Wnt Signalling and its Effects in Human – What, How, Where? A Comprehensive Review

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Abstract: *Wnt signalling is a fundamental cellular pathway that governs critical biological processes, including development, stem cell maintenance, and disease progression. This review offers a comprehensive exploration of Wnt signalling, detailing its canonical and non-canonical branches, their distinct molecular mechanisms, and resulting biological outcomes. The historical discovery of Wnt signalling is discussed, providing context for its central role in cellular functions. Advances in the field have identified natural Wnt inhibitors and mechanisms that amplify the pathway, enhancing both specificity and intensity. Beta-catenin stability, regulated by the cytoplasmic APC/Axin complex, plays a key role in modulating canonical signalling. The review further examines the diverse effectors of the Wnt cascade, highlighting their involvement in regulating cellular behaviours across various tissues. In human reproduction, Wnt signals are essential for gametogenesis and early embryonic development. Additionally, the modulation of Wnt signalling has become a vital tool in stem cell biology, with applications in organoid growth and regenerative medicine. However, aberrant Wnt signalling is implicated in several diseases, particularly cancer, where mutations in the Wnt pathway drive tumourigenesis. This review outlines the distinctions between ligand-dependent and ligand-independent mutations in the Wnt pathway and their roles in cancer, focusing on therapeutic strategies targeting these mutations. Furthermore, it explores emerging approaches to target the translational machinery downstream of Wnt signalling, offering potential new avenues for therapeutic intervention in Wnt-associated diseases, including cancer and degenerative disorders.*

Keywords*: Wnt, β-catenin, APC, trophoblast, endometrium, cancer, colon cancer, endometrial cancer, ligand-dependent, Ras, Metastasis*

1. Introduction

Wnt signalling is a complex network of pathways that regulate various cellular processes, including cell growth, differentiation, migration, and fate determination. It is crucial in development, tissue homeostasis, and disease progression. The pathway is activated when Wnt proteins bind to cell surface receptors, particularly the Frizzled (FZD) receptors, which then activate downstream signalling events. Wnt proteins are secreted signalling molecules that mediate cell-cell communication. For their proper function, O-acyltransferase Porcn in the Endoplasmic Reticulum (ER) adds palmitoleic acid to Wnts. The trafficking of Wnt proteins involves several key components: Wntless (WLS), which helps transport Wnts from the ER to the Golgi; GPR177, a sorting receptor regulating Wnt movement to the plasma membrane; and Evi, a transmembrane protein facilitating Wnt trafficking. After post-translational modifications, Wnts are transported to the plasma membrane, where they bind to the Frizzled receptor and LRP5/6 co-receptor, triggering a signalling cascade that stabilizes β-Catenin and activates target gene expression in the nucleus. There are two types of Wnt Signalling – Canonical and Non-Canonical. The Non-Canonical Wnt Signalling pathway is less studied than Canonical Wnt signalling. Canonical Wnt signalling, also known as the β-Catenin pathway, regulates cell proliferation and migration. This pathway influences genes like c-myc, cyclin D1, and CD44, and feedback mechanisms regulate its activity, with inhibitors like sFRPs and Dkk controlling Wnt signalling. The other type of Wnt Signalling is Non-Canonical Wnt Signalling that is lesser studied than the previous type which includes diverse receptors and downstream effectors and the key point here is, this type does not depend on β-Catenin. The Wnt signalling pathway was first linked to developmental defects in Drosophila in the 1930s with the discovery of the "glazed" eye mutant[1].

Wnt signalling is regulated by extracellular proteins that antagonize Wnt ligands. Notum, a carboxylesterase, removes palmitoleate from Wnt proteins, disrupting their signalling. Other Wnt antagonists include Dickkopf (DKK) and Sclerostin/SOST proteins, which bind to LRP5/6 and prevent Wnt-FZD-LRP6 dimerization. Secreted FZD-related proteins (sFRPs) and Wnt inhibitory factor (WIF) proteins directly bind to Wnt ligands, inhibiting signalling. Wnt signalling regulates stem cell behaviour, including in embryonic and adult stem cells, such as those in the intestine, hair follicles, and hematopoietic system. Wnt target genes like Lgr5 and Axin2 are used to trace and identify adult stem cells in different tissues. Mutations in key components like β-catenin, APC, and AXIN lead to stabilized β-catenin and aberrant Wnt signalling, driving tumorigenesis in various cancers. Targeted therapies for cancers driven by dysregulated Wnt signalling focus on inhibiting Wnt ligands, blocking receptor interactions, or targeting β-catenin and its downstream components. Porcupine inhibitors block Wnt ligand secretion, while Frizzled receptor blockers prevent Wnt binding. Anti-RSPO3 antibodies target R-spondin in cancers with RSPO mutations. For β-

catenin, therapies aim to disrupt its transcriptional activity, inhibit interactions with co-factors like BCL9/9L and Pygo, or stabilize AXIN using Tankyrase inhibitors.

These approaches offer personalized treatment options, particularly for cancers with specific Wnt pathway mutations, and are under ongoing clinical investigation.

2.WNT Signalling Pathway

Wnt proteins are such secreted proteins that mediates cell-cell communications and in this kind of cells, O-acyltransferase porcupine or Porcn is needed that does lipid modification of Wnts with palmitoleic acids in Endoplasmic Reticulum (ER). This pathway depends on multipass transmembrane and putative sorting receptor. This receptor has – 1) Wntless (WLS) – It interacts with Wnt protein and facilitate their transport from ER to golgi, 2) GPR177 – It is a putative sorting receptor that interacts with Wnt proteins and regulates their trafficking to the plasma membrane, 3) Evi – It is a transmembrane protein that interacts with Wnt and regulates their trafficking to plasma membrane. Thus, all these helps in the whole transport of Wnt proteins that include – Vesicular transport, Sort and packaging, Receptor mediated endocytosis and through all these Wnt proteins are transported from ER to golgi and then to plasma membrane and then they can be internalized by cells through receptor mediated endocytosis.

Wnt trafficking to the plasma membrane involves several steps like: Primarily Wnt proteins are synthesized in ER and then they undergo post-translational modifications like glycosylation and palmitoylation. Then these modified Wnt proteins are transported from ER to the Golgi apparatus where they are sorted into vesicles for transport into the plasma membrane. Then Wnt ligands bind to Frizzled receptor & LRP5/6 co-receptor. Phosphorylation of LR6 happens by GSK3 & CK1 and this phosphorylated LP6 disrupts the destruction complex and inhibits GSK 3 and CK1. By this, β-Catenin is stabilized and gets accumulated into cytoplasm and then translocated into nucleus where it interrupts with TCF/LEF factors and thus transcription of the target gene is activated and gene expression is regulated. That’s how the Wnt Signalling by ligand binding takes place.

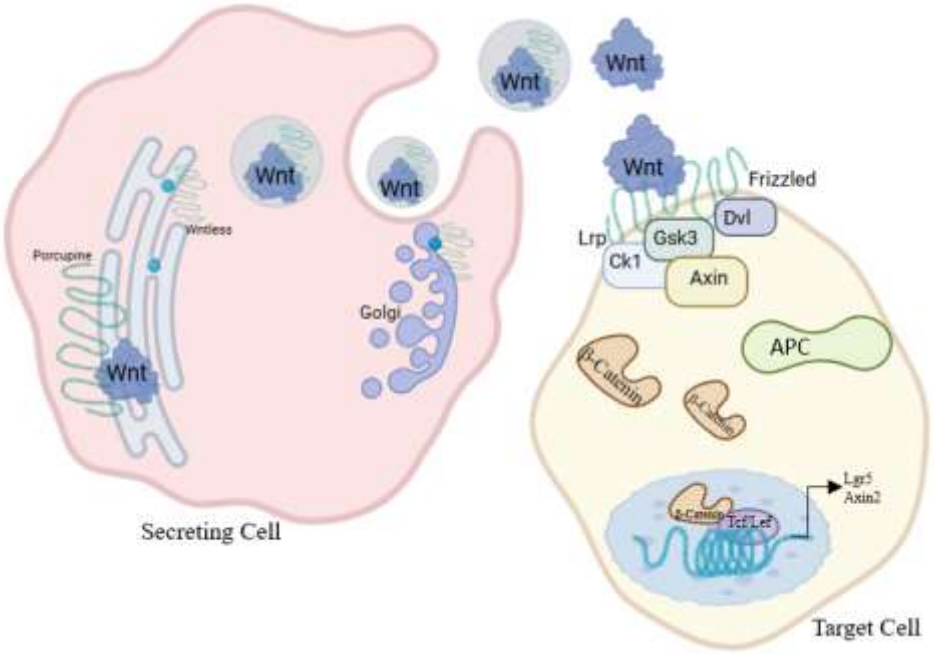

Fig 1: Summary of Wnt Signalling: A simplified model of Wnt signalling where the Wnt secreting cell is in pink (left) and the Wnt/Beta-catenin responsive cell are in yellow (right) and this features primary pathway components. See full text above for details. APC, adenomatous polyposis coli; Ck1, Casein kinase 1; Dvl, Dishevelled; Gsk3, Glycogen synthase kinase 3; Lgr5, Leucine-rich repeat-containing G-protein-coupled receptor 5; Tcf/Lef, T-cell factor/lymphoid enhancer factor. Created with BioRender.com.

There are two types of Wnt Signalling pathway– Canonical and Non-Canonical Wnt signalling.

