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The Role of DIX Domain Interactions in Downstream Regulation within WNT Signaling Pathway

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Abstract
The Wnt/β-catenin signaling is a highly conserved signaling pathway that controls the organism development and tissue homeostasis. This pathway plays a pivotal role in regulating cell differentiation during the cell's growth. Any abnormal signal transductions in this pathway have been linked to tumor initiation, growth, and malignant transformation. There are three DIX protein domains in this pathway: Disheveled, Axin, and Ccd1. Each serves distinct roles and functions in the initiating and controlling of the downstream Wnt signaling. This paper will review and summarize the effect of formation of homo- or heterodimers with the DIX domain and its role in regulating the inhibition or activation the downstream Wnt signaling. This paper also aims to address the on-going research strategies to identify the formation mechanism of such dimers and characteristics of the interactions between the protein and the DIX domains. In hope such study will pave the research direction for therapeutic approaches to address aberrant Wnt/β-catenin signaling associated with various diseases, including cancer.

Keywords: *Wnt signaling pathway; DIX domain; Disheveled; Axin; Ccd1*

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I. Introduction

The Wnt signaling plays a vital role in early embryonic development, organogenesis, tissue regeneration, and other physiological processes of animal embryos. This pathway, discovered decades ago for its critical role in development, has since emerged as a central player in the broader landscape of physiological regulation. However, its intricate molecular mechanisms, particularly those revolving around the Disheveled (Dvl) and Axin disruption complexes, have remained a subject of intense scrutiny and fascination. Mutations in key protein within this signaling pathway can trigger abnormal signal activation downstream, and create cascading problems, leading to a spectrum of disorders, including cancer, tumorigenesis, and developmental anomalies. At the heart of the Wnt signaling pathway lies a delicate balance between to key protein complexes: the Axin Disruption Complex and the Disheveled (Dvl) Signaling Complex, which exhibit opposing activities critical for Wnt signaling dynamics [1].

The role of Axin Disruption Complex will deactivate and degrade the downstream effector β-catenin protein, while the role of Disheveled (Dvl) Signaling Complex will inhibit Axin protein, resulting in the β-catenin protein stabilization and subsequently transcriptional activation within the nucleus. These complexes are assembled through dynamic head-to-tail polymerization of the DIX domains that are present in either Axin or Dvl, respectively. This will enhance their affinity for signal effectors, the β-catenin protein. It is interesting to point out that Axin itself also utilizes the DIX domain to interact with Dvl, which further complicates the intricate balance of molecular interactions. Moreover, a third protagonist, Coiled-Coiled-Dix (Ccd1) protein in the Wnt pathway featuring DIX domains can interact with both Dvl and Axin to activate the Wnt signaling pathway. However, the precise mechanisms governing the formation of these dimers and the characteristics of protein-DIX domain interactions remain areas of ongoing research.

This paper will systematically explore and review the assembly mechanisms governing homologous or heterologous polymers formation among Dvl, Axin, and Ccd1. The aim is to shed some lights on the intricate interaction entails the underlie Wnt signaling regulation and to unravel the enigmatic complexities of three proteins and the pathway. This prospect not only advances our fundamental understanding of Wnt signal transduction but also opens new avenues for drug research and development, holding promise for the treatment

of various diseases, including those with a prominent Wnt signaling component.

1 Wnt/β-catenin signaling pathway

1.1 Wnt signaling

In 1982, Nusse made a groundbreaking discovery when he identifies the Wnt gene in mouse mammary carcinoma [2]. This gene's activation was found to be dependent on the insertion of a mouse mammary carcinoma-associated viral gene and was named the Int1 gene, denoted as "integration 1". Subsequent studies revealed a critical role played by the Int1 gene in normal mouse embryonic development. Interestingly, it was observed that the introduction of Int1 gene could induce Drosophila to exhibit characteristics reminiscent of the wingless phenotype during their normal embryonic development. Hence the combination of word "Wingless" with "Int1 gene" created a new word of the "Wnt" gene [2].

During delicate embryonic development, the Wnt pathway emerges as an important signaling system that is intricately regulated by the Wnt gene. To date, nearly 100 Wnt genes have been isolated across various organisms, ranging from nematodes and humans. Within the human genome, a total of 19 Wnt genes have been identified, all of which encode for the secretory lipoproteins. These lipoproteins play a fundamental role in controlling cell size, cell-cell interactions, stem cell self-renewal, and tissue patterns during embryonic development [3]. This Wnt gene encodes a secretory glycoprotein of 350 to 400 amino acids with one or more glycosylation sites and 23 to 26 conserved cysteine residues [4]. The Wnt gene functions as the growth-stimulating factor. Unlike other growth factors, the Wnt gene distinguishes itself by allowing growth tissue to target differentiation while inducing cell proliferation. Through its dynamic influence, the Wnt pathway has the remarkable capacity to induce the differentiation of new cells, culminating in the formation of distinct tissues [5].

