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Review

A gust of WNT: analysis of the canonical WNT pathway

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Abstract. The Wnt pathway is a signal-transduction cascade that mediates communication between cells; the Wnt pathway is involved in key steps during embryological development and in the maintenance of adult tissue homeostasis. Mutational dysregulation of Wnt cascade components has been observed in diverse human pathological conditions and in oncogenic transformations. For these reasons, the Wnt signalling pathway has acquired growing interest in scientific and medical research over recent years. This review outlines the biochemical and functional features of the Wnt cascade with particular emphasis on a detailed functional analysis of all key players. In this instance, the regulations of the pathway have also been covered, emphasizing novelty in this regard. Furthermore, past and present studies on Wnt have been included, as well as a prediction of scientific progress, which may be made in this rapidly evolving field, in the near future; the review also embraces considerations on how further understanding of the Wnt pathway will provide important insight into managing human diseases. (www.actabiomedica.it)

Key words: Wnt canonical pathway, b-catenin, signal transduction, haematopoietic stem cells, carcinogenesis

Introduction

Multicellular organisms necessitate a communication system to grow and function; in complex multicellular organisms, like humans, cell-to-cell communication becomes the basis of life.

Intercellular communication in higher animals not only allows structural and functional coordination amongst cells, but also regulates cellular development, differentiation, organisation and division. The importance and complexity of cell-to-cell communication has attracted the attention of various areas of science over the last 50 years.

Although inter-cell communication occurs throughout adulthood, it has been greatly investigated during embryological development.

In the last 30 years, developmental biologists have proved and detailed the existence of signalling molecules which regulate the main steps of embryogenesis.

The Wnt family of signalling proteins has been found to be involved in embryonic patterning, in the homeostasis of adult tissue self-renewal and in the process of carcinogenesis. Such a diverse spectrum of action is due to the Wnt signal being pleiotropic; therefore, a vast array of cellular responses is coordinated by the Wnt proteins and their downstream effectors.

The Wnt proteins are secreted lipid-modified proteins (1) that, by binding to specific cell surface receptors, initiate diverse intracellular signalling pathways, which culminate in the activation of target genes and different cellular responses.

The deregulation of signalling pathways related to fundamental cellular functions, including Wnt, leads to alterations in several features of the cell, inducing tumourigenesis.

3. The WNT pathway

3.1 The Wnt pathway story: A tale 700 million years long

The last few decades have witnessed the discovery of numerous signalling families, since the bone morphogenetic proteins (BMPs) in 1977 (2). In the late 80s, the Wnt pathway was revealed. Prior to this, the wingless gene had already been identified in Drosophila, before its murine homolog, *int-1*, was detailed (3).

The *Wingless* gene (wg1) was discovered in *Drosophila melanogaster* with mutant phenotypes on wings and halteres 30 years ago (4).

Years later, new insights came from the analysis of the mouse mammary-tumour virus (MMTV), which integrates in a common site of the genome (5); the region was identified as the promoter of a gene called *Int-1* for integration (6). Sequence analysis demonstrated the murine proto-oncogene *Int-1* as an orthologue of the *Drosophila* segment polarity gene *Wingless* (7); the fusion of the two names gave rise to the term "Wnt", also used as acronym for "wingless-type" (7).

Since the discovery of *Int-1* (now Wnt1), many other Wnt genes have been identified in vertebrates and invertebrates, from hydra to humans. Nevertheless, they are absent from plants (8).

However, more downstream members of the Wnt signalling cascade, such as β-catenin, have been discovered in primitive organisms such as *Dictyostelium* (9) and plants (10). This suggests that an ancient Wnt-independent mechanism existed for the intracellular activation of Wnt transcriptional effectors like β-catenin, before the evolution of animals even started.

The evolutionary origin of the Wnt family has been traced back to primitive diploblasts (11); in members of the Cnidaria family, bona fide Wnt molecules have been found, emphasizing the amazing conservation of the Wnt signalling molecules throughout evolution (12, 13).

The evolutionary analysis of Wnt genes has been aided by the availability of genetic information from the sequencing of various organisms' genomes. Cur-