2.1. Canonical WNT Signalling: Canonical Wnt Signalling is a type of Wnt signalling pathway which is also known as β-Catenin pathway as the key component of this pathway is β-Catenin. β-Catenin generally stays in adherens junctions in unstimulated cells where it maintains epithelial integrity as it binds to E-cadherin and α-catenin. This signalling has two phases – 1) Off-State – where excess Wnt ligands are absent and then cytoplasmic β-Catenin binds to both Adenomatous Polyposis Coli (APC) and Axin and facilitates the phosphorylation of the protein by Casein Kinase Iα (CKIα) +and glycogen synthase kinase 3β (GSK 3β). Due to this there happens the degradation of β-Catenin by β-TrCP (β-transduction repeat containing protein) mediated ubiquitin/proteasome pathway that lowers the cytosolic levels. 2) On-State – When a Wnt ligand binds to the cysteine-rich domain (CRD) of FZD (Frizzled Receptors), that promotes FZD-LRP heterodimerisation that ultimately triggers a series of events that causes the disruption of Axin/APC/GSK-3β/CK1α destruction complex. When Wnt stimulation happens that induces recruitment of Dishevelled (Dsh) to the FZD receptor that forms a signalosome. catalysed by CK1γ and FZD co-receptor gets a GSK-3β-dependent phosphorylation. Then there happens sequestration of Axin of the destruction complex causing

activation of Dsh that ultimately results in impaired degradation and accumulation of β-Catenin in cytosol.

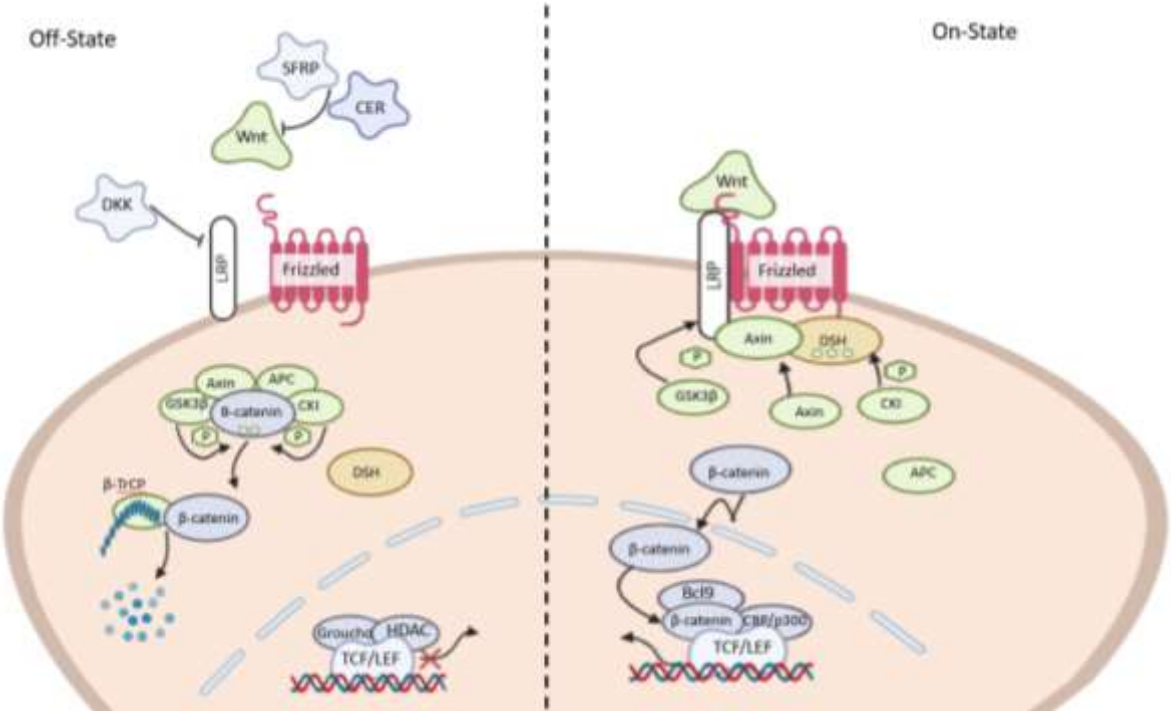

Fig 2: The Canonical Wnt/β-Catenin Pathway: In the absence of a Wnt ligand, the canonical Wnt/β-catenin pathway is in the "off-state". In this state, β-catenin is bound to a multiprotein degradation complex, which contains the scaffold protein Axin, the tumour suppressor gene product APC, as well as the kinases CK1 and GSK-3β, among others. Upon phosphorylation, β-catenin is ubiquitinated by the β-TrCP-E3-ligase complex and subsequently degraded by the proteasomes. When a Wnt ligand binds to the FZD and LRP-5/6 co-receptors, the pathway switches to the "on-state". This leads to the translocation of Axin to the plasma membrane through direct interaction with LRP-5/6 and Dsh/FZD. As a result, β-catenin is released from the multiprotein complex, accumulates in the cytoplasm in a non-phosphorylated form, and subsequently translocate into the nucleus. Here, β-catenin promotes transcription of Wnt target genes upon binding to TCF/LEF transcription factors. Created with BioRender.com.

Axin being a key negative regulator of β-Catenin stability gets translocated through the cytoplasmic tail of LRP that gets This β-Catenin is active and gets translocated into the nucleus and there it functions as co-regulator. Due to this the transcriptional inhibitors of Groucho protein family and histone deacetylases (HDACs) from T-Cell specific factors (TCFs)/ lymphoid enhancer binding factor 1 (LEF-1) gets displaced and that recruit histone acetylases, the legless family docking proteins (Bcl9) and thus the CBP/p300 converts TFC/LEF-1 into transcriptional activators. Axin, APC and other Wnt components enters the nucleus and thus modulates nuclear trafficking and transcription. APC here plays an important role in the exchange of co-activator and co-repressor complexes at Wnt target genes. Here several genes

like c-myc, c-jun, cyclin D1, CD44, etc, gets involved in cell proliferation and migration. Also, TCF/ β-Catenin dependent expression of Wnt pathway components indicates that feedback control is also a characteristic of Wnt signalling and Wnt signalling can be negatively affected by endogenous β-Catenin inhibitors (ICAT) or by soluble inhibitors like – secreted frizzled related protein (sFRPs), members of Dickkopf (Dkk) family.

2.2. Non-Canonical Wnt Signalling: Non-Canonical Wnt signalling is less studied than canonical Wnt signalling due to the presence of diverse receptors and downstream effectors who are the key components involved in this signalling process. Here β-Catenin is not the key component as this pathway does not operate through β-Catenin and this kind of Wnt signalling may also inhibit nuclear β-Catenin activity. There are two types of Non-Canonical Wnt Signalling – Wnt/planar cell polarity (PCP) and Wnt/Ca^{2+} and these are stimulated by non-canonical Wnt ligands like Wnt5a or Wnt11 and the signalling process is transduced by the binding of FZD and that causes activation of Dsh without the requirement of LRP as co-receptor.

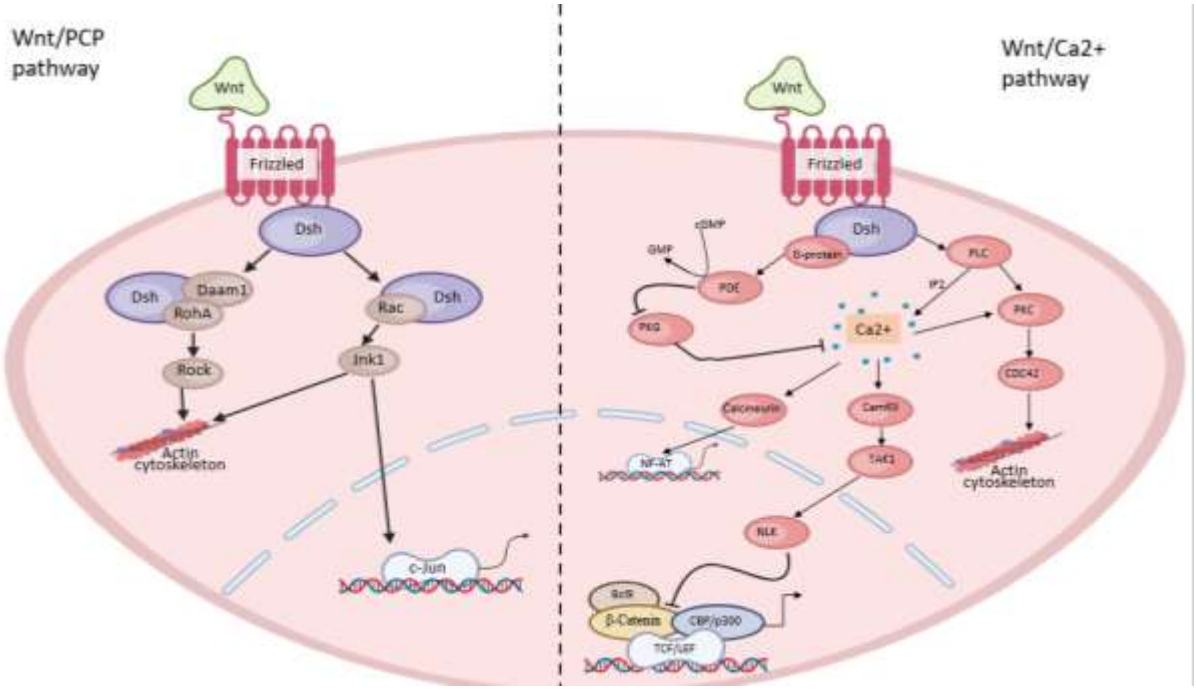

Fig 3: The non-canonical Wnt pathways, including the Wnt/PCP and Wnt/Ca2+ pathways, play crucial roles in regulating cellular processes.Wnt/PCP Pathway: This pathway is distinguished by the asymmetric distribution of Frizzled (FZD) receptors, leading to the establishment of cell polarity. The Wnt/PCP pathway activates RhoA/Rock GTPases and JNK through the actions of Dishevelled (Dsh) and DAAM1. Wnt/Ca2+ Pathway: The Wnt/Ca2+ pathway is triggered by the interaction between Wnts and Frizzled receptors, resulting in an increase in intracellular calcium levels. This rise in calcium activates a cascade of downstream effectors, including calcineurin, CAMKI, and PKC. Created with BioRender.com.