The Wnt signaling pathways can be generally divided into three types according to their molecular mechanisms: the Wnt/β-catenin signaling pathway, often referred to as the classical Wnt pathway, while the two non-classical Wnt signaling pathway, the Wnt/PCP signaling pathway and Wnt/Ca^{2+} signaling pathway. All three Wnt signaling pathways require the interaction with Frizzled, a transmembrane supracellular receptor essential for binding to the secretory glycoprotein Wnt. Frizzled is a 7-transmembrane protein with a cysteine-rich structural domain (CRD) located at the extracellular N-terminal end, serving as the binding site for Wnt molecules [6].

Activation of the Wnt signaling pathway occurs through the binding of extracellular Wnt ligands to membrane receptors, either through autocrine or paracrine process. This interaction initiates a cascade of events leading to the stable accumulation of β-catenin, followed by its translocation to the nucleus. Within the nucleus, β-catenin can then regulate the gene express, ultimately influencing the critical cellular processes such as proliferation, survival, differentiation, and migration. The intricate orchestration of these molecular events underscores the significance of Wnt signaling in cellular and developmental biology.

The classical Wnt signaling pathway comprises key components crucial for its functionality. These components include the Wnt protein, the transmembrane receptor protein known as Frizzled (Fz), low-density lipoprotein receptor-related protein 5/6 (LRP5/6), dishevelled protein (Dvl), intracellular casein kinase 1 (CK1), scaffolding protein also known as axin (Axin), glycogen synthase kinase 3β (GSK3β), colonic adenomatous polyp protein (APC), and β-catenin. Among these variety proteins, the classical Wnt/β-catenin pathway is activated by specific WNT1, WNT3, WNT3a, WNT7a, and WNT8. This pathway plays a pivotal role in cellular transformation.

1.2 Mechanism of canonical Wnt signaling pathway

The β-catenin protein is a central molecule within the Wnt signaling pathway, exerting direct control over the expression of target genes in the nucleus. It is regarded as the "switch molecule" of the Wnt signaling pathway [7]. When Wnt signal is missing, a series phosphorylation processes take place. First, CK1 phosphorylates Trp45 of β-catenin, followed by GSK3β phosphorylating Ser33, Ser37, and Thr47 of β-catenin. Once fully phosphorylated, β-catenin can be recognized and labeled by E3 ubiquitin ligase (β-TrCP), making it for degradation through the proteasome. This process ensures that the intracellular β-catenin levels remain low, preventing its entry into the nucleus. This marks the Wnt/β-catenin pathway is at the "off" State.

During the phosphorylation of β-catenin, Axin acts as a scaffolding protein and simultaneously interacts with APC, CKI, GSK3β, and β-catenin in the cytoplasm. This results in the formation of a large multi-protein complex known as the Axin disruption complex. Axin's presence in this complex brings GSK3β and β-catenin into spatial proximity, which significantly improves the phosphorylation process. In turn, it would improve β-catenin degradation proceeds.

Upon stimulation by Wnt signaling, the Wnt ligand binds to the Cysteine-Rich Doman (CRD) of the transmembrane receptor Fz and LRP5/6. Such binding triggers a conformational change in the membrane receptor that leads to phosphorylation of LRP5/6 to form the Wnt-Fz-LRP5/6 signaling complex. Phosphorylation of LRP5/6 leads to migration of the Axin destruction complex to the cell membrane near the Fz and LRP receptors. Meanwhile, Dvl in the cytoplasm is also recruited to the subcellular membrane by interacting with Fz. Dvl can phosphorylate GSK3β to detach it from Axin, preventing the formation of Axin destruction complex. Thus, the

degradation of β-catenin is blocked, allowing a large amount of free β-catenin to accumulate in the cytoplasm and enter the nucleus. This signifies activation of the Wnt/β-catenin pathway effectively turned "on".

The entry of β-catenin into the nucleus triggers the activation of downstream components of the Wnt signaling, primarily involving the transcription factors such as Lymphoid Enhancer Factor (LEF) and T-Cell Factor (TCF). LEF/TCF represents a class of transcription factors with bidirectional regulatory functions. In the absence of β-catenin, they form a repressor complex along with the transcriptional repressor Groucho and histonedeacetylase (HDAC), thereby suppressing the expression of Wnt target genes. In contrast, when β-catenin enters the nucleus, it directly replaces Groucho and binds to LEF/TCF instead. This allows LEF/TCF to be repressed and the expression of Wnt target genes [8]. These target genes encompass critical molecules such as oncogene Myc, cell cycle protein D1, and others. The expression of these genes plays an important role in various biological processes, including tumorigenesis, development, and metastasis.

The Wnt signaling pathway is subject to regulations by extracellular proteins, which adds another layer of complexity to its role. Outside the cell, the antagonistic proteins that modulate the Wnt signaling pathway are typically divided into two categories: sFRP (Frizzled Related Protein) and DKK (Dickkopf). The sFRP class includes sFRP family members, WIF1 (WNT Inhibitory Factor 1), and Cerberus. They directly bind to the Wnt ligands, preventing them from binding correctly to the receptor. In sessence, sFRPs and related proteins act as molecular roadblocks, preventing Wnt ligands from engaging with their receptros. On the other hand, the DKK proteins operate by binding directly to the membrane receptor LRP5/6. Such binding disrupts the mutual recognition of Wnt ligands and their receptors [9]. In this manner, DKK proteins function as key players in inhibiting Wnt signaling by intercepting the interaction between Wnt ligands and the receptors LRP5/6.