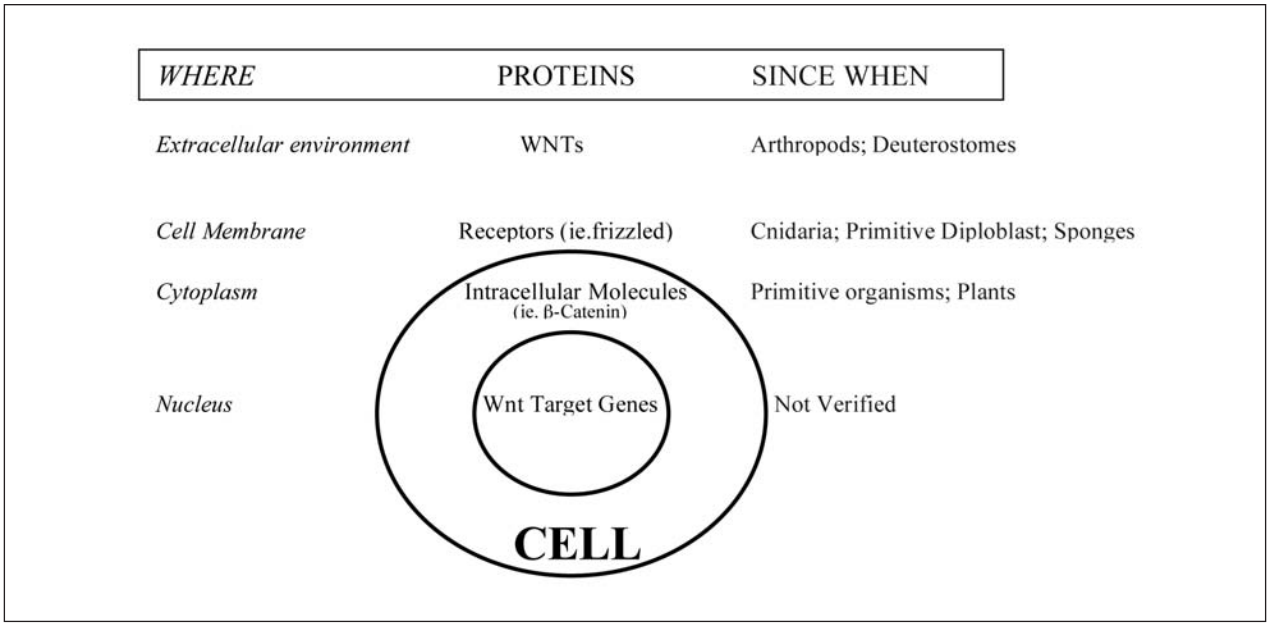

Figure 1. Evolutionary origins of selected components of the Wnt pathway and their location within the cell
A schematic representation of a cell, in which major components of the Wnt signalling cascade have been placed in their respective locations (in italics). The evolutionary origins of these molecules are shown on the right hand side.
It is interesting to notice that the most distal components of the Wnt pathway (ie. the intracellular molecule β-catenin) are highly conserved in very primitive organisms and plants

rently, 19 WNT genes have been found in human (Table 1) and other mammals; they reveal unique expression patterns and distinct functions, although some redundancies are observed (Table 1) (14). Between vertebrate species there is extensive conservation of Wnt gene sequence and structure (15). Also, between *Drosophila* and vertebrate Wnt molecules, an extensive conservation of biological and biochemical functions has been noticed, which permits the identification of orthologues. Furthermore, the conservation of clusters of genes between vertebrates and invertebrates (*Drosophila*) indicates the presence of a Wnt ancestral cluster (16) common to all bilateral animals (17), dating back 700 million years.

Table 1. Human Wnt Genes Details. Modified from Table 2 (46)

Gene name	Cancers (& *Human Diseases*)	Fetal expression	Adult expression
WNT1	Gastric, Pancreatic, Breast Cancer	/	Spleen, Bone, Uterus, Colon
WNT2	Breast Cancer	Placenta	Lung, Breast
WNT2B/13	Breast, Cervical, Gastric Cancer	Brain, Lung & Kidney	Heart, Brain, Lung, Prostate, Testis, Ovary, Intestine & Colon
WNT3	Leukemia; Breast Cancer; *Tetra-Amelia*	/	Breast
WNT3A	Breast Cancer	/	/
WNT4	Breast, Endometrial Cancer; *Mullerian-Duct Virilization*	Kidney, Liver	Breast, Endometrium, Adrenal Gland, Skeletal Muscle & Thyroid
WNT5A	Lung, Breast, Prostate Cancer	Heart, Lung	/
WNT5B	Leukemia; Gastric Cancer; Teratoma; *Diabetes type 2*	Brain	Prostate
WNT6	Colorectal, Cervical Cancer	Placenta	Spleen
WNT7A	Endometrial Cancer	Placenta, Lung, Brain Limbs Development	Kidney, Testis, Uterus, Brain
WNT7B	Teratocarcinoma; Breast, Lung, Gastric Cancer	Brain, Lung, Kidney	Brain, Lung, Prostate, Breast
WNT8A	Teratocarcinoma	Embryonic Stem Cells (ESC) (?)	/
WNT8B	Gastric, Breast Cancer	Brain (Forebrain) ESC (?)	/
WNT9A Previously WNT14	Leukemia; Breast, Gastric Cancer	/	Skeletal Muscle, Heart
WNT9B Previously WNT15	*Connective tissue diseases*	Neurons	Connective Tissues
WNT10A	Colorectal Cancer, Leukemia	Kidney, Placenta	Spleen
WNT10B	Breast, Gastric Cancer	/	Heart, Skeletal Muscle
WNT11	Prostate Cancer	Skeleton, Kidney, Lungs	Heart, Liver, Skeletal Muscle, Pancreas
WNT16 (A & B)	Leukemia	/	Spleen, Appendix, Lymph Nodes, Pancreas

(?) = Not yet confirmed
/ = Data not available in human
Human diseases are shown in italic

From the phylogenetic analysis, the Wnt molecular pathway has emerged as "*a quintessential aspect of organized multicellular life*" (18).