Wnt/PCP pathway: After stimulation of the pathway by Wnt ligands and binding of FZD and activation of Dsh, it activates GTPases RhoA and Rac and their downstream targets – ROCK (Rho Associated Kinase) and JNK respectively. This pathway plays a crucial role in

developmental processes like angiogenesis, bone morphogenesis or convergent extension movements of mesenchymal cells during gastrulation and this was first identified in Drosophila and it is also studied that non-canonical Wnt Ligands such as Wnt5a may also act in a canonical manner when binds to FZD4 which suggests that the effects of Wnt stimulation depends on cellular receptors.

Wnt/Ca^{2+} Pathway: This pathway first came in notice by the fact that the stimulation by non-canonical ligands intracellular Ca^{2+} gets released from Endoplasmic reticulum. This kind of Ca^{2+} increase is Wnt dependent and this process is achieved either by inhibition of cGMP-dependent protein kinase (PKG) which blocks Ca^{2+} release in unstimulated cells or by activation of PLC (Phospholipase C) that causes elevation of inositol-1,4,5-triphosphate (IP3). Due to this intracellular Ca^{2+} activates Protein Kinase C (PKC), Calcium/Calmodulin dependent kinase II (CamKII) and calcineurin. Due to this, CamKII got activated and thus it activates TGF-β-activated kinase (TAKI) and Nemo-like Kinase (NEMO). This can antagonize Wnt/ β-Catenin signalling by phosphorylation & inactivation of TCF. Nuclear localization sequences of nuclear factor of activated T-cells (NF-AT) is unmasked by the protein phosphatase which causes NF-AT to enter into the nucleus and activates gene expression. This pathway controls the dorsoventral axis formation in Xenopus embryos.

Other types of Non-canonical Wnt Pathway: Wnt ligands can signal through receptor tyrosine kinase Ryk and receptor tyrosine kinase-like orphan receptor (Ror) receptors by binding to their extracellular WIF and CRD domains. Activation of Ryk triggers multiple signalling pathways, including the canonical TCF/β-catenin pathway, Src family kinase activation, and nuclear translocation of the Ryk intracellular domain. Ryk plays a crucial role in neuronal development, regulating axon guidance, neurite outgrowth, and synaptogenesis. Ror activation by Wnt5a leads to polarized cell movement, involving Dsh and JNK signalling pathways. Ror-2 deficient mice exhibit similar abnormalities as Wnt5a mutant mice, highlighting the importance of non-canonical Wnt pathways like Wnt/PCP. Wnt ligands like Wnt3a and Wnt16b can activate alternative β-catenin-independent signalling cascades, such as ERK or PI3K/AKT pathways, demonstrating the complexity of the Wnt signalling network.

3. Discovery of Wnt Signalling

The Wnt-related phenotype was first discovered in 1936 by Thomas Hunt Morgan[1] and colleagues at Caltech where they discovered a Drosophila mutant with glazed eyes. After a few years, in India, scientists discovered wingless flies and the hypomorphic allele was named accordingly[2] (Sharma, 1973; Sharma and Chopra, 1976). Later it was revealed that glazed is a gain-of-function allele of wingless that was caused by a retrotransposon insertion. The advent of straightforward genetic screens in Drosophila enabled systematic searches for mutations causing developmental phenotypes. In 1980, a wingless (wg) null allele, later recognized as Nobel Prize-winning, was rediscovered among genes causing lethal segmentation defects in developing fly embryos. Specifically, wg belongs to a subclass of genes affecting polarity[3] within individual body segments (Nüsslein-Volhard and Wieschaus, 1980). Over the following decade, more refined genetic screens identified additional segment polarity mutants, including

arrow (Lrp), armadillo (β-catenin), dishevelled (Dvl), porcupine (Porcn), and shaggy/zeste-white 3 (Gsk3) (Perrimon and Mahowald, 1987; Wieschaus and Riggleman, 1987; Wieschaus et al., 1984). Like wg, these mutations disrupted embryonic patterning, but their interconnections—and their link to a mammary tumour oncogene in mice—were initially unclear. Parallel research on the mouse int1 oncogene[1], linked to mammary tumours (Nusse and Varmus, 1982), traced its origins back to the 1930s, when Morgan and colleagues described the glazed mutant in flies and other researchers investigated a hereditary "milk factor" in mammary gland carcinoma (Bittner et al., 1945; Korteweg, 1936). Unbeknownst to them, these tumours arose from integration of the mouse mammary tumour virus (MMTV) near int1, a mechanism resembling the transposon-driven activation of wg in the glazed mutant. It wasn't until 1987 that int1 was identified as the mouse homolog of Drosophila wg (Cabrera et al., 1987; Rijsewijk et al., 1987). Following the discovery of related genes in mice (Gavin et al., 1990), the Wnt gene family (short for wingless-type MMTV integration site) was established (Nusse et al., 1991). However, identifying one gene was just the beginning.

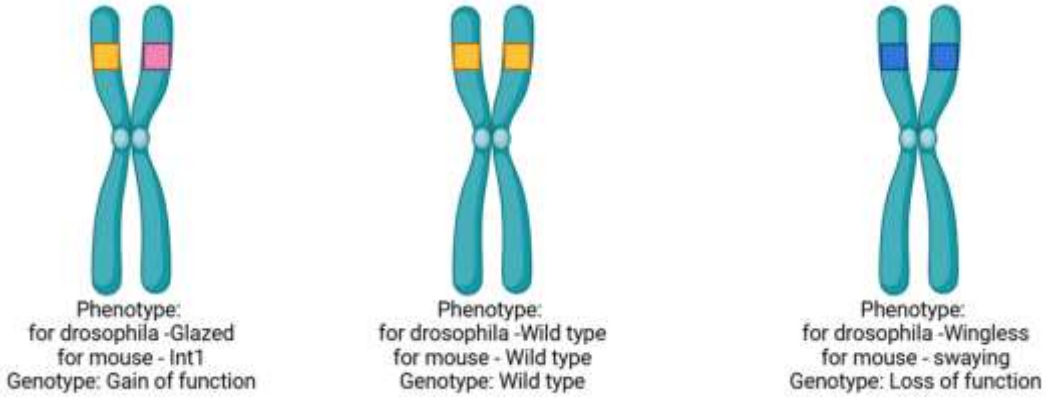

Fig 4: From phenotype to genotype in flies and mice. Gain- and loss-of- function alleles of Wnt were independently discovered based on diferent phenotypes in flies and mice. Interesting parallels can be observed in these vastly different model organisms. The wild-type allele is depicted in orange. Gain of-function mutations are shown in pink, whereas loss- of-function mutations are shown in blue. Created with BioRender.com.

Elucidating the entire signalling cascade required detailed analysis of functional dependencies and relationships between genes. While much biochemical knowledge emerged from other model systems, core interactions in the Wnt pathway were first mapped through epistasis analyses. These experiments, which compare the phenotypes of double mutants to single mutants[4], are a powerful tool in developmental biology (Fig. 3). For instance, to determine the role of the Gsk3 homolog Zw3/Sgg in the Wnt pathway, researchers examined Engrailed (En) expression in wg single mutants, zw3 single mutants, and wg/zw3 double mutants in Drosophila. En expression was destabilized in wg mutants (suggesting wg is a positive regulator), while zw3 mutants showed enhanced En expression (suggesting Zw3 is a negative regulator). Double-mutant phenotypes resembled those of zw3 single mutants, demonstrating that wg acts upstream of Zw3 to antagonize its repression of En (Siegfried et al., 1992). Further investigations into segment polarity mutants in transgenic wg fly strains revealed additional essential components, such as Dsh (vertebrate Dvl) and Arm (vertebrate β-catenin), placing Arm downstream of wg and Zw3, with wg positively and Zw3 negatively

regulating Arm abundance (Oosterweel et al., 1994; Peifer et al., 1994). Today, the Wnt pathway is recognized as a complex network of ligands, co-receptors, effectors, and feedback regulators, with its intricacy vastly expanded since these initial studies. High-throughput genome-wide screenings now allow the discovery[5] of novel genetic and functional interactions (DasGupta et al., 2005; Lebensohn et al., 2016). Yet, the foundational invertebrate genetic analyses laid a crucial blueprint for understanding one of the most essential developmental signalling cascades in the animal kingdom.

4. Natural Wnt Inhibitors

Wnt activity is controlled by extracellular proteins who antagonizes the ligand. For example, Notum is a carboxylesterase[6], that was discovered in Drosophila as an enzyme, can remove palmitoleate modification of Wnt and this palmitoleate is essential for signalling and participates during the binding of Wnt to FZD. Notum has a hydrophobic pocket in its structure where there happens accumulation of palmitoleate moiety. Hydrolysis of this palmitoleate by Notum may produce a Wnt protein outside of the cells that acts there as a dominant interfering molecule. Other Wnt antagonists include proteins from the Dickkopf (DKK) and Sclerostin/SOST families (Cruciat and Niehrs, 2013). These proteins inhibit Wnt signalling by binding to LRP5/6, potentially disrupting the dimerization of Wnt-induced FZD-LRP6 (Cruciat and Niehrs, 2013). Additionally, secreted FZD-related proteins (sFRPs) and Wnt inhibitory factor (WIF) proteins interfere with Wnt signalling by directly binding to Wnt ligands. Collectively, these findings depict a complex extracellular environment composed of Wnt-modifying and Wnt-binding factors that fine-tune the signalling intensity (Niehrs, 2012).

