Wnt-Dickkopf antagonism existed in the last common origin of cnidarians and bilaterians, while it was lost in Caenorhabditis and insects [61].

3.4 The LRP family

Low-density lipoprotein receptors (LDLRs) are a protein family that is found in a variety of tissues and have a variety of cellular functions. LRP5 and LRP6 are Wnt ligand co-receptors in canonical Wnt signaling that significantly influence various physiological and pathological procedures in adult tissues. The dysregulation of LRP5 and LRP6 has been associated with various diseases ranging from bone, cardiac, neurodegenerative, diabetes, and hypercholesterolemia to cancer. Though impaired LRP6 signaling is associated with human diseases, studies suggest a predominant role of LRP6 during development, where loss-of-function of LRP6 in mice was embryonically lethal [62–65].

3.4.1 Structure

The LRP5/6 are single-pass transmembrane receptors with an extracellular domain that comprises four tandem YWTD-type b-propeller (BP)-epidermal

growth factor (EGF)-like domains, which are named P1E1-P2E2, and P3E3–P4E4. These regions are followed by three LDLR type (A) repeats (**Figure 5**). The extracellular regions of LRP5/6 are responsible for interacting with Wnt molecules and antagonists, including Dickkopf-related protein 1 (DKK1) and Sclerostin [66]. The domains of P1E1-P2E2 primarily interact with Wnt1, Wnt2, Wnt2b, Wnt6, Wnt8a, Wnt9a, Wnt9b, and Wnt10b, while Wnt3 and Wnt3a bind to P3E3-P4E4 domains. The cytoplasmic region of LRP5/6 comprises 200 amino acids and five signature PPPSPxS motifs labeled A through E. The cytoplasmic domains of LRP5/6 have a binding site for Axin, which is required for LRP6 signaling. Though these proteins have been highly conserved during evolution, the amino acid sequences of LRP5/6 proteins share 71% identity [67]. The crystal structure of LRP5 has not been assessed yet. Although the whole structure of LRP6 does not exist, the structures of functional domains of LRP6, which interact with Wnt proteins, exist in the PDB bank (**Figure 6A** and **C**). Also, conservation analysis demonstrated that functional domains of LRP6 were nearly conserved during evolution (**Figure 6B** and **D**).

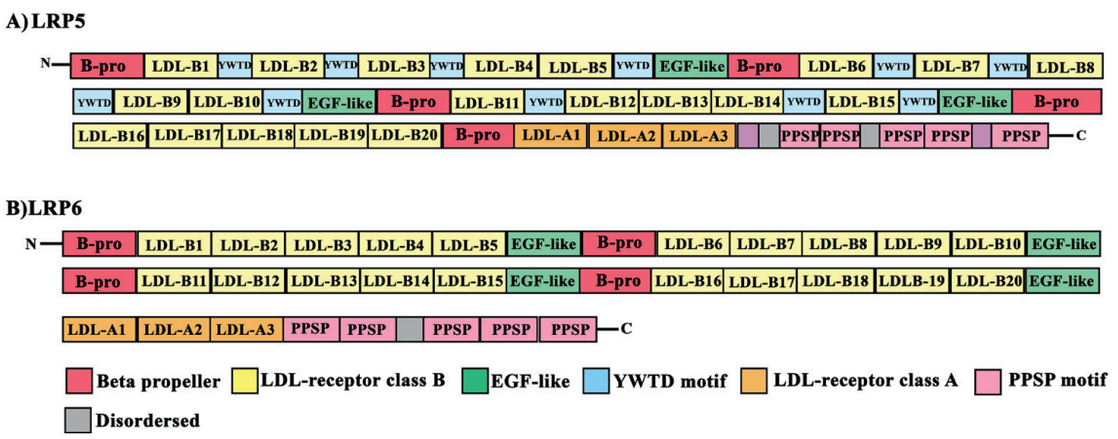

Figure 5.
Schematic view of LRP5/6 domains and regions in humans.