Also, the implication of Wnt in carcinogenesis during the discovery of the first Wnt gene marked the early onset of extensive Wnt research. Data have been accumulated over the last decades, which allow this literature review to be able to cover details of the Wnt pathway, as well as clinical considerations about the strong relationship between the Wnt signalling cascade and human pathologies. At present, several questions are being addressed, whose answers will allow the design of effective therapeutic strategies to be applied in curing diseases like cancer, in the near future.

3.2 Molecular mechanisms of the Wnt signal transduction pathway

Wnts are lipid-modified, cystein-rich glycoproteins secreted by signalling cells. The Wnt proteins are notoriously insoluble; the *porcupine* gene, in the Wnt producing cells of *Drosophila* (19), is responsible for the increase of Wnts hydrophobicity, through palmitoylation on a conserved cystein residue (Figure 2) (1). This feature of Wnt molecules is critical for signalling, but has hindered structural and biochemical characterization of the proteins. It is known that human Wnt proteins have very similar molecular weights, from 39 kDa (Wnt7a) to 46kDa (Wnt10a).

The diffuse expression of Wingless in *Drosophila* imaginal discs indicates that Wnt ligands function as concentration-dependent long-range morphogenetic cues (20, 21). In order to relay the Wnt patterning signal on distant cells, the proteins may be transported via a vesicle-based mechanism, outside the cell (22). Indeed, other transporting systems have been proposed (23), yet it is still not clear how Wnts are shuttled between cells and which molecules are involved.

Once released into the extracellular milieu, the Wnt ligands interact with multiple secreted proteins, which modulate Wnt activity. This is a pivotal control step to ensure the specificity of the activity of the Wnt signal.

Wnt proteins can interact with the secreted Heparin-sulfated proteoglycans (HSPGs), such as *Dally* (*division abnormally delayed*) in *Drosophila* (24-26) and the murine *syndecan-1* (27). Although HSPGs have been postulated to act as co-receptors present on target cells (26), experiments suggest the presence of additional roles in the stabilization of Wnt proteins and in aiding their transport between cells (28).

Molecules like SFRP (secreted frizzled-related proteins) (29, 30) and WIF (Wnt inhibitory factor) (31) are thought to be extracellular inhibitors of Wnt signalling, because, by binding to the secreted Wnt ligands, they sequester them and prevent them from binding to the receptor of responding cells (32-35). Nevertheless, SFRPs and WIFs may play a less unilateral role than assumed, by changing and adapting their functions depending on the Wnt expression level and the cellular context; moreover, they could act to prevent degradation of the Wnt ligands and facilitate their secretion and movement (36).

Two other related extracellular antagonists of the Wnt molecules are Cerberus (37) and Coco (38); in *Xenopus*, these two inhibitors can complex and act to sequester Wnt ligands from their receptors.

The Wnt signalling pathway is initiated by the Wnt molecules upon binding to seven-transmem-

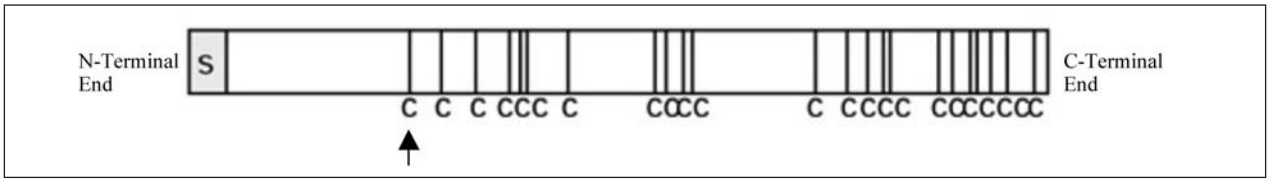

Figure 2. Schematic diagram illustrating the structural elements of Wnt
Wnt molecules all contain a signal sequence at the amino terminus (S), followed by 23 (or 24) cysteines, whose distribution is highly conserved; this latter characteristic may indicate that several intramolecular disulfide bonds aid Wnt proteins' folding (15). The arrow indicates the most N-terminal cysteine for the palmitate modification, identified via mass spectrometry by (45) and mapped to C77 in the murine Wnt3a and C51 in *Drosophila*'s Wnt8. This cysteine is conserved in all Wnt proteins (1)

Table 2 - Gene nomenclature of selected Wnt pathway members. The numerous components of the Wnt cascade may present different names for diverse organisms, still referring to the same molecule. Modified from Table 1 (46)