1.3 Models of Wnt pathway initiation

Currently, there are three prevailing models regarding the initiation of Wnt signals, each offering distinct insights into the intricacies of this critical pathway

The first model, known as the initiation-amplification model, proposes a sequence of events starting with the binding of Dvl to the membrane receptor and interact with Axin [10]. This attracts GSK3β to the vicinity of the membrane where LRP5/6 is phosphorylated. The phosphorylated LRP5/6, in turn, binds to Axin, further leading to form more Axin-GSK3β complexes. This process creates a positive feedback loop and promoting self-phosphorylation process. Additionally, CK1 can also phosphorylate the LRP5/6, and further enhances the interaction between LRP5/6 and GSK3β. This model suggests that the membrane receptors Fz and LRP5/6 play different roles in transmitting Wnt signals: Frizzled is mainly responsible for initiating the signal through Dvl, while LRP5/6 amplifies the extracellular signal through a series of phosphorylation processes.

The second mechanism model, known as the receptor-clustering model [14], suggests that Dvl forms multimers near the membrane through the DIX structural domain, which promotes the polymerization of Wnt receptor Fz and LRP5/6 on the membrane surface. It can significantly increase the local concentration of the protein and enhance the weak interactions between signaling molecules. In turn, it can directly and effectively promote the phosphorylation process of membrane receptors. Schwarz-Romond et al., through their 2007 resolved crystal structure of the Axin-DIX structural domain, showed that Axin-DIX and Dvl-DIX are dynamic multimers, that Axin and Dvl form the characteristic structure of an oligomeric puncta in the cell through the DIX structural domain. This puncta is also dynamic in that it is in the process of constant cytoplasmic protein exchange [11-13]. It is worth mentioning that Bilic et al. found that Fz, LRP5/6, Dvl, Axin, and GSK3β formed large complexes called 'Signalsome' on the cell membrane surface under Wnt-3a stimulation by cellular localization and other methods. This finding provides a model for receptor polymerization a solid experimental basis for the receptor aggregation model [15].

A third mechanism model suggests that the role of Phosphatidylinositol 4,5-bisphosphate (PIP2) in mediating receptor multimerization. In this model, Dvl binds to and activates PI4K IIα and PIP5K I near the cell membrane through its DIX and PDZ structural domains. Activation of PIP5K I generates PIP2, which promotes receptor oligomerization and signals [16].

II. Research progress of the Dvl-DIX domain

The first Dvl gene was discovered and cloned in 1959 by Fahmy in his study of mutants in Drosophila [17]. Dvl proteins constitute a highly conserved class of cytoplasmic proteins, exhibiting variations in sequence and function across the evolutionary spectrum, from lower nematodes and Drosophila to higher mammals. Members of the Dvl protein family include Dvl1, Dvl2, and Dvl3 where Dvl2 accounts for 80%-95% of total Dvl protein expression. Dvl2 is generally considered as the most critical Dvl molecule for signaling. Nevertheless, if Dvl1 and Dvl3 were knocked out, the intracellular Wnt signal transmission would have been greatly affected [18]. Therefore, it is believed that Dvl1 and Dvl3 have unique means to promote the Wnt signal pathway.

Dvl proteins typically contain 600-700 amino acids and are structurally divided into three highly conserved structural domains: an N-terminal DIX domain, a central PDZ domain, and a C- terminal DEP. Dvl acts

as a critical intermediate signaling molecule to activate different Wnt signaling pathways. The DIX and PDZ structural domains are required for Dvl to activate the classical Wnt signaling pathway, while PDZ and DEP activate the non-classical Wnt signaling pathways [19].

On the other hand, the DIX domain contains a ubiquitin-like fold consisting of five β chains (β1-β5) and an α helix. DIX interacts with neighboring molecules through the β bridge between β2 and β4 and forms filaments in crystals through head-to-tail self-interactions of the β bridge. It is noted that purified DIX structural domains self-associate in vitro and gradually polymerize reversibly in a concentration-dependent manner to form fibers. One mechanism of Wnt signaling, namely reversible polymerization signaling, is that the DIX structural domain mediates the formation of a dynamic interaction platform with a high local concentration of transient signaling ligand binding sites [20]. That is, if the binding of a ligand to a protein depends on the polymerization of that protein, and if the polymerization, in turn, depends on the concentration of the protein, the binding between that protein and the ligand will be highly concentration-dependent. Dvl polymers are highly dynamic, and the DIX domain of Dvl2 mediates dynamic polymerization, which is required for the signaling activity of Dvl2 and highlights the potential mechanistic principle of "signaling by reversible polymerization [11]. "Overexpressed Dvl tends to form large polymers in the form of cytoplasmic puncta, whereas biologically active Dvl forms low molecular weight oligomers [21].