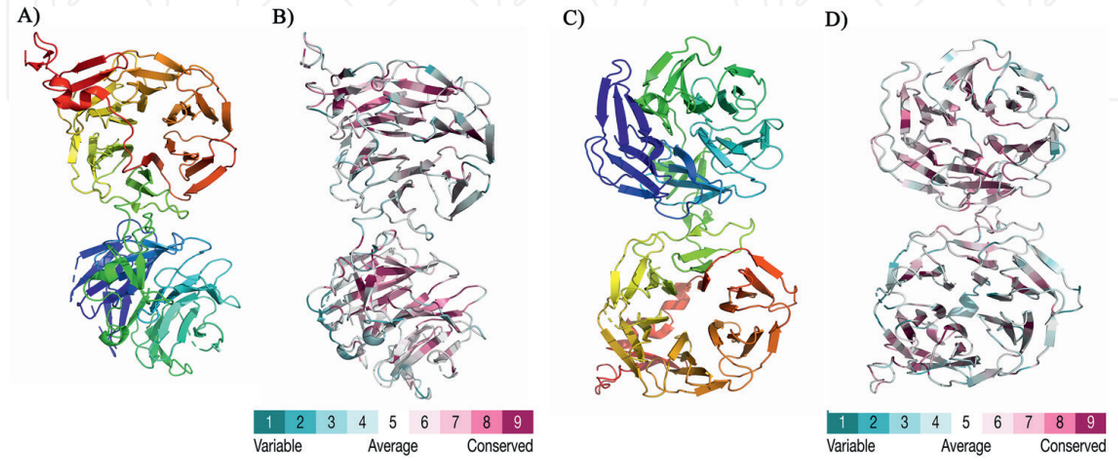

Figure 6.
The tertiary structure of LRP6. (A) the crystal of domains E1 and E2 of LRP6 (PDB ID: 3S94), (B) ConSurf web server conservation analysis of E1 and E2 regions of LRP6, (C) the crystal of domains E3 and E4 of LRP6 (PDB ID: 3S8Z), and (D) ConSurf web server conservation analysis of E3 and E4 domains of LRP6.

3.4.2 Role and function

LRP5 and LRP6 are co-receptors for canonical Wnt signaling, though LRP6 is more active than LRP5 in the Wnt canonical pathway. Binding the Wnt ligand to the cell surface receptors Frizzled (FZD) and LRP5/6 induces phosphorylation of the intracellular domain of LRP5/6, dissociation of the destruction complex, stabilization of cytosolic β-catenin, translocation of β-catenin into the nucleus, and eventually, activation of Wnt target gene transcription [68]. Wnt ligands are classified according to their preference for binding to LRP5 or LRP6. Wnt3, Wnt3A, Wnt6, Wnt8A, and Wnt8B only activate Wnt signaling through LRP6, whereas Wnt2 primarily promotes Wnt signaling through LRP5. Wnt1, Wnt7B, and Wnt9A interact similarly with LRP5 and LRP6 [66].

Several proteins, such as DKK1, Sclerostin, PEDF, and Kallistatin, inhibit Wnt binding by interacting with LRP6. However, besides the Wnt ligands, proteins such as the R-Spondin (Rspo) family, Norrin, parathyroid hormone (PTH), transglutaminase 2 (TG2), Cripto-1, Biglycan, and single-span membrane protein TMEM59 stimulate the Wnt pathway by interacting with LRP6. These findings demonstrate that LRP5/6 may also operate as the co-receptor of other ligands in initiating the Wnt/β-catenin signaling [66].

Although LRP6 expression varies between human tissue types, LRP6 is essential in regulating cell proliferation, differentiation, migration, and stem cell homeostasis. Regarding the unique function of LRP5/6 as the co-receptor for several extracellular cues, LRP5/6 has been an attractive target for designing a drug to abrogate/activate the Wnt signal pathway in recent years (**Table 1**) [69].

3.4.3 Evolution of LRP5/6

Several studies showed conservation evolutionarily in the Lrp5/6 gene among all animals. Investigation of the LDLR family in *Drosophila* indicated that the arrow gene, which defines LDL-receptor-related protein as a required protein for signaling

Company/University	Category	Targeting mechanism	Disease
Surrozen INC, USA	Antibodies	LRP5/6	Melanoma cancer
Yale University, USA	Antibodies	Interaction between DKK2 and LRP5	
Agency for Science, Technology, and Research (A*STAR), Singapore	Small molecule	phosphorylated LRP6 (Ser1490)	Human pancreas HPAF-II cancer cells
Boehringer Ingelheim international GMBH, Germany	Polypeptides	LRP5/6	Cancer
Nantbio Inc., USA	Small molecule	Phosphorylation of LRP6	—
Surrozen INC, USA	Antibody	LRP6	Osteoporosis
Surrozen INC, USA	Antibody	LRP6	Retinopathy

Table 1.
List of released patents for targeting LRP6 in various diseases.

in the Wingless pathway, is structurally similar to LRP5 and LRP6 in vertebrates. Despite having 45% homology with the LRP5 and LRP6 proteins, arrow exhibits phenotypic similarities with Wg/Wnt mutants. Furthermore, there is a 98% amino acid sequence similarity between human and mouse LRP6. Furthermore, at the protein level, LRP6 shares more than 93% identity with humans and chickens [63].