Protein	Human	Mouse	*D. melanogaster*	*C. elegans*
β-catenin	CTNNB1	Catnb	Armadillo	Bar-1
β -catenin; plakoglobin	JUP	Jup	Armadillo (?)	Wrm-1; hmp-2
APC	APC; APCL	Apc	dApc; E-Apc	Apr-1
Axin	AXIN (1 & 2)	Axin	dAxin	Pry-1
Dickkopf	DKK	Dkk	Dickkopf	?
Dishevelled	DVL	Dvl	Disheveled	Mig-5; dsh (1 & 2)
Frizzled	FZD	Fzd	Frizzled; Dfz	Mom-5; lin-17; mig-1; cfz-4
Groucho	TLE	Grg	Groucho	Unc-37
GSK3β	GSK3B	Gsk3b	Shaggy; Zeste white	Sgg-1
Legless	BCL9	Lgl	Legless	?
LRP	LRP (5 & 6)	Lrp	Arrow	Lrp-1
Pygopus	PYGO	Pygo	Pygopus; Pygo	?
TCF/LEF	TCF; LEF	Tcf/Lef	dTcf/pangolin	Pop-1
Wnt	Wnt	Int; Wnt	Wingless (Wg)	Mom (1 & 2); Lin-44; Egl-20 Cwn (1 & 2)

? = Not yet identified

brane receptors called Frizzled (Fz; (39, 40)). Fzs are a family of serpentine receptors with seven-transmembrane-spanning domains and with a long amino-terminus called CRD (cystein rich domain) to which Wnt ligands directly bind (39, 41).

At this point, divergence between the different Wnt signalling cascades occurs. If the Wnt ligands, as well as binding to a member of the Fz family of receptors, simultaneously interact with co-receptors LRP-5 or LRP-6, which are part of the low-density lipoprotein receptor related protein family (LRP; (42)), they will activate the intracellular Wnt canonical signalling pathway. On the other hand, if LRP is not expressed on the responding cell's surface, or it has been down-regulated by inhibitory secreted factors, Wnt molecules will only bind to the Fz receptor, triggering the so-called non-canonical pathways; the planar cell polarity and the Wnt/Ca^{2}+ non-canonical cascades will not be discussed in this review (for further reading, refer to recent reviews by (43, 44)).

3.2.1 The canonical Wnt signalling pathway

The best characterized and understood Wnt signalling cascade is the so-called β-catenin/Wnt canonical pathway. The key transcriptional effector of this pathway is β-catenin (45), whose accumulation within the cytoplasm mediates diverse cellular responses.

The canonical pathway follows a linear genetic cascade, but it involves numerous players (Table 2) and interactions, which, for the sake of simplicity, will not be quoted in detail.

Since Wnt molecules mediate paracrine, non-cell autonomous signals, several control steps are indispensably scattered throughout the Wnt cascade, to ensure a spatially restricted pattern of Wnt morphogens.

As described above, the initiation of the canonical pathway is marked by the binding of Wnt proteins to both Frizzled transmembrane receptors and to LRP-5 or LRP-6 (42). Upon binding, the Wnt signal is transduced into the cell. The engagement of the Wnt ligands to the Fz receptors induces differential phosphorylation of the cytoplasmic mediator, Dishevelled (Dsh; (47)), an event regulated by numerous protein kinases, including Par1 (48). Notably, suggestions have been forwarded that the interaction between Dsh and Fz may be mediated by a heterotrimeric G protein, such as Gαo (49). Nevertheless, existing evidences do not fully support the mechanistic basis for these protein interactions.

The distinguishing characteristic of canonical Wnt pathway activation is the augmentation of cytoplasmic levels of β-catenin. The available evidence indicates that the phosphorylated Dsh undergoes conformational changes which mediate the dissociation of

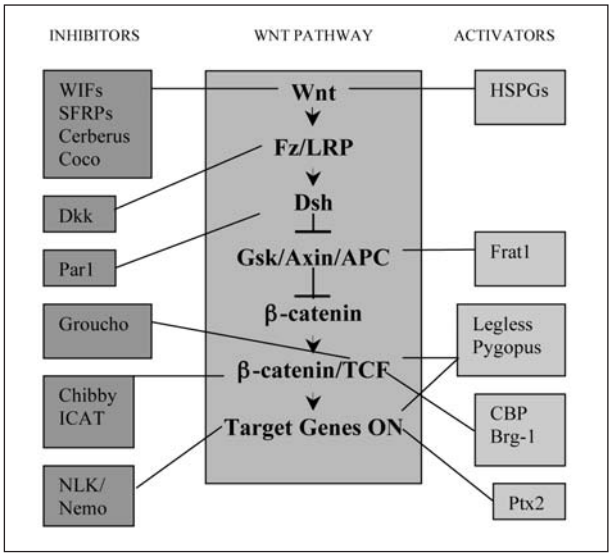

Figure 3. Schematic outline of the major components and modulators of the Wnt pathway
The central box in the diagram above indicates the main components of the Wnt pathway and their interactions. The boxes on the right hand side contain selected Wnt inhibitors, whereas the boxes on the left hand side show major molecules promoting the Wnt cascade.
NB. The lines locate the proteins within a specific step or to a specific molecule of the Wnt pathway on which they act

glycogen synthase kinase 3β (GSK3β), preventing β-catenin degradation (50, 51); Frat-1 may also be involved in the process (52). Thereby, the elevation of cytoplasmic β-catenin levels initiates its translocation into the cell nucleus.