III. Research progress of the Axin-DIX domain

Axin is a somatic axis repressor that was discovered in 1997 [22]. Axin proteins are widely present in all types of organisms, from nematodes to humans, and are likewise highly conserved. It is also widely expressed in various tissues and organs of the organism and is actively involved in physiological processes such as growth and development and tumorigenesis. The Axin protein is 862 amino acids in length, with an N-terminal RGS domain, a C-terminal DIX domain, and a GSK3 kinase binding site [23]. Axin plays a dual role in the regulation of Wnt signaling: on the one hand, Axin acts as a scaffolding protein with multiple structural domains to form a β-catenin disruption complex (APC-Axin-GSK-3β), which promotes β-catenin degradation and effectively regulates β-catenin, keeping it at a low concentration in normal cells, thereby inhibiting Wnt signaling. On the other hand, Axin interacts with LRP5/6 and promotes GSK3 recruitment to the plasma membrane to facilitate LRP5/6 phosphorylation and Wnt signaling [24]. Axin binds directly to APC through its N-terminal RGS structural domain, which contains a DIX structural domain at its C-terminus that mediates Axin homodimerization and is also required for the interaction of Axin with Dvl. Two members of the Axin family, Axin (Axin1) and the homolog Axil (Axin-like, also known as Conductin or Axin2), were found to have 44% homology with Axin [22]. Recent studies have shown that Axin 2 has a lower inhibitory activity on Wnt signaling than Axin 1 [25].

In the presence or absence of the Wnt stimulus, excessive degradation of Axin will lead to abnormal activation of the Wnt/β-catenin signaling pathway. In contrast, efficient assembly of the β-catenin disruption complex and β-catenin degradation require homologous aggregation of Axin mediated by head-to-tail aggregation of its C-terminal DIX structural domain[26]. We note that some studies have proposed a model of Axin's self-inhibition, which can be mediated by the interaction between its N- and C-terminal domains, suggesting that self-inhibition is an effective mechanism for Axin to regulate its function in Wnt/ beta-catenin signaling [27]. Overexpressed Axin typically forms highly dynamic cytoplasmic patches during polymerization-mediated processes of its C-terminal DIX structural domain [11] [28]. Compared to Axin2, Axin1 can polymerize more efficiently, leading to the formation of axin spots. The Axin1 RGS domain allowed Dix-mediated polymerization, while the Axin2 RGS domain prevented polymerization [25]

IV. Research progress of the Ccd1-DIX domain

Ccd1 is a new gene identified and isolated from zebrafish embryonic brain tissue in 2003, and its homologous protein is only found in vertebrates and is highly conserved [29]. Structural domain analysis revealed that a Coiled-Coil domain of about 120 amino acids is located at the N-terminal end of Ccd1. In contrast, the DIX domain is located at its C-terminal end, making Ccd1 the third protein in the Wnt signaling pathway containing a DIX domain.

The most crucial structural domain of Ccd1 is the DIX domain, which regulates typical Wnt signaling by interacting with Dishevelled and Axin to form a heterodimeric complex [29]. The DIX domain is involved in the Wnt signaling pathway and in the function of Ccd1 to inhibit JNK signaling. ccd1-DIX binds to Dvl and inhibits Dvl-activated JNK. The DIX and Coiled-Coil domains of Ccd1 synergize to bind Axin and thus block the interaction of Axin with MEKK1 and MEKK4. Therefore, Ccd1 is a promoter of the Wnt signaling pathway and a suppressor of the JNK signaling pathway [30]. Notably, Ccd 1 forms a heterodimeric complex with Dvl through the "head" of Dvl-DIX and the "tail" of Ccd 1DIX to depolymerize Dvl homodimers, thus controlling the size of Dvl multimers [21].

V. Advances in DIX domain research

5.1 Dynamic features of the DIX domain

The DIX structural domain is located at the N-terminal end of Dvl 1/2/3, while the Ccd1 and Axin 1/2 structural domains are located at the C-terminal end. To indicate the distinction, the DIX structural domain of Dvl is referred to as DIX, the DIX structural domain of Axin is referred to as DAX, and the DIX structural domain of Ccd1 is referred to as DIXDC1 in the following. Both DIX and DAX are polymers in vitro. Density gradient centrifugation of DIX expressed in vitro shows that the molecular weight of the polymer increases with the increase of protein concentration, suggesting the deepening degree of DIX's self-polymerization. This also suggests that the self-oligomerization mediated by the DIX domain is not a stable state but a dynamic and reversible process [11-13].

The DIX structural domain mediates the self-association of Dvl and Axin and participates in the interaction between Dvl, Axin, and Ccd1. Although DIX alone cannot interact with DAX in vitro, DIX is critical for the mutual recognition of Dvl and Axin. Dvl and Axin cannot be combined without DIX, but they can still be combined without DAX[31]. In contrast, the interaction between Dvl and Ccd1 is much simpler, and the interaction is carried out directly through heterodimerization of the DIX structural domain[29][30]. In the crystal structure, the DIX structural domains from Axin and Dvl form head-to-tail helical polymers [21][32]. Dvl DIX has been observed to form thin filaments in negative-staining electron microscopy[11].