3.5 The frizzled family

The Frizzled (FZD) family is characterized as G-protein-coupled receptors (GPCRs) that contribute significantly to embryogenesis. According to robust evidence, this family controls tissue homeostasis in various adult organs [70]. Any dysregulation of this family causes multiple types of disease in adults or during embryonic development. These problems include cancer, cardiac hypertrophy, familial exudative vitreoretinopathy, brain synapses, tissue and cell polarity, proliferation control, and schizophrenia in human and animal models [70, 71]. This family could activate three main signaling pathways: the FZD/β-catenin pathway, the FZD/Ca2+ pathway, and the FZD/PCP (planar cell polarity) pathway. Many secreted ligands, including all forms of Wnt protein, the SFRP family, R-Spondin, and Norrin, could interact directly with Frizzled and initiate the downstream signaling pathway [70].

Based on sequence analysis, humans have 10 Frizzled genes, comprising four primary classes. According to sequence classification, FZD1, FZD2, and FZD are classified into one group, which has 75% of the amino acids are identical; the second group consists of FZD5 and FZD8 with 70% amino acid identity to each other; the third group consists of FZD4, FZD9, and FZD10 with 65% identical amino acids in sequences. The last group is FZD3 and FZD6, sharing 50% amino acid identity [71].

3.5.1 Structure

Frizzled proteins (FZD) have different lengths ranging from 500 to 700 amino acids. They are composed of an extracellular region at the N-terminus, comprising a cysteine-rich domain (CRD), a hydrophilic linker region of 40–100 amino acids, and a transmembrane area is compromised of the seven-pass transmembrane domain receptors in alpha helix form. The intracellular domain at the C-terminus region is highly variable among different Frizzled family members (**Figure 7A**) [71]. The CRD domain of Frizzled protein is responsible for its interaction with Wnt protein, consisting of 120–125 residues and a fully conserved disulfide bond. The only crystal structure of Frizzled 2 (PDB ID: 6C0B), 4 (PDB ID: 5CM4), 5 (PDB ID: 5O39), 7 (PDB ID: 5 T44), and 8 (PDB ID: 5UN5) of humans has been discovered yet belongs to the CRD domain. The CRD region crystal structure analysis of Frizzled proteins revealed that the dominant structure of this region is the alpha helix, which is highly conserved during evolution (**Figure 7B**) [37]. Conservation analysis of the human Frizzled CRD region showed that this domain is highly conserved during evolution (**Figure 7C**). Sequence similarity search reveals that conservation of transmembrane domain of Frizzled compared to other GPCR is low and limited to the hydrophobic residues. According to sequence similarity search, this family might be the evolution from the Taste2 subfamily of taste receptors. After the transmembrane domain, the highly conserved KTXXXW motif is essential for the Wnt/β-catenin pathway activation. Apart from this motif, other regions in the carboxy-terminus domain are not utterly conserved among Frizzleds [71].