Two highly homologous Wnt target genes, Rnf43 and Znrf3, were recently identified as potent negative-feedback regulators of Wnt signalling[7]. Initially recognized as markers specific to Wnt-dependent Lgr5 stem cells in intestinal crypts (Koo et al., 2012) and enriched in colon cancer cells with activating Wnt pathway mutations (Hao et al., 2012), these proteins belong to a family of single-pass transmembrane E3 ligases featuring intracellular RING domains. Similar to the founding family member Grail, Rnf43 and Znrf3 mediate multi-ubiquitination of lysines within the cytoplasmic loops of the 7TM domain of FZDs (Figure 2) (Hao et al., 2012; Koo et al., 2012). This triggers rapid endocytosis and lysosomal degradation of Wnt receptors. Likewise, the orthologous E3 ligase PLR-1 in C. elegans reduces FZD surface expression (Moffat et al., 2014). While the structural basis for how these E3 ligases recognize FZDs as specific substrates remains unclear, DVL proteins are hypothesized to act as intermediaries in the recognition process (Jiang et al., 2015). The loss of Rnf43 and Znrf3 is expected to increase cellular responsiveness to endogenous Wnt signals. Indeed, the co-deletion of these two Wnt modulators in murine intestinal epithelium leads to an adenomatous expansion of crypts, which can be reversed by treatment with small-molecule inhibitors targeting the Porcupine enzyme required for Wnt lipid modification (Koo et al., 2015). Mutations in Rnf43 and Znrf3 have also been observed in various human cancers, rendering malignant cells dependent on significantly lower levels of Wnt signalling than normal cells and particularly sensitive to inhibition at the Wnt receptor-ligand interface.

5. Amplification of Wnt Signals

The vertebrate genome encodes four secreted R-spondin proteins, each characterized by two N-terminal furin domains and a thrombospondin domain. Initially identified by Kazanskaya et al. (2004) as enhancers of Wnt Signalling in Xenopus embryos, R-spondin-1 was later shown to robustly stimulate[8] Wnt-dependent intestinal crypt proliferation both in vivo (Kim et al., 2005) and in vitro (Sato et al., 2009). Three members of a small family of seven-transmembrane (7-TM) receptors—Lgr4, Lgr5, and Lgr6—bind R-spondins with high affinity and are critical for amplifying Wnt Signalling at low doses (Carmon et al., 2011; de Lau et al., 2011; Glinka et al., 2011). These Lgr proteins interact with R-spondins via their N-terminal ectodomains and do not appear to rely on G-proteins for their Signalling (Carmon et al., 2011; de Lau et al., 2011).

The prototypical Lgr family member, Lgr5, is known to mark adult stem cells in several actively self-renewing tissues, particularly in the intestine (Barker et al., 2007). A significant genetic interaction between Lgr4 and Lgr5 has been identified, which is essential for maintaining Wnt signal strength in intestinal crypts of double mutant mice (de Lau et al., 2011). Recent studies have shown that while Wnts alone are insufficient to drive self-renewal of Lgr5-positive stem cells, they facilitate this process by maintaining R-spondin receptor expression, ultimately promoting stem cell expansion (Yan et al., 2017). Similarly, Lgr6 marks stem cells in the skin (Füllgrabe et al., 2015; Snippert et al., 2010). Thus, the role of Lgr proteins as R-spondin receptors underscores the close relationship between Wnt Signalling and adult stem cell biology. The mechanism by which R-spondins and Lgrs amplify Wnt Signalling was elucidated by Hao et al. (2012) through biochemical studies. They demonstrated that R-spondins, in an Lgr-dependent manner, counteract the Rnf43/Znrf3-mediated removal of Wnt receptors from the membrane. A weak but specific interaction between R-spondins and Znrf3 was observed. X-ray crystallography studies (de Lau et al., 2014) further refined this model, revealing that R-spondin’s high-affinity binding to Lgr5 via its Furin-2 domain allows its other Furin domain to interact with Rnf43/Znrf3. This interaction inhibits the E3 ligases, preserving Wnt/FZD/LRP receptor complexes on the plasma membrane, thereby enhancing Wnt Signalling strength and duration.

Although homologs of Rnf43/Znrf3 are present in invertebrates, the R-spondin/Lgr5/Rnf43 module represents a relatively recent evolutionary adaptation found exclusively in vertebrates. This mechanism is largely dedicated to adult stem cell function. The emergence of this novel Wnt signal amplification strategy likely played a key role in the evolution of more complex stem cell and transit-amplifying cell compartments, aligning with the increase in vertebrate body size and complexity.

6. β-Catenin Stability

The canonical Wnt pathway is primarily regulated by the cytoplasmic protein β-catenin, as shown in Figure 4. β-catenin's stability is managed by the destruction complex (DC), where Axin, a tumour suppressor protein, acts as a scaffold. Axin interacts with β-catenin, another

tumour suppressor protein (APC), and two constitutively active serine-threonine kinases (CK1a/8 and GSK3a/β). APC, a large protein, contains three Axin-binding motifs interspersed among 15- and 20-amino acid repeats that bind β-catenin. Studies on colorectal cancer have confirmed APC's critical role in DC function, although its exact molecular mechanism remains only partially understood (Figure 4). In the absence of Fz/LRP receptor activation by ligands, CK1 and GSK3 sequentially phosphorylate Axin-bβound β-catenin at specific N-terminal Ser/Thr residues. CK1 initially phosphorylates β-catenin at Ser45, followed by GSK3-mediated phosphorylation at Thr41, Ser37, and Ser33 (Liu et al., 2002). The resulting phosphorylated "degron" motif serves as a docking site for the F-box-containing E3 ubiquitin ligase β-TrCP, leading to ubiquitination and subsequent proteasomal degradation of β-catenin (Aberle et al., 1997; Kitagawa et al., 1999) (Figure 4). When Wnt ligands engage the Fz/LRP receptors, the destruction complex relocates to the cell membrane, disrupting its activity and causing a rapid increase in free β-catenin levels. Simultaneously, phosphorylated LRP receptors may inhibit GSK3 directly, stabilizing β-catenin (Stamos et al., 2014). Biochemical analysis of the endogenous DC has shown that phosphorylated β-catenin ubiquitination is inhibited within the intact complex. Consequently, the complex becomes saturated with phosphorylated β-catenin, allowing newly synthesized β-catenin to accumulate, translocate to the nucleus, and activate target genes (Lij et al., 2012; Azzolin et al., 2014).

An alternative model suggests that Wnt receptor activation dynamically regulates β-catenin phosphorylation (Hernández et al., 2012) and promotes Axin-complex disassembly (Kim et al., 2013). A highly conserved regulatory domain in APC, known as the β-catenin inhibitory domain (CID), is located between the second and third 20-amino acid repeats (Kohler et al., 2009; Roberts et al., 2011). This domain is crucial for reducing β-catenin levels and suppressing Wnt transcriptional activity. Notably, the CID corresponds to the mutation cluster region where APC truncations frequently occur in cancer. The CID is thought to enhance β-catenin ubiquitination by stabilizing its interaction with APC and to suppress β-catenin/TCF transcription in the nucleus (Choi et al., 2013). Another model proposes that GSK3 phosphorylation near the CID region induces a conformational change in APC, facilitating access of the E3 ligase to phosphorylated β-catenin (Pronobis et al., 2015). The analysis of β-catenin's roles is further complicated by its second major function in epithelial tissues. β-catenin serves as a critical binding partner for the cytoplasmic tails of cadherins, such as E-cadherin, in adhesion junctions (Peifer et al., 1992). While the signalling pool of β-catenin has a half-life of only a few minutes, the adherens junction pool is highly stable. These adhesive and signalling functions of β-catenin are likely independent. In C. elegans, these roles are carried out by distinct homologs of β-catenin (Korswagen et al., 2000).

7. Effectors of Wnt Cascade

Canonical Wnt signalling induces a specific cellular response by activating β-catenin/TCF target genes (Figure 4). When the Wnt pathway is activated, β-catenin accumulates in the cytoplasm and nucleus, where it interacts with DNA-bound TCF transcription factors (Behrens et al., 1996; Molenaar et al., 1996). The recognized TCF binding motif is 5'-AGATCAAAGG-3' (van de Wetering et al., 1997). Widely used Wnt/TCF reporters, such as pTOPflash, contain

multiple copies of this motif (Korinek et al., 1997). In the Wnt "off" state, TCFs associate with Groucho proteins to mediate transcriptional repression (Cavallo et al., 1998; Roose et al., 1998). Conversely, in the Wnt "on" state, β-catenin binds TCFs, temporarily transforming them into transcriptional activators. While many Wnt target genes are specific to particular cell types and developmental stages, the Axin2 gene serves as a universal transcriptional target and is commonly used as a marker for canonical Wnt pathway activity (Lustig et al., 2002).

Active Wnt signalling can lead to an increase in overall β-catenin levels, even without noticeable nuclear accumulation. It has been proposed that relative changes in β-catenin levels, rather than absolute amounts, are critical for target gene activation. This suggests that low nuclear β-catenin levels may suffice for gene activation (Goentoro and Kirschner, 2009). While alternative transcription factors have been suggested as non-TCF effectors of β-catenin, these findings often lack independent validation. Recent genome-wide studies in mammalian cells (Schuijers et al., 2014) and Drosophila (Franz et al., 2017) indicate that all direct activation of β-catenin target genes ultimately depends on TCFs as the final mediators. β-catenin, upon recruitment to promoter and enhancer regions, drives gene transcription through its C-terminal transcriptional activation domain (van de Wetering et al., 1997). It also interacts with chromatin modifiers, such as CBP and Brg-1 (Städeli et al., 2006), as well as Parafibromin/Hyrax, which are homologs of yeast Cdc73 (Mosimann et al., 2006).