A structural study showed that the hetero complex has a stronger affinity than the homodimer DAX-DAX (KD = 45 μM) (KD 9 μM and 24 μM for DIX-DAX and DAX-DIX, respectively). The DAX-DIX interface shows two contact sites, one formed by hydrophobic interactions (involving Y 787 and F801 for DAX and Y27) and the other formed by hydrogen bonding between charged residues (involving K789 and K821 for DAX and D23, D24 and T25 for DIX) [1]. However, Wei et al. showed that DIX forms antiparallel double-stranded oligomers in vitro, whereas intracellular Dvl forms oligomers of typically less than ten molecules at the endogenous expression level; DAX can form small single-stranded oligomers through the head-to-tail interface, but is more self-associative than DIX [33]. They found that the affinity of the heterotypic DIX-DAX interaction was the same as that of DIX-DIX. In contrast, the homotypic DAX-DAX interaction was approximately one order of magnitude more substantial than the DIX-DIX or DIX-DAX interaction, contrary to the estimate previously reported by Yamanishi et al. In this way, stronger DAX-DAX homotypic interactions could prevent Dvl from disrupting the degradation complex in the absence of Wnt signaling compared to DIX-DAX; Dvl DIX oligomerization may increase the affinity of Dvl for binding to Axin, which would be required to overcome stronger homotypic DAX-DAX interactions and thus recruit the disrupted complex to the activated receptor [33].

5.2 Dvl and Axin polymerize themselves through the DIX domain

Dvl tends to form distinct cytoplasmic patches through its DIX structural domain, also known as "signal vesicles," that contribute to forming multimers and possible phase separation, containing multiple copies of the receptor complex and its associated cytoplasmic components [34][35]. Wnt-activated Fzd recruits Dvl to the membrane and activates Dvl oligomerization as the first step in signaling vesicle formation[36-38]. FRAP experiments show that they represent highly dynamic proteomes whose formation depends on the DIX structural domain of Dvl, i.e., punctate Dvl coexists with diffuse Dvl and is in rapid exchange, thus undergoing a process similar to phase separation [39][40]. Like Dvl, the DIX domain of Axin is also required for Axin polymer and spot formation [41-43].

The binding affinity between the two DIX structural domains is low (in the moderate micromolar range), and the cellular concentration of Axin is well below the micromolar concentration, which means that monomeric Dvl does not bind properly to Axin in cells [22]. However, it has been shown that the ability of the DIX structural domain of Dvl to dynamically and reversibly self-associate in vitro and in vivo is essential for the signaling activity of Dvl2 and is critical because Dvl2 polymerization generates a fluid interaction platform with a high local concentration of ligand binding sites, thereby increasing the affinity of Dvl2 for its low-affinity ligand [26]. This suggests that when Wnt signals bind to their receptors, it triggers or stimulates DIX-dependent Dvl polymerization, increasing its local concentration and, thus, its binding affinity for Axin. This promotes effective interaction between the two proteins and the recruitment of Axin degradosomes to the receptor complex [22]. This explains the need for Dvl polymerization during Wnt signaling.

5.3 Role of DIX domain in Dvl-Axin mutual recognition

DIX and DAX are essential in mediating the Dvl-Axin interaction, which is required for Wnt/ β-catenin signaling [26] [42][44][45]. Deleting the DIX structural domain would severely affect their binding. However, no direct interaction occurs between DIX alone. Hence, the DIX structural domain's function will likely promote interprotein recognition by mediating its multimerization and significantly increasing the local protein concentration. Meanwhile, besides the DIX structural domain, the C-terminal region of Dvl-DIX and the N-terminal region of Axin-DIX may also play a vital role in the overall recognition process [34][36]. It has been

shown that after Wnt signaling, Dvl self-conjugates through dimerization of its DIX structural and DEP structural domains and is subsequently recruited through co-polymerization of DIX and DAX structural domains into filamentous oligomers [44][46].

Fiedler et al. showed that upon stimulation with Wnt signaling, Dvl interacts with Axin to recruit Axin to the plasma membrane, where Dvl assembles a stable signaling vesicle that stimulates phosphorylation of multiple motifs in the cytoplasm of the LRP6 co-receptor tail [26]. One of these phosphorylated motifs acts as a direct competitive inhibitor of GSK3β, blocking its activity on β-catenin and thus allowing unphosphorylated β-catenin to accumulate in the nucleus and operate the transcriptional switch. They suggest that DIX-dependent aggregation of Dvl may be triggered or stimulated by Wnt-induced dimerization or aggregation of Frizzled receptors with their LRP6 co-receptors [26]. At the same time, the DAX structural domain of Axin also blocks DIX aggregation in vitro and Dvl2 self-assembly in vivo. It attenuates signalosome assembly at the plasma membrane and phosphorylation of the LRP6 cytoplasmic tail.

In addition to using its DIX structural domain to stimulate LRP6 phosphorylation and signalosome assembly to inhibit GSK3β, Dvl could also use this structural domain to interfere with the effector function of Axin by competing with its polymerization. Thus, it is proposed that Dvl could act as a "natural" dominant inhibitor, interfering with the self-assembly of Axin by binding to the polymerization interface of the DIX structural domain in Axin, thereby blocking its effector function [1][26].