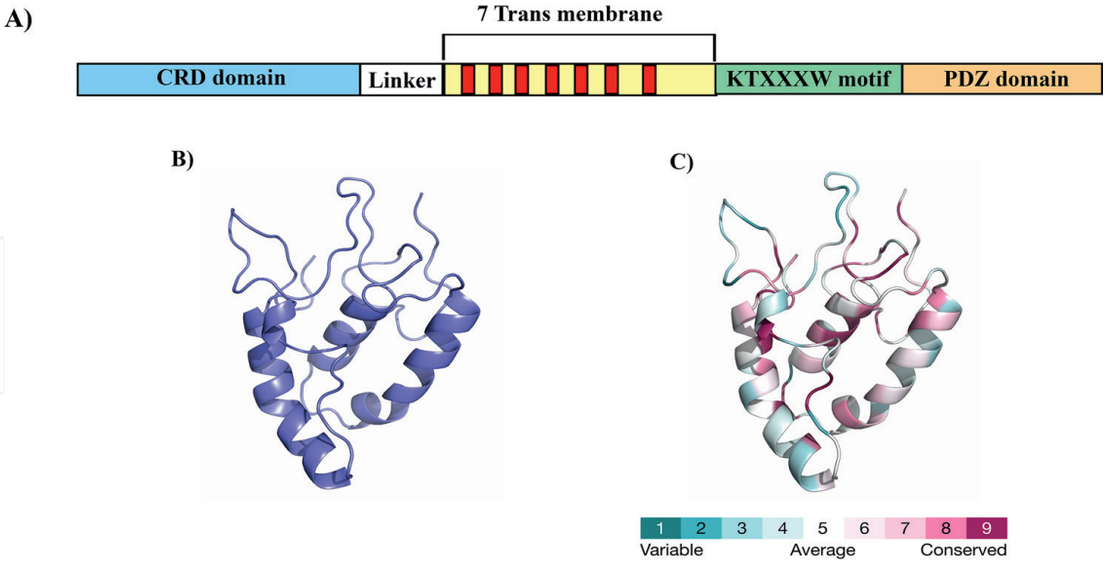

Figure 7.
Schematic view of the frizzled sequence and tertiary structure analysis of the CRD domain. (A) Frizzled protein domains, (B) tertiary structure of frizzled 4's CRD domain (PDB ID: 5CM4), revealing that most of the CRD domain consists of the alpha structure, and (C) conservation analysis of the CRD domain by the ConSurf web server reveals that this domain was highly conserved during evolution.

3.5.2 Role and function

Frizzled proteins play an essential role in the Wnt signaling pathway. This protein family controls various signaling pathways, including PCP, canonical Wnt, and Wnt-calcium signaling, which could be activated by Wnt ligands interacting with Frizzled receptors. Targeted mutation analysis in the Frizzled family illustrates this family's role in a vast range of development and homeostatic processes. This process includes morphogenetic movements responsible for the palate, ventricular septum, ocular furrow, and neural tube closure; the existence of thalamic neurons; osteogenesis; angiogenesis of the central nervous system (CNS); generation and conservation of the blood-brain barrier; and a huge range of procedures that are responsible for cellular, subcellular, and multicellular orientation constructions that are related to the body's orientations [72]. Also, upregulated FZD receptor expression in several cancer malignancies affects patient outcomes (survival and recurrence), followed by activation of the Wnt signaling pathway [73].

3.5.3 Evolution of frizzled

The Frizzled genes were discovered in Drosophila while looking for mutations that could affect the polarity of adult fly epidermal cells. Later, Frizzled proteins were found in various metazoans but not in protozoans. The early metazoans, such as the sponge *Suberites domuncula* and *Hydra vulgaris* have Frizzled genes. *Caenorhabditis elegans* as a roundworm and *Drosophila* have three and four Frizzled genes, respectively. At least 10 Frizzled genes have been identified in vertebrates [71]. Sequence analysis of Frizzled genes in various species shows that around 20–40% of their sequence is identical. Furthermore, genomic organization analysis of Frizzled genes in humans and invertebrates shows that Frizzled genes do not appear to be evolutionarily conserved across a wide range of species, such as the lack of introns region in human FZD1, FZD2, and FZD7 to FZD10, whereas other FZD genes, such as human

FZD5, have one intron like Drosophila Frizzled 2 (Dfz2). Even though the lack of introns in Frizzled genes seems to happen in *Drosophila*, it also occurs in humans. This data shows that Frizzled genes are derived from a common ancestor [71].

4. Conclusion

Though the three Wnt signaling pathways are initiated by the identical initial events of interacting Wnt molecules with Frizzled receptors, various proteins are involved in providing the specificity of function downstream that defines each pathway. The canonical pathway has been broadly studied as a significant factor in animal life, from protists to vertebrates, to achieve tissue homeostasis and development. In contrast, limited knowledge is available on the evolutionary distribution and the molecular evolutionary preservation of the Wnt/PCP and the Wnt/calcium pathway components during evolution.