8. Effects & Roles of WNT Signalling in Human Reproduction

Although research involving human tissues is primarily conducted through in vitro studies, emerging evidence indicates that Wnt signalling plays a significant role in endometrial and placental functions. Alterations in this pathway are linked to various endometrial and gestational disorders. Additionally, Wnt signalling has been shown to influence ovarian development and function. However, this review will focus on its role in the endometrium and placenta.

8.1. Endometrium, Decidualization, and Associated Diseases: Studies have identified several Wnt ligands and components within the endometrium, suggesting their involvement in the diverse functions of uterine cells. Wnt2, Wnt3, Wnt4, Wnt5a, Wnt7a, Wnt8b, and FZD1, FZD4, FZD6, and FZD10 mRNAs have been detected in endometrial tissues and cells using various molecular techniques. Hormonal regulation of certain Wnt genes (e.g., Wnt2, Wnt3, Wnt4, Wnt5a) appears limited, but their expression is often altered in endometrial carcinoma cell lines.

Wnt7a is expressed in luminal epithelial cells, while sFRP4, Dkk1, and FZD are found in uterine glands and stroma. Gene expression analyses during the menstrual cycle revealed regulation of Wnt pathway components by steroid hormones. For example, Dkk1 and Wnt10b levels increase during the luteal phase, while sFRP1 and sFRP4 are downregulated around the LH surge, suggesting a role in implantation and decidual differentiation. Elevated sFRP4 levels have been observed in estrogen-dependent cancers, indicating its potential role in regulating endometrial cell proliferation. Conversely, reduced sFRP1 and sFRP4 expression in

endometrial carcinomas correlates with enhanced cancer cell proliferation. Progesterone and Wnt Signalling: Progesterone induces Dkk1 expression in endometrial stromal cells, inhibiting Wnt signalling and potentially facilitating decidualization. This repression may also be linked to impaired decidualization in conditions like endometriosis, where Dkk1 expression is reduced.

8.2. Placental Function, Trophoblast Differentiation, and Gestational Disorders: Wnt signalling is crucial for blastocyst activation and implantation. It is also involved in trophoblast adhesion to maternal uterine tissues. For instance, Dkk1 has been shown to impair trophoblast attachment in vitro, highlighting its regulatory role.

Wnt signalling supports trophoblast differentiation and is implicated in the transformation of these cells. For example, nuclear β-catenin expression is observed in invasive trophoblasts and in pathological conditions like hydatidiform moles. Transcription factors TCF-3 and TCF-4 are expressed in extravillous trophoblasts (EVTs), with TCF-4 linked to non-proliferative trophoblast phenotypes. Recombinant Wnt3a promotes trophoblast migration, invasion, and outgrowth, suggesting activation of the canonical Wnt pathway and possibly AKT signalling.
Gestation-Dependent Wnt Expression: Early-stage trophoblasts express Wnt1, Wnt7b, Wnt10a, and Wnt10b, which are downregulated in term placentas, pointing to their roles in early placental function. Wnt10 and Wnt10b are localized to villous cytotrophoblasts, implicating their involvement in proliferation rather than differentiation.

Wnt Inhibitors and Methylation: Epigenetic changes, such as methylation of Wnt inhibitors (e.g., sFRP2), are observed in first-trimester trophoblasts, potentially activating Wnt signalling. Aberrant methylation patterns in conditions like choriocarcinomas further highlight the pathway's role in cancer progression. Lack of Dkk1 in choriocarcinoma cells correlates with tumour formation, while its overexpression induces apoptosis and growth arrest.

In summary, Wnt signalling plays a multifaceted role in the regulation of human reproductive tissues, influencing processes such as implantation, differentiation, and pathological transformations. Further research is needed to explore its therapeutic potential in treating related disorders. Wnts may also modulate other trophoblast processes such as phospholipid uptake and transport. StarD7, a member of the StAR1 lipid transfer proteins, was identified as direct target gene of TCF/β- catenin in trophoblasts. Moreover, in addition to Wnt, other ligands and receptors may contribute to β-catenin/TCF-dependent signalling in trophoblasts. Gene silencing of protease activated receptor-1, PAR1, provoked β-catenin destabilisation and reduced trophoblast motility.

9. Effect of Wnt Signalling in Growth

Wnt signalling plays a crucial role in influencing target cells during development and has become a key focus in understanding its involvement in healthy stem cells and cancer. Stem cells, whether embryonic stem (ES) cells or adult stem cells, are defined by their ability to self-renew while producing specialized cells. Their fate and behaviour are primarily governed by

short-range signals originating from the stem cell niche (Losick et al., 2011). The role of Wnt in adult stem cell biology was first demonstrated when disrupting the TCF4 gene in mice resulted in the loss of intestinal stem cells[11] and subsequent epithelial breakdown (Korinek et al., 1998). Since then, Wnt signalling has been found essential for almost all stem cell types. For instance, the ES cell phenotype can be maintained in culture[9] using two small molecules, including the Wnt-activating GSK3 inhibitor CHIR[9] (Silva et al., 2008). Similarly, purified Wnt proteins have been shown to maintain ES cell pluripotency[9] (ten Berge et al., 2011). In the hair follicle, Wnt signalling regulates stem cell[10] and progenitor cell behaviour (DasGupta and Fuchs, 1999; Lim et al.,2016). Overexpression of Dkk, a Wnt pathway inhibitor, eliminates hair follicles and other skin appendages such as mammary glands (Andl et al., 2002). In the hematopoietic system, Axin1 overexpression reduces transplantable stem cell numbers, whereas treatment with Wnt3a protein enhances self-renewal in stem cells, as shown through clonogenic assays and long-term reconstitution experiments in irradiated mice (Reya et al., 2003; Willert et al., 2003).

Stem cell-specific Wnt target genes, such as Lgr5 and Axin2, have been used to develop genetic tools for tracing known and novel adult stem cells. Lgr5 is expressed in small cycling cells at the base of intestinal crypts, identified by Paneth (1887) and later suggested to represent intestinal stem cells (Cheng and Leblond, 1974). Lineage tracing using Lgr5-CreERT2 mice[10] demonstrated that these Lgr5+ stem cells are long-lived, multipotent adult stem cells (Barker et al., 2007). Similar studies revealed Lgr5 as a marker for stem cells in other tissues, including the hair follicle (Jaks et al., 2008), stomach (Barker et al., 2010), pancreas (Huch et al., 2013a), liver (Huch et al., 2013b), and many others. Lineage tracing using Axin2-CreERT2[11] and related tools has further demonstrated Wnt-responsive stem cell functions across various tissues, including the mammary gland (van Amerongen et al., 2012), interfollicular epidermis (Lim et al., 2013), nail (Takeo et al., 2013), and periportal liver regions (Wang et al., 2015).

Growth of Organoids: Organoids, three-dimensional structures grown from stem cells, consist of organ-specific cell types and self-organize through spatially restricted lineage commitment. Purified Wnt proteins expand the number of clonogenic cells derived from mammary gland stem cells while retaining their developmental potential upon transplantation (Zeng and Nusse, 2010). By refining growth factor cocktails, researchers established culture systems that enable single Lgr5+ stem cells to grow into epithelial organoids, such as "mini-guts" (Sato et al., 2009). These organoids, suspended in Matrigel and stimulated with R-spondin1, EGF, and the BMP inhibitor Noggin, develop into polarized, differentiated epithelial structures. They represent all gut epithelial cell types in normal proportions and remain genetically and phenotypically stable for years (Grün et al., 2015; Sato et al., 2009). Organoids grown from single Lgr5+ colon stem cells[8] have demonstrated functional integration and long-term stability in experimental colitis models, forming epithelial patches indistinguishable from host tissue (Yui et al., 2012). The addition of small-molecule inhibitors, such as Alk and p38, has extended organoid culture to human small intestine and colon (Jung et al., 2011; Sato et al., 2011). Similar systems incorporating mesenchymal elements[8] have been developed from induced pluripotent stem cells (Spence et al., 2011).

This methodology has been adapted to grow organoids from Wnt-dependent adult stem cells across various mouse and human tissues of ectodermal, mesodermal, and endodermal origins. Essential components include a Wnt source, a tyrosine kinase receptor activator (e.g., EGF), BMP/TGF-ß inhibitors, and Matrigel. Organoid protocols have been successfully reported for tissues including the stomach (Barker et al., 2010), liver (Huch et al., 2013b, 2015), pancreas (Boj et al., 2015), prostate (Karthaus et al., 2014), and others. Advances, such as potent "surrogate" Wnt proteins that bypass the need for lipid modification (Janda et al., 2017) and the replacement of Matrigel with synthetic hydrogels[8] (Gjorevski et al., 2016), have further improved organoid culture systems. It now appears that most mammalian epithelia rely on Wnt-dependent Axin2/Lgr5+ stem cells for self-renewal and repair, enabling the establishment of long-term organoid cultures.

Table 1: Diseases Associated with Wnt Signalling Components

Disease	Gene
Bone Density Defects	LRP5, LGR4, SOST, WNT16, WNT1, WTX
Familial Exudative vitreoretinopathy	LRP5, FZD4, Norrin, TSPAN12
Robinow syndrome	WNT5A, DVL1, ROR2
Tooth development defects	LRP6, WNT10A, WNT10B, AXIN2

Based on http://web.stanford.edu/group/nusselab/cgi-bin/wnt/human_genetic_diseases (selected for diseases with multiple pathway components implicated).