5.4 Drug therapy strategies targeting the WNT/β-catenin pathway through the DIX domain

Given the correlation between aberrant Wnt/β-catenin signaling, the pathogenesis of multiple cancers, and the occurrence of Wnt pathway-related gene mutations in many cancers, this pathway has emerged as an important target for cancer drug discovery [47]. The N-terminal DIX structural domain is required for concentration-dependent auto-oligomerization of Dvl, leading to cytoplasmic speckling, and for Axin recruitment to Dvl and proper Wnt signaling. Dvl and Axin oligomerization and the Dvl-Axin oligomeric structure may be potent targets for regulating the Wnt pathway [26][48]. Analysis of the DIX structural domain provides a possible mechanistic basis for the disruption of Axin polymerization by Dvl oligomers and offers the possibility of developing novel small molecules to disrupt the DAX-DIX complex by exploiting the structural properties of the interface and its differences from the DAX-DAX and DIX-DIX homotypic interfaces; Dvl forms a heterodimeric complex through the DIX structural domain and the DAX structural domain of Axin, the hydrophobic interactions and hydrogen bonding and other forces formed at the DAX-DIX contact interface provide a structural basis for the development of small molecule drugs, but the dynamic process of Dvl-Axin interactions needs to be further revealed [47][49].

Investigating these dynamic interactions is complex and generally requires traditional structural biology methods, such as X-ray crystallography and NMR, which are usually time, energy, and money-consuming, and the efficiency is not apparent. However, artificial intelligence has shown great promise in medicine, biology, and pharmaceuticals in recent years, especially in protein design, such as the recent sensational AlphaFold source code in life science and human proteome three-dimensional structure data disclosure, two significant events [50][51]. AlphaFold, for example, has provided structural biologists with a convenient and efficient alternative to crystallography, cryoelectron microscopy, and NMR to uncover the molecular mechanisms by which biological macromolecules function [52]. The field of AI drug development is growing at a rapid pace, although the impact of AI in drug discovery has yet to be evaluated.

VI. Conclusion

The Wnt signaling pathway is closely related to cell genesis, development, growth, and differentiation and controls many life processes. Among currently known cancers, more than a dozen high-incidence carcinomas arise from dysregulation of Wnt signaling [53]. Therefore, studying critical regulatory proteins in Wnt signaling can provide new drug directions for the clinical treatment of tumors. Despite the variety of Wnt/β-catenin-targeted therapies, there are still many challenges, such as off-target effects and toxic side effects, with the continuous development of targeted drugs and combination drug strategies [49]. Therefore, further exploration is needed to identify attractive novel drug molecular targets that inhibit aberrant classical Wnt pathways in tumors without or with minimal adverse effects. Here, the state of research on the interaction mechanism of Disheveled, Axin, and Ccd1 in the Wnt/β-catenin signaling pathway is reviewed and summarized, and we can better understand the additional structural possibilities of dynamic complexes in the Wnt pathway through the DIX domain assembly mechanism itself and the way the DIX identify each other.

In conclusion, the binding events between the DIX structural domains of the three proteins (Axin 1/2, Dishevelled 1/2/3 and Coiled-coil-DIX1) are critical for the mechanism of action downstream of Wnt signaling. Describing protein-protein interactions and specificity is a matter of key importance for understanding the mechanism of action of Wnt signaling. Domains are structural and functional regions of proteins that play important roles during protein interactions. And the study of protein-protein interactions is important not only for elucidating the mechanism of regulation of life activities, but also for disease prevention, diagnosis and drug

design. The analysis of DIX structural domains helps us to better understand the mechanism of action regarding dynamic complexes in the Wnt/β-catenin signaling pathway, such as Dvl-Axin interactions, which can be used to precisely design relevant targeting inhibitors in conjunction with drug studies of protein-protein interactions. Although effective drugs targeting and regulating Wnt signaling through the DIX domain have not been prepared yet, given the critical role of the DIX domain in the Wnt signaling pathway, it is essential to study the structural biology of the DIX domain, especially the interaction between DIX domains. It also can provide some important implications for promoting or inhibiting the Wnt signaling pathway.

References

[1]. Yamanishi K, Fiedler M, Terawaki S, et al. A direct heterotypic interaction between the DIX domains of Dishevelled and Axin mediates signaling to β-catenin[J]. Science signaling, 2019, 12(611): eaaw5505.

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

[3]. Staal F J T, Clevers H C. WNT signalling and haematopoiesis: a WNT–WNT situation[J]. Nature Reviews Immunology, 2005, 5(1): 21-30.

[4]. Coudreuse D, Korswagen H C. The making of Wnt: new insights into Wnt maturation, sorting and secretion[J]. 2007.

[5]. Chen X, Liu H, Li D, et al. Dual Role of Wnt5a in the Progression of Inflammatory Diseases[J]. Chinese Medical Sciences Journal, 2022, 37(3): 265-275.

[6]. Chen C, Struhl G. Wingless transduction by the Frizzled and Frizzled2 proteins of Drosophila[J]. Development, 1999, 126(23): 5441-5452.