Author details

Elham Rismani[1], Nasrin Haghighi-Najafabadi[1,2], Babak Elyasi Far[3], Behzad Shahbazi[1] and Ladan Mafakher[4]*

1 Molecular Medicine Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

2 Virology Department, Pasteur Institute of Iran, Tehran, Iran

3 Department of Physiology and Pharmacology, School of Medicine, Dezful University of Medical Sciences, Dezful, Iran

4 Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

*Address all correspondence to: ladan.mafakher@gmail.com

IntechOpen

© 2022 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

References

[1] Srivastava M, et al. The Amphimedon queenslandica genome and the evolution of animal complexity. Nature. 2010;**466**(7307):720-726

[2] Nusse R, Varmus HE. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell. 1982;**31**(1):99-109

[3] Rao TP, Kühl M. An updated overview on Wnt signaling pathways: A prelude for more. Circulation Research. 2010;**106**(12):1798-1806

[4] Janssen R et al. The evolution and expression of panarthropod frizzled genes. Frontiers in Ecology and Evolution. 2015;**3**:96

[5] Nusse R, Clevers H. Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. Cell. 2017;**169**(6):985-999

[6] Perugorria MJ et al. Wnt–β-catenin signalling in liver development, health and disease. Nature Reviews Gastroenterology & Hepatology. 2019;**16**(2):121-136

[7] Kitagawa M et al. An F-box protein, FWD1, mediates ubiquitin-dependent proteolysis of β-catenin. The EMBO Journal. 1999;**18**(9):2401-2410

[8] Aberle H et al. β-Catenin is a target for the ubiquitin–proteasome pathway. The EMBO Journal. 1997;**16**(13):3797-3804

[9] Liu J et al. Wnt/β-catenin signalling: Function, biological mechanisms, and therapeutic opportunities. Signal Transduction and Targeted Therapy. 2022;**7**(1):3

[10] Seifert JRK, Mlodzik M. Frizzled/PCP signalling: A conserved mechanism regulating cell polarity and directed motility. Nature Reviews Genetics. 2007;**8**(2):126-138

[11] Gordon MD, Nusse R. Wnt signaling: Multiple pathways, multiple receptors, and multiple transcription factors. The Journal of Biological Chemistry. 2006;**281**(32):22429-22433

[12] Komiya Y, Habas R. Wnt signal transduction pathways. Organogenesis. 2008;**4**(2):68-75

[13] Sugimura R, Li L. Noncanonical Wnt signaling in vertebrate development, stem cells, and diseases. Birth Defects Research Part C: Embryo Today: Reviews. 2010;**90**(4):243-256

[14] Petersen CP, Reddien PW. Wnt signaling and the polarity of the primary body axis. Cell. 11 Dec 2009;**139**(6):1056-1068

[15] Clevers H, Loh Kyle M, Nusse R. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. Science. 2014;**346**(6205):1248012

[16] Steinhart Z, Angers S. Wnt signaling in development and tissue homeostasis. Development. 2018;**145**(11):dev146589

[17] Bovolenta P et al. Beyond Wnt inhibition: New functions of secreted frizzled-related proteins in development and disease. Journal of Cell Science. 2008;**121**(6):737-746

[18] Gurley KA, Rink JC, Sánchez Alvarado A. Beta-catenin defines head versus tail identity during planarian regeneration and homeostasis. Science. 2008;**319**(5861):323-327

[19] Jin T. Current understanding on role of the Wnt signaling pathway effector TCF7L2 in glucose homeostasis. Endocrine Reviews. 2016;**37**(3):254-277

[20] Lim X, Nusse R. Wnt signaling in skin development, homeostasis, and disease. Cold Spring Harbor Perspectives in Biology. 2013;**5**(2):1-25

[21] Lorzadeh S et al. Autophagy and the Wnt signaling pathway: A focus on Wnt/β-catenin signaling. Biochimica et Biophysica Acta (BBA)-molecular. Cell Research. 2021;**1868**(3):118926

[22] Holstein TW. The evolution of the Wnt pathway. Cold Spring Harbor Perspectives in Biology. 2012;**4**(7):1-17

[23] Bodine PV. Wnt signaling control of bone cell apoptosis. Cell Research. 2008;**18**(2):248-253

[24] Du SJ et al. Identification of distinct classes and functional domains of Wnts through expression of wild-type and chimeric proteins in Xenopus embryos. Molecular and Cellular Biology. 1995;**15**(5):2625-2634