Precisely how β-catenin is translocated into the cell nucleus is not clear, since it does not contain a clear nuclear localization sequence. Several studies have been carried out in order to identify the proteins responsible for β-catenin nuclear import. APC (adenomatous polyposis coli) has been suggested as a possible candidate (53-56); alternatively, or in addition, a recent report has proposed a plausible model in which pygopus, along with the protein Bcl9/legless, complexes with β-catenin and imports it into the cell nucleus (57).

In the nucleus, β-catenin interacts with the TCF/LEF family of transcription factors (58); perhaps surprisingly, these DNA-binding proteins, rather than β-catenin itself, bind to the DNA through a high mobility group (HMG) box and promote activation of target genes (59).

The final outcome of the Wnt/β-catenin canonical pathway is the activation of specific genes. The panoply of genes which are targeted by β-catenin is constantly growing. The importance of these genes becomes crucial because of the deep connection between the Wnt cascade activation and neoplastic transformation (Table 3).

Significantly, many Wnt genes encode transcription factors that are able to regulate Wnt activity by feedback loops. An example of this fine-tuning of the Wnt pathway at the cell surface is the regulation, by Wnt genes, of the levels of the Frizzleds receptors, LRPs and HSPGs (20, 42, 60).

In addition, the cytoplasmic negative regulators of the Wnt cascade, *naked cuticle* (*naked*) and *Axin2*, are direct targets of Wnt signalling.

It is surprising how the rigid Wnt pathway regulation steps clash with the wide range of transcriptional targets and outputs in multiple cells and tissue types. In fact it is thought to be the cell, rather than the Wnt signal itself, which determines the nature of the response depending on environmental or intrinsic aspects; therefore, the up or down-regulation of Wnt genes and subsequent transcriptional effects are cell-type specific.

The intricacies of the Wnt pathway regulation do not simply relate to individual steps of the cascade itself, but also reconnect to the multiple interactions of the Wnt cascade with other cell signalling pathways. The interaction between Wnt and other signalling pathways, for example the Hedgehog cascade, does not only have an antagonizing role, but may also promote a cellular collaboration which gives rise to different genetic outcomes. For further details, the reader is referred to several reviews on the topic (61-67).

Another important aspect that needs to be mentioned is the convergence of the Wnt/β-catenin cascade and Cadherin pathway (68). Notably, cytoplasmic β-catenin is not only associated with Wnt signalling, but it is also connected to type I cadherin adhesion molecules, mediating structural organization and interactions between cells. The signalling regulation of gap junction through β-catenin as an adher-

ance-junction protein has been shown to have profound implications in angiogenic processes in development and disease (69). Furthermore, the link between cadherins and β-catenin may not only be related to adhesion, but can also be used as a control tool for the Wnt cascade; cadherin can act to sequester β-catenin, preventing it from functioning in the Wnt signalling pathway, and vice versa, cadherin may release it from the cell membrane and thus make β-catenin available for Wnt signalling (70).

In the next section the role of Wnt signalling in development, stem cell maintenance and cancer will be elucidated. It is crucial to remember that to perform these functions the Wnt pathway may intersect and synergize with the cadherin cascade and other signalling pathways involved in cell adhesion and migration.

3.3 Development, adulthood and diseases: The variety of functions (and malfunctions) of the Wnt signalling pathway

At the onset of Wnt research, model organisms such as *Drosophila* and *Xenopus* were powerful experimental tools for the investigation of Wnt pathway components and functions. The need to relating the information coming from these organisms to humans called for the adoption of mice models to bridge the evolutionary gap for signalling pathway analysis. *Zebrafish* has recently acquired a major role as vertebrate model for the study of Wnt-related human pathologies and cancer.

In the Wnt field, a plethora of discoveries have been made using these models; developmental functions and pathological transformations driven by Wnt molecules and downstream effectors have been discovered via genetic experiments in lower organisms. Mutant phenotypes, outcome of experimental methods including gene knock-out, gene gain-of function, mutant and transgenic lines, chemical mutagenesis, RNA interference and morpholino antisense oligonucleotides injections, targeting Wnt pathway components have been detailed, interpreted and related to the functional role of individual Wnt molecules. Their functions have been a paradigm to obtain a global picture of the Wnt signalling pathway.