[7]. James R G, Conrad W H, Moon R T. β-catenin-independent Wnt pathways: signals, core proteins, and effectors[J]. Wnt signaling: Pathway methods and mammalian models, 2008: 131-144.

[8]. Mosimann C, Hausmann G, Basler K. β-Catenin hits chromatin: regulation of Wnt target gene activation[J]. Nature reviews Molecular cell biology, 2009, 10(4): 276-286.

[9]. KWANOY K. Secreted antagonist of the Wnt signaling pathway[J]. Journal of Cell Science, 2003, 116(13): 2627P2634.

[10]. Zeng X, Huang H, Tamai K, et al. Initiation of Wnt signaling: control of Wnt coreceptor Lrp6 phosphorylation/activation via frizzled, dishevelled and axin functions[J]. 2008.

[11]. Schwarz-Romond T, Fiedler M, Shibata N, et al. The DIX domain of Dishevelled confers Wnt signaling by dynamic polymerization[J]. Nature structural & molecular biology, 2007, 14(6): 484-492.

[12]. Schwarz-Romond T, Merrifield C, Nichols B J, et al. The Wnt signalling effector Dishevelled forms dynamic protein assemblies rather than stable associations with cytoplasmic vesicles[J]. Journal of cell science, 2005, 118(22): 5269-5277.

[13]. Schwarz-Romond T, Metcalfe C, Bienz M. Dynamic recruitment of axin by Dishevelled protein assemblies[J]. Journal of cell science, 2007, 120(14): 2402-2412.

[14]. Zhang B, Liang C, Bates R, et al. Wnt proteins regulate acetylcholine receptor clustering in muscle cells[J]. Molecular brain, 2012, 5(1): 1-8.

[15]. Bilic J, Huang Y L, Davidson G, et al. Wnt induces LRP6 signalosomes and promotes dishevelled-dependent LRP6 phosphorylation[J]. Science, 2007, 316(5831): 1619-1622.

[16]. Zimmermann P, Meerschaert K, Reekmans G, et al. PIP2-PDZ domain binding controls the association of syntenin with the plasma membrane[J]. Molecular cell, 2002, 9(6): 1215-1225.

[17]. Fahmy O G, Fahmy M. New mutants report[J]. Dros. Inf. Serv, 1959, 33: 85.

[18]. Lee Y N, Gao Y, Wang H. Differential mediation of the Wnt canonical pathway by mammalian Dishevelleds-1,-2, and-3[J]. Cellular signalling, 2008, 20(2): 443-452.

[19]. Wallingford J B, Habas R. The developmental biology of Dishevelled: an enigmatic protein governing cell fate and cell polarity[J]. 2005.

[20]. Shibata N, Schwarz-Romond T, Fiedler M, et al. Structural basis of dynamic polymerization of DIX domains: A revised model of Wnt signaling[C]//ACTA CRYSTALLOGRAPHICA A-FOUNDATION AND ADVANCES. 2 ABBEY SQ, CHESTER, CH1 2HU, ENGLAND: INT UNION CRYSTALLOGRAPHY, 2008, 64: C319-C319.

[21]. Liu Y T, Dan Q J, Wang J, et al. Molecular basis of Wnt activation via the DIX domain protein Ccd1[J]. Journal of Biological Chemistry, 2011, 286(10): 8597-8608.

[22]. Zeng L, Fagotto F, Zhang T, et al. The mouse Fused locus encodes Axin, an inhibitor of the Wnt signaling pathway that regulates embryonic axis formation[J]. Cell, 1997, 90(1): 181-192.

[23]. Noutsou M, Duarte A M S, Anvarian Z, et al. Critical scaffolding regions of the tumor suppressor Axin1 are natively unfolded[J]. Journal of molecular biology, 2011, 405(3): 773-786.

[24]. Song X, Wang S, Li L. New insights into the regulation of Axin function in canonical Wnt signaling pathway[J]. Protein & cell, 2014, 5(3): 186-193.

[25]. Bernkopf D B, Brückner M, Hadjihannas M V, et al. An aggregon in conductin/axin2 regulates Wnt/β-catenin signaling and holds potential for cancer therapy[J]. Nature Communications, 2019, 10(1): 4251.

[26]. Fiedler M, Mendoza-Topaz C, Rutherford T J, et al. Dishevelled interacts with the DIX domain polymerization interface of Axin to interfere with its function in down-regulating β-catenin[J]. Proceedings of the national Academy of Sciences, 2011, 108(5): 1937-1942.

[27]. Kim S E, Huang H, Zhao M, et al. Wnt stabilization of β-catenin reveals principles for morphogen receptor-scaffold assemblies[J]. Science, 2013, 340(6134): 867-870.

[28]. Jin L H, Shao Q J, Luo W, et al. Detection of point mutations of the Axin1 gene in colorectal cancers[J]. International journal of cancer, 2003, 107(5): 696-699.

[29]. Shiomi K, Uchida H, Keino-Masu K, et al. Ccd1, a novel protein with a DIX domain, is a positive regulator in the Wnt signaling during zebrafish neural patterning[J]. Current biology, 2003, 13(1): 73-77.