[25] van Ooyen A, Kwee V, Nusse R. The nucleotide sequence of the human int-1 mammary oncogene; evolutionary conservation of coding and non-coding sequences. The EMBO Journal. 1985;**4**(11):2905-2909

[26] Miller JR. The Wnts. Genome Biology. 2002;**3**(1):REVIEWS3001

[27] Janda CY et al. Structural basis of Wnt recognition by frizzled. Science. 2012;**337**(6090):59-64

[28] Mikels AJ, Nusse R. Wnts as ligands: Processing, secretion and reception. Oncogene. 2006;**25**(57):7461-7468

[29] He S et al. Wnt3a: Functions and implications in cancer. Chinese Journal of Cancer. 2015;**34**(3):1-9

[30] Somorjai IM et al. Wnt evolution and function shuffling in liberal and conservative chordate genomes. Genome Biology. 2018;**19**(1):1-17

[31] Miller JR. The wnts. Genome Biology. 2001;**3**(1):1-15

[32] Bazan JF, Janda CY, Garcia KC. Structural architecture and functional evolution of Wnts. Developmental Cell. 2012;**23**(2):227-232

[33] Kawano Y, Kypta R. Secreted antagonists of the Wnt signalling pathway. Journal of Cell Science. 2003;**116**(13):2627-2634

[34] Li X et al. Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. Journal of Biological Chemistry. 2005;**280**(20):19883-19887

[35] Liang C-J et al. SFRPs are biphasic modulators of Wnt-signaling-elicited cancer stem cell properties beyond extracellular control. Cell Reports. 2019;**28**(6):1511-1525. e5

[36] Bu Q et al. The crystal structure of full-length sizzled from Xenopus laevis yields insights into Wnt-antagonistic function of secreted frizzled-related proteins. Journal of Biological Chemistry. 2017;**292**(39):16055-16069

[37] Dann CE et al. Insights into Wnt binding and signalling from the structures of two frizzled cysteine-rich domains. Nature. 2001;**412**(6842):86-90

[38] Leyns L et al. Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. Cell. 1997;**88**(6):747-756

[39] Lin K et al. The cysteine-rich frizzled domain of Frzb-1 is required and sufficient for modulation of Wnt signaling. Proceedings of the National Academy of Sciences. 1997;**94**(21):11196-11200

[40] Wang S et al. Frzb, a secreted protein expressed in the Spemann organizer, binds and inhibits Wnt-8. Cell. 1997;**88**(6):757-766

[41] Chong JM et al. Disulfide bond assignments of secreted frizzled-related protein-1 provide insights about frizzled homology and netrin modules. Journal of Biological Chemistry. 2002;**277**(7):5134-5144

[42] Bányai L, Patthy L. The NTR module: Domains of netrins, secreted frizzled related proteins, and type I procollagen C-proteinase enhancer protein are homologous with tissue inhibitors of metalloproteases. Protein Science. 1999;**8**(8):1636-1642

[43] Fernandez-Catalan C et al. Crystal structure of the complex formed by the membrane type 1-matrix metalloproteinase with the tissue inhibitor of metalloproteinases-2, the soluble progelatinase a receptor. The EMBO Journal. 1998;**17**(17):5238-5248

[44] Muskett FW et al. High resolution structure of the N-terminal domain of tissue inhibitor of metalloproteinases-2 and characterization of its interaction site with matrix metalloproteinase-3. Journal of Biological Chemistry. 1998;**273**(34):21736-21743

[45] Tuuttila A et al. Three-dimensional structure of human tissue inhibitor of metalloproteinases-2 at 2.1 Å resolution. Journal of Molecular Biology. 1998;**284**(4):1133-1140

[46] Semënov MV, He X. Secreted Antagonists/Modulators of Wnt Signaling, in Madame Curie Bioscience Database [Internet]. Landes Bioscience. 2013

[47] Bhat RA et al. Structure–function analysis of secreted frizzled-related protein-1 for its Wnt antagonist function. Journal of Cellular Biochemistry. 2007;**102**(6):1519-1528

[48] Zhong X et al. Regulation of secreted frizzled-related protein-1 by heparin. Journal of Biological Chemistry. 2007;**282**(28):20523-20533

[49] Uren A et al. Secreted frizzled-related protein-1 binds directly to wingless and is a biphasic modulator of Wnt signaling. Journal of Biological Chemistry. 2000;**275**(6):4374-4382