10. WNT Signalling and Diseases

Wnt signalling plays a critical role in epithelial stem cell activity, and mutations in this pathway are frequently linked to various cancers, including colorectal cancer, melanoma, and hepatocellular carcinoma. Key mutations involve the APC gene, β-catenin, Axin1/2, and other components, leading to abnormal signalling and tumourigenesis. In rare cases, mutations or gene fusions in R-spondins or E3 ligase genes like RNF43 and ZNRF3 further highlight the pathway's complexity. Wnt-related degenerative diseases, such as sclerosteosis, osteoporosis, and retinal disorders, underscore its importance in human health and developmental mechanisms. Therapeutically, targeting Wnt signalling is challenging due to potential side effects, but advances include bone-specific SOST inhibitors like Romosozumab and small molecules targeting Wnt secretion (e.g., Porcupine inhibitors) or β-catenin stabilization. Promising experimental and clinical trials suggest Wnt pathway modulation could benefit cancer treatment, regenerative medicine, and neurodegenerative diseases such as Alzheimer's. Although early in development, these approaches provide hope for effective therapies.

Table 2: Small molecules to activate or inhibit Wnt Signalling

Compound	Target	Inhibitor/Activator of the target	Effect on Wnt Signalling
IWP	Porcupine	Inhibitor	Inhibits
LGK974	Porcupine	Inhibitor	Inhibits
C59	Porcupine	Inhibitor	Inhibits
Apicularen and bafilomycin	vacuolar ATPase	Inhibitor	Inhibits
XAV939	tankyrase, Axin	Activates Axin	Inhibits
IWR	tankyrase, Axin	Activates Axin	Inhibits
G007-LK, G244-LM	tankyrase, Axin	Activates Axin	Inhibits
IQI	PP2A	Activator	Activates
QS11	ARFGAP1	Activator	Activates
SB-216763	GSK3	Inhibitor	Activates
CHIR99021	GSK3	Inhibitor	Activates
BIO (6-bromoindirubin-3'-oxime)	GSK3	Inhibitor	Activates
L807mts	GSK3	Inhibitor	Activates
LY2090314	GSK3	Inhibitor	Activates
ICG-001	CREB-binding protein	Inhibitor	Inhibits

Based on http://web.stanford.edu/group/nusselab/cgi-bin/wnt/smallmolecules .

11. WNT Signalling & Cancer – Transitional Relevance

The first mammalian Wnt gene, Wnt1, was initially discovered as an oncogene that was held responsible for driving mammary tumour formation in mice. This discovery came from efforts to identify host cell genes activated by insertional mutagenesis of the mouse mammary tumour virus (MMTV). After two years of meticulous mapping, the int1 gene (short for "first common integration site") was identified (Nusse and Varmus, 1982). Unlike other proto-oncogenes such as Myc and Ras, no dominant activating mutations or alterations in the int1 locus were observed in human cancers. While its discovery initially classified int1 as a proto-oncogene (Tsukamoto et al., 1988), research gradually shifted toward understanding its developmental functions, leading to its rebranding as Wnt1. Over a decade later, the connection between Wnt signalling and cancer resurfaced. A region on human chromosome 5q21 was linked to familial adenomatous polyposis[12] (FAP) and other colon cancers. In 1991, the APC gene (adenomatous polyposis coli) was cloned (Groden et al., 1991; Kinzler et al., 1991; Nishisho et al., 1991) and found to interact with β-catenin (Rubinfeld et al., 1993; Su et al., 1993). The significance of this interaction became clear when it was shown that APC is part of a destruction complex that degrades β-catenin in the absence of Wnt ligands (Korinek et al., 1997; Morin et al., 1997;

Munemitsu et al., 1995). Now recognized as a "gatekeeper" gene, APC is vital for regulating cell expansion during early colorectal carcinogenesis (Kinzler and Vogelstein, 1996). Mutations in APC and deregulation of the Wnt pathway have been identified as common drivers in various human cancers. Both β-catenin-dependent and independent Wnt Signalling are now promising therapeutic targets (Daulat and Borg, 2017; Zhang and Hao, 2015). After four decades of developmental research, the first clinical trials involving Wnt inhibitors are underway (www.clinicalTrials.gov). Despite this progress, translating scientific discoveries into clinical therapies has been challenging. Drug development initially focused on inhibiting β-catenin/Tcf complexes, but results have been largely disappointing, reflecting the difficulty of targeting transcription factors. Efforts continue, however, and new drugs are being developed. Notably, targeting upstream Wnt signalling events has shown promise. For instance, decoy receptors or receptor-blocking antibodies have yielded encouraging preclinical results across various tumour types (Gurney et al., 2012; Takebe et al., 2015). Wnt secretion depends on palmitoyl groups added by the Porcn enzyme (Janda et al., 2012; Takada et al., 2006). In 2009, Lum and colleagues developed the first Porcn inhibitor, WP2, and in 2013, LGK974 was identified (Chen et al., 2009; Liu et al., 2013). LGK974 is now in phase 1/II clinical trials. While clinical efficacy is yet to be confirmed, these developments highlight the widespread involvement of Wnt pathway deregulation in cancer, where the pathway is effectively hijacked to promote tumour growth. Given the complexity of Wnt signalling, additional drugs with targeted mechanisms, such as the Wnt5a hexapeptide Foxy5 (Säfholm et al., 2008), may be necessary to modulate specific signalling branches. This is critical as evidence suggests that the balance between β-catenin-dependent and independent signalling is key to regulating cell proliferation and differentiation during development, tissue maintenance, aging, and regeneration (Stoick-Cooper et al., 2007). Since cancer can be seen as a disruption of normal developmental processes, the fate of cancer cells is likely shaped by the interplay of these signals. Moving forward, new therapeutic insights will likely emerge from fundamental developmental biology research and the curiosity-driven questions it inspires.

12. Role of Wnt Signalling and Signalling Elements in the activation of Mutations in Cancer

Mutations in CTNNB1, which encodes β-catenin, are less common in certain cancers, such as colorectal cancer (CRC), compared to other mutations like APC. In contrast, CTNNB1 mutations are found in about 25% of hepatocellular carcinoma (HCC) cases, whereas APC mutations are rare in HCC. The tissue specificity of these mutations may be influenced by the homeostatic levels of Wnt signalling, which can affect the fitness of mutant cells relative to normal ones. In CRC, high levels of E-cadherin expression limit the transforming potential of CTNNB1 mutations by sequestering β-catenin to the cell membrane, making APC mutations more dominant in driving CRC. Both APC and CTNNB1 mutations are found in 12% and 30% of endometrial cancers, respectively, with CTNNB1 mutations likely being the dominant drivers of Wnt activation. Notably, APC mutations in endometrial cancer often do not result in truncated proteins, and the loss of Apc alone does not induce malignant transformation unless combined with Pten loss. This suggests that different Wnt pathway mutations may have varying transforming potentials in endometrial cancer. While this review focuses on DNA mutations in

Wnt pathway genes, it also acknowledges the role of epigenetic alterations. For example, the Wnt antagonist DKK1 is epigenetically downregulated in CRC, and Secreted Frizzled-Related Proteins (SFRPs), another class of Wnt antagonists, are epigenetically inactivated in gastric cancer.

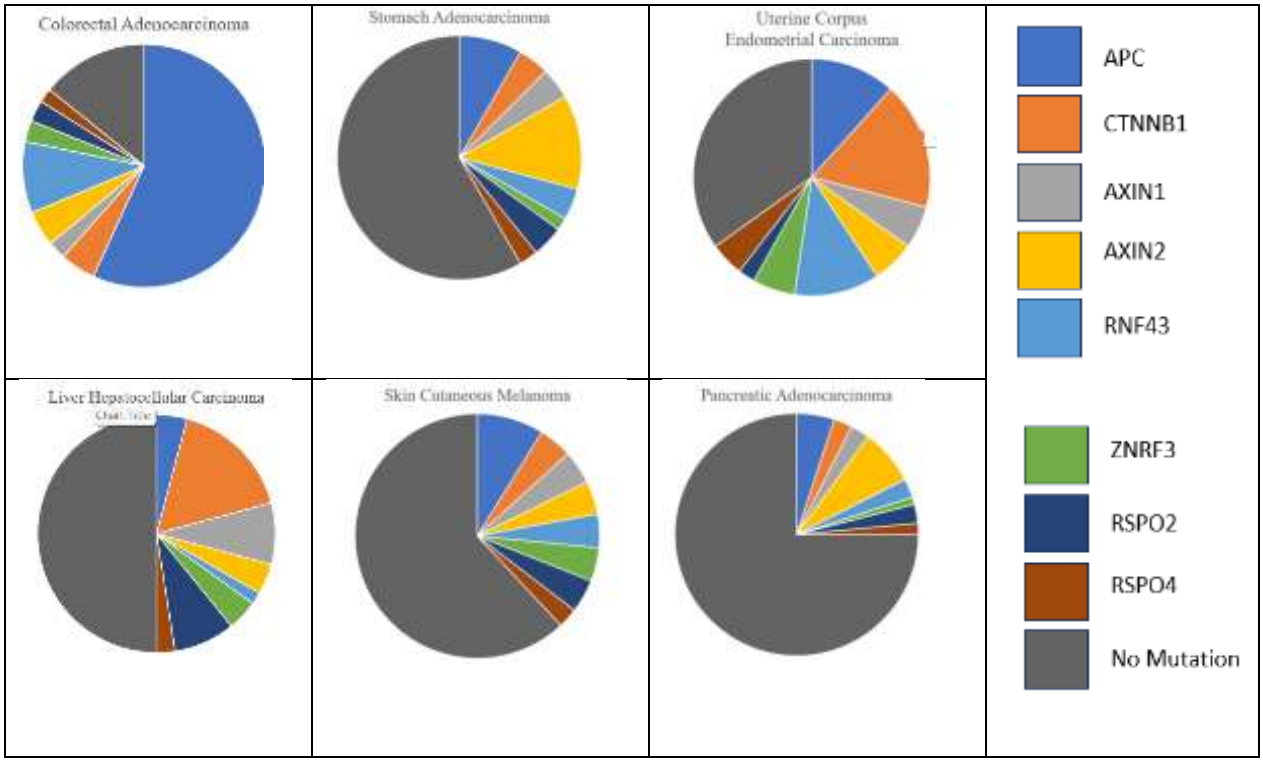

Fig 5: An overview of the frequency of certain WNT pathway mutations found in colon, liver, endometrial, gastric, pancreatic and skin cancer. Data taken from Cancer Genome Atlas's PanCancer Atlas on cBioportal as of February 2023. No mutation indicates patients in the queried datasets that did not have a mutation in any of the genes shown above.