3.3.1 Wnt signalling pathway harnesses the secret of life

In the light of the developmental studies undertaken on the Wnt pathway, the functions of the Wnt cascade in adulthood have been investigated.

One characteristic of the Wnt pathway that encompass both embryogenesis and adulthood is the ability of the Wnt signal to augment a progenitor cell population by simultaneously endorsing cell division and inhibiting apoptosis and cellular commitment. This observation has been made in central nervous system development and function, as well as in gut and lung development and intestinal and bronchial epithelia maintenance (71).

These findings, together with the data from studies in human haematopoiesis (72), prompt for the establishment of a role for the Wnt pathway in stem cell biology.

Much information about stem cells comes from studies of the haematopoietic stem cells (HSC) (73), that sustain the production of blood cells and cells of the immune system during the lifetime of an individual. Although Wnt signalling is important in the maintenance of multiple stem cells types (gut, epithelial, etc.), as well as pluripotent human and mouse embryonic stem cells, HSC will be used as example to define the role of the Wnt cascade in the preservation of adult tissue homeostasis via control of stem cell proliferation and differentiation.

HSC use Wnt ligands in an autocrine and paracrine manner, by producing Wnt molecules themselves and by responding to the Wnt signals present in the HSC native microenvironment (the so-called stem cell niche). *In vitro* experiments in mice suggest that Wnt proteins promote the growth of haematopoietic progenitors and maintain them in an undifferentiated state. In addition, they are able to promote murine progenitor cells and HSC self-renewal, enabling repopulation of the haematopoietic system after lethal irradiation (72, 74). Furthermore, T-cell development and B-cell survival are mediated by the Wnt signalling cascade (75, 76).

Intriguingly, it is not yet clear whether the role of Wnt in stem cell expansion is mediated by the canonical or non-canonical Wnt cascades, since β-catenin has been demonstrated to be dispensable for

haematopoiesis and lymphopoiesis (77). Although β-catenin has been found to be not-essential for various Wnt pathway roles in development and adulthood, it does not necessarily mean that the canonical Wnt cascade is not implicated in those functional activities; rather, there could be compensatory mechanisms and redundancies at the molecular level.

Other stem cell niche-derived signalling pathways exist, such as the Hedgehog (Hh), BMP and Notch cascades; it would be interesting to investigate the extent of the cross-talk and convergence between the pathways and their distinct roles in stem cells expansion.

3.3.3 Wnt signalling and diseases: "With great power can come dire consequences"

Loss of control of a signalling cascade with such diverse functions as Wnt certainly has disastrous consequences on human health. While Wnt signals simultaneously promote growth and prevent differentiation of stem cells, one small insult can disrupt the balance from a pool of undifferentiated stem cells to one of aberrant cancer cell.

Following on from the previous chapter, considering the influence of Wnt pathway components on HSC, Wnt dysregulation has been directly associated with the development of leukemias. The same parallel can be drawn in several other cases: colorectal cancer and proliferating progenitors in the colonic epithelium, adenomas and intestinal crypt progenitor stem cells and, in regard to certain developmental abnormalities, a connection could be made going all the way back to embryonic stem cells.

The molecular events responsible for oncogenic transformation by altered Wnt signalling occur upon its aberrant activation.

Constitutive activation of the Wnt cascade confers to the cell two essential requirements for carcinogenesis: selective growth advantage for the onset of clonal expansion and genetic instability to allow further mutations to be accumulated by the cancer cells, facilitating its advancement and spreading. β-catenin can therefore be seen as a proto-oncogene; its ectopic activation may be caused by direct mutation of the β-catenin gene CTNNB1, often preventing β-catenin degradation, or by mutation to the Wnt pathway's intracellular antagonists APC and Axin; both events lead to the ectopic accumulation of β-catenin within the cell and the overexpression of several Wnt target genes responsible for cell malignancy and modulators of metastatis.

Hence, the real mediators of oncogenesis are the target genes of the Wnt cascades; examples of genes transcribed when the Wnt cascade is activated include the proto-oncogenes *c-myc*, which stimulate G1/S phase progression, *cyclinD1* that also promotes progression through the cell cycle, *survivin*, an anti-apoptotic gene that leads to the accumulation of transformed cells, and *c-Met*, the receptor for an epithelial growth factor that promotes cell migration and invasion. Thus, direct connections can be drawn between the function of Wnt target genes and specific features of cancer cells. Moreover, each Wnt target gene affects numerous cellular processes and may contribute to further neoplastic changes.

Similarly, a crucial role in tumourigenesis is played by the genes silenced by the activation of the Wnt pathway; the inhibition of such genes has been associated with loss of terminal differentiation and insensitivity to growth inhibitors, both key features of many tumours.

Loss-of-function of APC is a significant cause of colorectal cancer (CRC), accounting for 85% of all sporadic CRC. It is also responsible for a rare human inherited autosomal dominant condition, called familial adenomatous polyposis (FAP; (78)). FAP consists of multiple colorectal adenomas and increased risk of malignancies affecting the brain and the thyroid. This is a well-studied example of degenerative disease caused by misregulation of the Wnt pathway.