[50] Berndt T, Kumar R. Phosphatonins and the regulation of phosphate homeostasis. Annual Review of Physiology. 2007;**69**:341-359

[51] Berndt T, Kumar R. Novel mechanisms in the regulation of phosphorus homeostasis. Physiology. 2009;**24**(1):17-25

[52] Yan J et al. The evolutionary analysis reveals domain fusion of proteins with frizzled-like CRD domain. Gene. 2014;**533**(1):229-239

[53] Bastin BR et al. Structure, phylogeny, and expression of the frizzled-related gene family in the lophotrochozoan annelid Platynereis dumerilii. EvoDevo. 2015;**6**(1):1-24

[54] Niehrs C. Function and biological roles of the Dickkopf family of Wnt modulators. Oncogene. 2006;**25**(57):7469-7481

[55] Li L et al. Second cysteine-rich domain of Dickkopf-2 activates canonical Wnt signaling pathway via LRP-6 independently of dishevelled. The Journal of Biological Chemistry. 2002;**277**(8):5977-5981

[56] Chen L et al. Structural insight into the mechanisms of Wnt signaling antagonism by Dkk. Journal of Biological Chemistry. 2008;**283**(34):23364-23370

[57] Baetta R, Banfi C. Dkk (Dickkopf) Proteins. Arteriosclerosis, Thrombosis, and Vascular Biology. 2019;**39**(7):1330-1342

[58] Semënov MV et al. Head inducer Dickkopf-1 is a ligand for Wnt coreceptor LRP6. Current Biology. 2001;**11**(12):951-961

[59] González-Sancho JM et al. The Wnt antagonist DICKKOPF-1 gene is a downstream target of β-catenin/TCF and is downregulated in human colon cancer. Oncogene. 2005;**24**(6):1098-1103

[60] Wu W et al. Mutual antagonism between dickkopf1 and dickkopf2 regulates Wnt/beta-catenin signalling. Current Biology. 2000;**10**(24):1611-1614

[61] Guder C et al. An ancient Wnt-Dickkopf antagonism in hydra. Development. 2006;**133**(5):901-911

[62] Singh R et al. LRP6 enhances glucose metabolism by promoting TCF7L2-dependent insulin receptor expression and IGF receptor stabilization in humans. Cell Metabolism. 2013;**17**(2):197-209

[63] Alrefaei AF, Abu-Elmagd M. LRP6 receptor plays essential functions in development and human diseases. Genes. 2022;**13**(1):120

[64] Nie X et al. LRP5 promotes cancer stem cell traits and chemoresistance in colorectal cancer. Journal of Cellular and Molecular Medicine. 2022;**26**(4):1095-1112

[65] Zhang L et al. LRP6 is involved in the proliferation, migration and invasion of trophoblast cells via miR-346. International Journal of Molecular Medicine. 2020;**46**(1):211-223

[66] Ren Q, Chen J, Liu Y. LRP5 and LRP6 in Wnt signaling: Similarity and divergence. Frontiers in Cell and Developmental Biology. 2021;**9**:1-11

[67] Kaur P et al. Membrane targeting of disheveled can bypass the need for arrow/lrp5. Scientific Reports. 2017;**7**(1):1-13

[68] Singh HD, Ma J-X, Takahashi Y. Distinct roles of LRP5 and LRP6 in Wnt signaling regulation in the retina. Biochemical and Biophysical Research Communications. 2021;**545**:8-13

[69] Goswami VG, Patel BD. Recent updates on Wnt signaling modulators: A patent review (2014-2020). Expert Opinion on Therapeutic Patents. 2021;**31**(11):1009-1043

[70] Schulte G, Bryja V. The frizzled family of unconventional G-protein-coupled receptors. Trends in Pharmacological Sciences. 2007;**28**(10):518-525

[71] Huang H-C, Klein PS. The frizzled family: Receptors for multiple signal transduction pathways. Genome Biology. 2004;**5**(7):1-7

[72] Wang Y et al. Frizzled receptors in development and disease. Current Topics in Developmental Biology. 2016;**117**:113-139

[73] Ueno K et al. Frizzled homolog proteins, microRNAs and Wnt signaling in cancer. International Journal of Cancer. 2013;**132**(8):1731-1740