12.1. Role of APC Mutations:

The APC protein is a large scaffold protein with multiple domains that are crucial for its interactions with binding partners within the destruction complex and β-catenin. Specifically, the 15-amino-acid repeats (15AARs) and 20-amino-acid repeats (20AARs) are involved in β-catenin binding, while the Ser-Ala-Met-Pro (SAMP) repeats interact with AXIN. In colon cancer, the majority of APC mutations occur in the 5' region of exon 15, leading to truncated proteins. A well-defined mutation hotspot known as the Mutation Cluster Region (MCR) is observed. These truncated proteins typically retain only 1–3 functional 20AARs, while the SAMP motifs are lost, resulting in altered, but not maximal, WNT signalling. Consequently, the predominant APC mutation in CRC still allows for some β-catenin binding, preventing full activation of WNT signalling. This has given rise to the "just right WNT signalling hypothesis," suggesting that an optimal level of WNT signalling is necessary for CRC transformation. Evidence shows that the number of retained 20AARs correlates with the location of CRC tumours: tumours in the proximal colon tend to retain more 20AARs than those in the distal colon, likely reflecting distinct levels of WNT signalling. This pattern could be influenced by

the gradient of WNT signalling, which decreases from the proximal to the distal colon. It is proposed that high basal WNT signalling in the proximal colon may be unfavourable for tumours with elevated pathological WNT signalling, thus promoting tumourigenesis in the distal colon. Mouse models of intestinal tumourigenesis support this theory. For example, the $Apc^{Min/+}$ mouse, which carries germline mutations in Apc that result in a truncated APC protein lacking β-catenin binding, typically develops tumours in the distal small intestine. In contrast, the $Apc^{1322T/+}$ mouse, which retains one functional 20AAR, develops proximal intestinal tumours with lower nuclear β-catenin levels compared to the $Apc^{Min/+}$ model. The reduced WNT signalling in $Apc^{1322T/+}$ tumours is consistent with tumour formation in the higher basal WNT environment of the proximal intestine. Beyond colon cancer, APC mutations are also observed in approximately 13–15% of cases of uterine endometrial, stomach, and skin cutaneous melanoma.

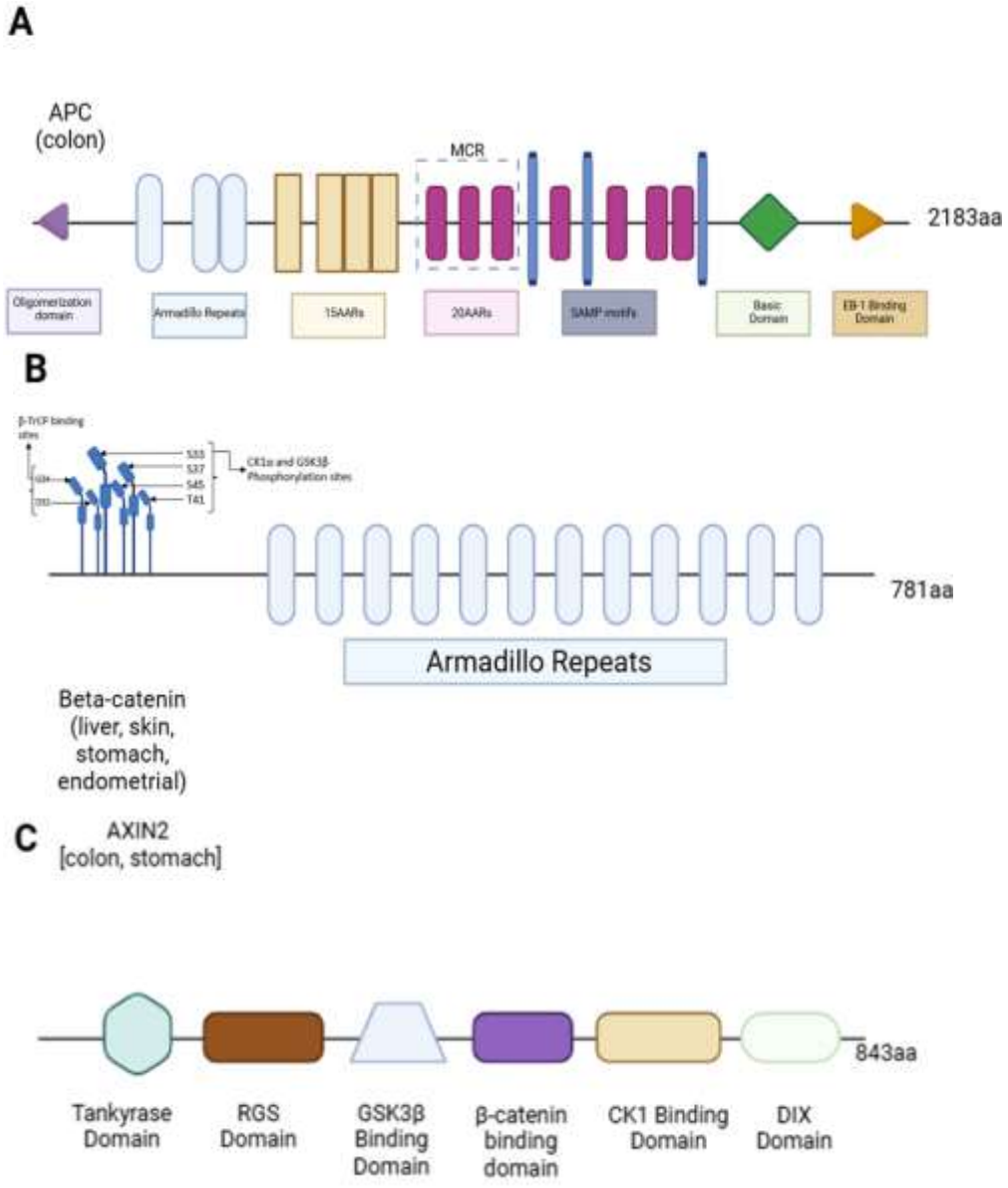

Figure 6: Schematic representations of proteins -A)APC, B) β-Catenin, C)AXIN2 : Mutations in the APC, CTNNB1, and AXIN2 genes significantly impact protein functionality in various cancers. The APC protein, for instance, plays a crucial role in binding to β-catenin. However, mutations in the mutation cluster region (MCR) of APC, commonly found in colorectal cancer (CRC), lead to truncated proteins with reduced β-catenin and AXIN binding capacity. Similarly, mutations in the CTNNB1 gene, which encodes β-catenin, can stabilize β-catenin in the cytoplasm, leading to its accumulation and subsequent activation of WNT target genes. These mutations are commonly found in liver, skin, endometrial, and stomach cancers. The AXIN2 protein, another key component of the WNT signalling pathway, is also affected by mutations. A recurring frameshift mutation in AXIN2, found in colon and stomach cancers, disrupts the protein's ability to bind to CK1α, leading to impaired destruction complex function. Understanding these mutations and their impact on protein functionality is crucial for developing effective therapeutic strategies against WNT-driven cancers.

12.2. Role of CTNNB1 Mutations:

While APC mutations often result in truncated proteins, particularly in colorectal cancer (CRC), activating mutations in CTNNB1 lead to the stabilization of β-catenin through point mutations in cancers such as hepatocellular carcinoma (HCC), endometrial cancer, and pancreatic cancer. These mutations commonly occur in exon 3 of CTNNB1, focusing on phosphorylation sites targeted by the destruction complex. Key phosphorylation sites such as Ser33, Ser37, Thr41, and Ser45 are altered in these mutations, preventing phosphorylation by CK1 and GSK3, and thereby stabilizing β-catenin. Additionally, mutations in Asp32 and Gly34 impair β-TrCP binding, further preventing β-catenin ubiquitination. This mutational hotspot in exon 3 is crucial for β-catenin's stabilization. Outside of this region, two rarer mutations—N387K and K335I/T—have been identified in HCC patients. These mutations occur within the armadillo repeats of β-catenin, which are involved in binding interactions with APC and AXIN. Notably, although these mutations lead to β-catenin stabilization, they activate the WNT pathway to varying degrees. For instance, the N387K and K335I/T mutations outside the exon 3 hotspot generate weaker signals in WNT reporter assays compared to mutations at Ser33, Thr41, and Ser45. Interestingly, while the Ser45 mutation also produces a weaker signal than Ser33 and Thr41 mutations, it is frequently duplicated in HCC, leading to high β-catenin activity. Recurrent mutations in CTNNB1 exon 3 are also observed in endometrial cancer, but no direct correlation has been found between nuclear β-catenin levels and specific CTNNB1 mutations in these cases.