Loss-of-function mutation in the LRP5 receptor gene, due to a single amino-acid substitution, makes it refractory to Dkk-mediated Wnt canonical cascade inhibition, leading to bone abnormalities and to familial exudative vitreopathy (FEVR), a retinal hereditary disorder (79, 80). Alzheimer's disease and cardiovascular pathologies have also been attributed to a malfunctioning Wnt pathway (Table 3) (81, 82).

The widespread occurrence of the Wnt pathway in human physiological and pathological processes makes this pathway one of the most pivotal cellular signalling cascades; it can be said that proper function

Table 3. Wnt pathway genes are linked to diverse human pathologies. The spatial and functional eclecticism of the Wnt signalling cascade is mirrored by the wide range of diverse diseases caused by the pathway's misregulation. Therefore, the Wnt pathway finds itself not only implicated in carcinogenesis, but also in numerous other diseases, which vary in their diffusion, intensity and pathology. Table from Table 1 of a comprehensive review by (83); also refer to the paper for references

WNT signalling components	Conditions/diseases
Wnt1	Schizophrenia
Wnt3	Tetra-amelia
Wnt4	Intersex phenotype Kidney damage and Polycystic kidney disease
SFRP3	Osteoarthritis
Fz4	Familial Exudative Vitreopathy (FEVR)
LPR5	FEVR Low or high bone mass
Axin2	Tooth agenesis
β-catenin	Aggressive fibromatosis Pulmonary fibrosis

and tight control of the Wnt pathway are essential requirements for physiological "immortality".

3.4 The forthcoming "WNT Storm": Therapeutical applications and future perspectives

The collaboration between medicine and science has initiated a new era for cancer treatments and therapies. Chemotherapy, an indispensable step in the treatment of almost all cancers in advanced stage, is going through a paradigm change. The cancer drugs and combinational therapies currently used, which act on fast dividing cells by non-selective inhibition of DNA replication, have enhanced patient survival over the past decades. Yet these cytotoxic agents, administrated daily to thousand of patients all over the world, result in substantial toxicity causing heavy side effect. New agents against cancer- and cell-specific targets represent the future weapons in the battle against cancer. In this perspective, the investigation of the Wnt pathway becomes an invaluable tool for the search of new anticancer therapeutic targets and for the emerging translational therapy.

Imatinib Mesylate (IM, Gleevec) is probably the best example of a rationally-designed "molecular" drug, manufactured on the basis of research data available on cancer. IM inhibits the oncogenic fusion protein product of the leukemogenic BCR-ABL translocation involved in chronic myelogenous leukemia (CML). This molecular event causes the uncontrolled growth of white blood cells. Recently, it has been observed that IM also leads to down-regulation of β-catenin in human colorectal cancer cells. Although this is not the primary effect of IM, it indicates that targeting tyrosine kinases alters the phosphorylation steps required for correct Wnt pathway signalling (84).

Endostatin is a protein able to inhibit blood vessel formation, leading to a reduced blood supply to the tumour and consequential apoptosis of cancer cells. Similarly to Glivec, the specificity of this drug moderates its side effects. Furthermore, the compound has been implicated in modulating the Wnt cascade via stimulation of β-catenin degradation (85).

Other drugs that target β-catenin, promoting its degradation, are Indomethacin, Sulindac and Aspirin; these non-steroidal anti-inflammatory drugs (NSAIDs) also prevent the transcription of target genes, like *Cyclin D1*, halting tumour growth (see review by (86)).

Therapies designed against Wnt pathway genetic targets are not the only option for "molecular medicine". Alternative approaches, based on biological experimental methods, include the use of oncolytic viruses to selectively lyse malignant cells, and of gene therapy, using non-replicating viruses to introduce a non-viral toxic gene, causing the death of target cells. However, there is a major concern over the use of these biologically-based mechanisms, due to the possible bio-hazard that the release of viruses represents, and regarding the cost and duration of their production, which is extremely high compared to other therapeutical methods.

The application of low molecular weight organic molecules to prevent the activation of TCF/β-catenin target genes may offer a new therapeutic strategy, yet it still requires refinement regarding toxicity and efficacy of the compounds.

Numerous considerations need to be pondered before the clinical application of the above anti-cancer

therapeutics will be confirmed and achieved. However, being able to target components of the Wnt pathway aberrantly modulated during carcinogenesis has profound implications on medical oncology and scientific research in general, albeit no "anti-Wnt pathway" cancer drugs are currently in use.

Nevertheless, the progress and improvement of therapies based on the Wnt pathway strictly depend on the advancements made in the understanding of the Wnt signalling cascade and on the prompt translation of the scientific data into clinical implements.