[30]. Wong C K, Luo W, Deng Y, et al. The DIX domain protein coiled-coil-DIX1 inhibits c-Jun N-terminal kinase activation by Axin and dishevelled through distinct mechanisms[J]. Journal of Biological Chemistry, 2004, 279(38): 39366-39373.

[31]. Kishida S, Yamamoto H, Hino S, et al. DIX domains of Dvl and Axin are necessary for protein interactions and their ability to regulate β-catenin stability[J]. Molecular and cellular biology, 1999, 19(6): 4414-4422.

[32]. Madrzak J, Fiedler M, Johnson C M, et al. Ubiquitination of the Dishevelled DIX domain blocks its head-to-tail polymerization[J]. Nature communications, 2015, 6(1): 6718.

[33]. Kan W, Enos M D, Korkmazhan E, et al. Limited dishevelled/Axin oligomerization determines efficiency of Wnt/β-catenin signal transduction[J]. Elife, 2020, 9: e55015.
[34]. Gammons M, Bienz M. Multiprotein complexes governing Wnt signal transduction[J]. Current opinion in cell biology, 2018, 51: 42-49.
[35]. Schaefer K N, Peifer M. Wnt/Beta-catenin signaling regulation and a role for biomolecular condensates[J]. Developmental cell, 2019, 48(4): 429-444.
[36]. Gammons M V, Renko M, Johnson C M, et al. Wnt signalosome assembly by DEP domain swapping of dishevelled[J]. Molecular cell, 2016, 64(1): 92-104.
[37]. Gammons M V, Rutherford T J, Steinhart Z, et al. Essential role of the Dishevelled DEP domain in a Wnt-dependent human-cell-based complementation assay[J]. Journal of cell science, 2016, 129(20): 3892-3902.
[38]. Xiao H, Xiong L, Song X, et al. Angelica sinensis polysaccharides ameliorate stress-induced premature senescence of hematopoietic cell via protecting bone marrow stromal cells from oxidative injuries caused by 5-fluorouracil[J]. International journal of molecular sciences, 2017, 18(11): 2265.
[39]. Chong P A, Forman-Kay J D. Liquid–liquid phase separation in cellular signaling systems[J]. Current opinion in structural biology, 2016, 41: 180-186.
[40]. Dill K, Bromberg S. Molecular driving forces: statistical thermodynamics in biology, chemistry, physics, and nanoscience[M]. Garland Science, 2010.
[41]. Chie S, Lewis T W. Functional Domains of Axin[J]. Journal of Biological Chemistry, 1999, 274(20): 14090-14093.
[42]. Smalley M J, Sara E, Paterson H, et al. Interaction of axin and Dvl-2 proteins regulates Dvl-2-stimulated TCF-dependent transcription[J]. The EMBO journal, 1999, 18(10): 2823-2835.
[43]. Yamanishi K, Sin Y, Terawaki S, et al. High-resolution structure of a Y27W mutant of the Dishevelled2 DIX domain[J]. Acta Crystallographica Section F: Structural Biology Communications, 2019, 75(2): 116-122.
[44]. Cliffe A, Hamada F, Bienz M. A role of Dishevelled in relocating Axin to the plasma membrane during wingless signaling[J]. Current Biology, 2003, 13(11): 960-966.
[45]. Julius M A, Schelbert B, Hsu W, et al. Domains of axin and disheveled required for interaction and function in wnt signaling[J]. Biochemical and biophysical research communications, 2000, 276(3): 1162-1169.
[46]. Bienz M. Signalosome assembly by domains undergoing dynamic head-to-tail polymerization[J]. Trends in biochemical sciences, 2014, 39(10): 487-495.
[47]. Zhang Y, Wang X. Targeting the Wnt/β-catenin signaling pathway in cancer[J]. Journal of hematology & oncology, 2020, 13: 1-16.
[48]. Kan W, Enos M D, Korkmazhan E, et al. Limited dishevelled/Axin oligomerization determines efficiency of Wnt/β-catenin signal transduction[J]. Elife, 2020, 9: e55015.
[49]. Chatterjee A, Paul S, Bisht B, et al. Advances in targeting the WNT/β-catenin signaling pathway in cancer[J]. Drug Discovery Today, 2022, 27(1): 82-101.
[50]. Jumper J, Evans R, Pritzel A, et al. Highly accurate protein structure prediction with AlphaFold[J]. Nature, 2021, 596(7873): 583-589.
[51]. David A, Islam S, Tankhilevich E, et al. The AlphaFold database of protein structures: a biologist's guide[J]. Journal of molecular biology, 2022, 434(2): 167336.
[52]. Nussinov R, Zhang M, Liu Y, et al. AlphaFold, artificial intelligence (AI), and allostery[J]. The Journal of Physical Chemistry B, 2022, 126(34): 6372-6383.
[53]. MacDonald B T, Tamai K, He X. Wnt/β-catenin signaling: components, mechanisms, and diseases[J]. Developmental cell, 2009, 17(1): 9-26.