12.3. Role of AXIN Mutations:

Both AXIN1 and AXIN2 function as scaffold proteins within the destruction complex, playing key roles in regulating β-catenin levels. AXIN1 mutations occur in about 8% of hepatocellular carcinoma (HCC) cases, and deletion of Axin1 in the murine liver can lead to the development of hepatic tumours. Mutations in both AXIN1 and AXIN2 are found in approximately 14% of uterine endometrial cancers, while AXIN2 mutations are present in about 5% of colorectal cancer (CRC) cases. Despite their high degree of homology, the mutation patterns of these two

proteins differ significantly across cancers. AXIN1 mutations are spread throughout the entire coding sequence, with varying mutations across different tumour types. In contrast, AXIN2 frequently exhibits a recurring frameshift mutation in exon 7, particularly in CRC and stomach cancer, leading to the production of a truncated protein. This results in stabilized β-catenin and the activation of WNT signalling.

12.4. Role of RNF43/ZNRF3 Mutations:

Both AXIN1 and AXIN2 act as scaffold proteins in the destruction complex, crucial for regulating β-catenin levels. Mutations in AXIN1 are observed in around 8% of hepatocellular carcinoma (HCC) cases, and deleting Axin1 in the mouse liver can lead to the formation of hepatic tumours. Mutations in both AXIN1 and AXIN2 are found in approximately 14% of uterine endometrial cancers, while AXIN2 mutations occur in about 5% of colorectal cancer (CRC) cases. Although AXIN1 and AXIN2 share significant structural similarity, their mutation patterns in cancer differ considerably. AXIN1 mutations are scattered across the entire coding region, showing a variety of changes depending on the tumour type. In contrast, AXIN2 often has a recurring frameshift mutation in exon 7, particularly in CRC and stomach cancer, resulting in a truncated protein. This leads to the stabilization of β-catenin and the activation of WNT signalling.

12.5. Role of RSPO Mutations:

The RSPONDIN family consists of four members, with RSPO2 being the most commonly mutated in cancer. Mutations in RSPO2 and RSPO3 are observed in approximately 10% of colon cancers and typically occur in a mutually exclusive manner with APC mutations. Studies have identified recurring fusion mutations, such as PTPRK-RSPO3 and EIF3E-RSPO2, as well as PIEZO1-RSPO2 fusion mutations in colorectal cancer. These fusion events result in elevated levels of RSPO2 and RSPO3 in the tumours, and mice with Rspo2 or Rspo3 fusion mutations develop intestinal tumours. RSPO2 mutations are also recurrent in hepatocellular carcinoma (HCC), often due to a large deletion on chromosome 8q23.1. Although a similar EIF3E-RSPO2 fusion mutation, as seen in CRC, was speculated, it could not be confirmed due to issues with DNA and RNA fragmentation in the formalin-fixed paraffin-embedded samples used in the study. In these HCC tumours, high levels of RSPO2 are detected, and immunohistochemical analysis reveals strong nuclear β-catenin and glutamine synthetase staining, indicating significant activation of the WNT pathway. RSPO2 fusion mutations have also been reported in gastric and prostate cancers, while overexpression of all four RSPONDIN proteins has been observed in a wide range of other cancer types.

13. Therapeutic Options against mutations of Wnt Signalling components that causes Cancer

Although mutations that activate the WNT pathway leads to increased nuclear β-catenin levels, their dependence on WNT ligands to drive WNT signalling varies depending on where they occur within the signalling cascade. Some mutations, such as those in APC, CTNNB1,

and other components of the destruction complex, are considered ligand-independent, meaning they can activate the pathway without requiring WNT ligands. In contrast, mutations in genes like RNF43 or RSPO2 are typically ligand-dependent, meaning they rely on WNT ligands to enhance signalling. This distinction has significant implications for the development of WNT-targeted therapies, as treatment strategies may differ based on whether the mutations are ligand-dependent or independent. The following section will explore therapeutic options tailored to specific WNT-activating mutations at various levels of the WNT signalling pathway.

13.1. Based on WNT ligands:

Currently, there are no approved WNT-targeting therapies for cancer, but several potential treatments are being tested in clinical trials, targeting WNT ligands or the receptor complex, like – Porcupine Inhibitors, Wnt ligands, Extracellular methods. A major focus has been on inhibiting WNT ligand secretion through PORCN inhibitors, such as WNT974 (LGK974), which blocks the palmitoylation of canonical WNT ligands, a process essential for their secretion. These inhibitors have shown effectiveness in cancer models with RNF43 or RSPO mutations. For example, WNT974 inhibited growth in RNF43-mutant pancreatic tumours and regressed intestinal tumours with RSPO2 or RSPO3 fusion mutations. Another PORCN inhibitor, Wnt-C59, has demonstrated efficacy in both intestinal and breast cancer models. However, PORCN inhibitors may not be effective in cancers with mutations in the destruction complex or CTNNB1, as these mutations bypass the need for WNT ligands. Additionally, one side effect of PORCN inhibition is a loss of bone density, but strategies are being explored to mitigate this, such as combining treatment with osteoporosis drugs. Other strategies aim to block WNT ligand binding to Frizzled (FZD)[14] receptors. For instance, OMP-54F28 is a truncated FZD8 receptor fused to an antibody that can bind free WNT ligands and block WNT signalling. Similarly, OMP-18R5 (Vantictumab) targets Frizzled receptors[14], inhibiting downstream WNT signalling. This approach has shown promise in breast, pancreatic, lung, and colon cancer models. However, it may not be effective in cancers with mutations in the destruction complex or CTNNB1, as indicated by its limited impact in certain models, such as those treated with GSK3 inhibitors.

Another approach focuses on targeting R-spondin signalling, which is crucial in certain WNT-driven cancers. The anti-RSPO3 antibody OMP-131R10[14] is being tested in clinical trials, with early evidence suggesting it can inhibit tumour growth in colorectal cancer models with PTPRK-RSPO3 fusion mutations. However, it appears ineffective in APC-mutant models, highlighting the need to tailor therapies based on mutational profiles. In colorectal cancer, particularly in patients with Familial Adenomatous Polyposis[12] (FAP) and germline APC mutations, Notum, a WNT deacylase secreted by Apc-null cells[6], suppresses WNT signalling in nearby wild-type cells, promoting tumour growth. Inhibiting Notum has shown promise in reducing tumour formation in mouse models, offering a potential strategy for treating FAP patients prone to colorectal cancer.

13.2 Based on β-Catenin Targeting therapies:

The previous section focused on therapies targeting the extracellular components of WNT signalling, which have shown efficacy in ligand-dependent cancer models but limited success in ligand-independent models, such as those with mutations in CTNNB1 or APC. Now, attention shifts to strategies targeting β-catenin directly or downstream components of the WNT signalling pathway. One approach targets β-catenin-mediated transcription by disrupting its interaction with transcriptional co-activators, such as CBP and p300. Small molecules like ICG-001[14] have been shown to inhibit this interaction, reducing WNT target gene expression and tumour growth in APC-mutant colon cancer models. A related compound, PRI-724[14], has also demonstrated effectiveness in hepatocellular carcinoma (HCC) cell lines and successfully entered phase I clinical trials in combination with Gemcitabine for advanced pancreatic cancer. In addition, targeting components of the WNT enhanceosome, such as BCL9, BCL9L, and Pygo, has shown promise in pre-clinical models. Deletion of these components has extended survival in APC-mutant colon cancer mice and reduced WNT signalling in various models. Small molecule inhibitors of BCL9, including carnosic acid (found in rosemary) and E722-2648, have proven effective in reducing WNT signalling and tumour growth in colon cancer models. These findings suggest that targeting the BCL9/9L and Pygo1/2 complexes could be a potential therapeutic strategy for WNT-driven cancers.

Another approach focuses on boosting the activity of the WNT destruction complex to lower β-catenin levels. Tankyrase inhibitors[14], which stabilize AXIN by preventing its degradation, have shown promise in colon cancer models with APC mutations, although their toxicity in the intestine limits their clinical potential. Similarly, Pyrvinium, which activates the destruction complex by modulating CK1, has demonstrated efficacy in both colon cancer and HCC models, reducing tumour growth and WNT target gene expression[14]. Pyrvinium is currently undergoing clinical trials for pancreatic cancer, highlighting its potential as a therapeutic agent targeting the WNT pathway at the level of the destruction complex.

14. Conclusion

Thus, from the studies it is seen that the Wnt signalling pathway plays a crucial role in the development of various cancers, and mutations in key components such as CTNNB1, APC, AXIN, and RSPO contribute to the aberrant activation of this pathway. Understanding the specific mutations and their effects on Wnt signalling has led to the development of targeted therapeutic strategies. These therapies include inhibitors of Wnt ligand secretion (e.g., PORCN inhibitors), Frizzled receptor blockers, and agents that directly target β-catenin or components of the Wnt destruction complex. While these approaches have shown promise in pre-clinical models, their effectiveness varies depending on the mutational profiles of the cancer. Cancers driven by ligand-dependent mutations, such as those in RNF43 or RSPO, may respond to therapies targeting Wnt ligands, while those driven by mutations in the destruction complex or CTNNB1 may require strategies that directly target β-catenin or modulate the downstream signalling cascade. As these therapies continue to be tested in clinical trials, the potential for

more personalized and effective treatments for Wnt-driven cancers is becoming increasingly clear.

Acknowledgement

I would like to thank Prof. Nandan Kumar Jana for his constant support & guidance towards the whole work done. I would also like to thank Head of the Department, Department of Biotechnology, Heritage Institute of Technology, Kolkata and Principal sir of Heritage Institute of Technology, Kolkata for their constant support.

Fundings

No fundings were raised & required during this work.

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