Several pivotal questions still remain to be addressed regarding crucial steps and mechanisms of the Wnt pathway. Firstly, there are still uncertainties about steps of the molecular Wnt cascade. The mechanisms by which the frizzled receptor transfers the Wnt signal into the cell via activation of Dishevelled, and the subsequent deactivation of the β-catenin destruction complex by Dsh remain unclear. The phosphorylation events occurring throughout the pathway are vital to the functions of the cascade, but the modalities of control over these steps remain elusive. In this regard, it will also be of great importance to determine how β-catenin is transported into the nucleus, since the process could be an ideal target to halt the Wnt target genes transcriptional activation.

Another major challenge will be to shed light on the specificity of the Wnt pathway. Various Wnt ligands can interact with different Frizzled receptors, untimately leading to the transcriptional activation of several target genes leading to diverse outcomes in different cell types. It will therefore be interesting to ascertain whether the different ligand-receptor pairs are responsible for the pathway specificity or else the outcome is determined solely by the cellular contexts. Furthermore, taking into account that some target genes can be promoted in multiple cell types and tissues, it will be of key importance to establish if these genes are "universal" Wnt pathway targets. The answer to these questions will provide a better understanding of how the Wnt cascade is able to control the complex balance between differentiation, proliferation and migration.

In the near future, new models of gene activation may be found for Wnt target genes that do not contain a TCF/LEF binding site in the gene promoter region. In this instance, nuclear β-catenin has been shown to mediate gene expression through other molecules, such as Sox17 in the specification of endoderm (87); it is likely that a similar mechanism applies to other cellular situations.

The identification of unknown players related to the Wnt cascade will give new important insights, in a near future.

One of the main aspects regards the Wnt connection to tumourigenesis. Predictive screening strategies can be designed by the identification of function-altering polymorphisms in different tumour types and by measurement and detection of nuclear β-catenin levels. In addition, the analysis of the endogenous differences between the responses that cancer cells have to selected anti-Wnt compounds may provide new clues about the cellular activity of the Wnt cascade.

The many unanswered questions suggest that, despite incredible progress has been made in the present-day understanding of this key pathway, the view to which we have currently arrived is simply the beginning of the story. A path has now been found, but the road is still long, and a network of ways needs to be explored to learn more about the functional and mis-functional roles the Wnt pathway in humans. The scientific knowledge gathered along these roads may improve therapeutic modalities and optimise prevention, endeavouring to fight many diseases, above all, cancer.

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References

1. Willert K, et al. Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* 2003; 423 (6938): 448-52.

2. Urist MR, et al. Transmembrane bone morphogenesis across multiple-walled diffusion chambers. New evidence for a diffusible bone morphogenetic property. *Arch Surg* 1977; 112 (5): 612-9.
3. McMahon AP, RT. Moon, int-1-a proto-oncogene involved in cell signalling. *Development* 1989; 107 Suppl: 161-7.
4. Sharma RP, Chopra VL. Effect of the Wingless (wg1) mutation on wing and haltere development in Drosophila melanogaster. *Dev Biol* 1976; 48 (2): 461-5.
5. Nusse R, Varmus HE. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. *Cell* 1982; 31 (1): 99-109.
6. Nusse R, et al. Mode of proviral activation of a putative mammary oncogene (int-1) on mouse chromosome 15. *Nature* 1984; 307 (5947): 131-6.
7. Rijsewijk F, et al. The Drosophila homolog of the mouse mammary oncogene int-1 is identical to the segment polarity gene wingless. *Cell* 1987; 50 (4): 649-57.
8. Nusse R, Varmus HE. Wnt genes. *Cell* 1992. 69(7): 1073-87.
9. Grimson MJ, et al. Adherens junctions and beta-catenin-mediated cell signalling in a non-metazoan organism. *Nature* 2000; 408 (6813): 727-31.
10. Amador V, et al. Gibberellins signal nuclear import of PHOR1, a photoperiod-responsive protein with homology to Drosophila armadillo. *Cell* 2001; 106 (3): 343-54.
11. Hobmayer B, et al. WNT signalling molecules act in axis formation in the diploblastic metazoan Hydra. *Nature* 2000; 407 (6801): 186-9.
12. Wikramanayake AH, et al. An ancient role for nuclear beta-catenin in the evolution of axial polarity and germ layer segregation. *Nature* 2003; 426 (6965): 446-50.
13. Adell T, Nefkens I, Muller WE. Polarity factor "Frizzled" in the demosponge Suberites domuncula: identification, expression and localization of the receptor in the epithelium/pinacoderm(1). *FEBS Lett* 2003; 554 (3): 363-8.
14. Wodarz A, Nusse R. Mechanisms of Wnt signaling in development. *Annu Rev Cell Dev Biol* 1998; 14: 59-88.
15. Miller JR, The Wnts. *Genome Biol* 2002; 3 (1): Reviews3001.
16. Nusse R. An ancient cluster of Wnt paralogues. *Trends Genet* 2001; 17 (8): 443.
17. Prud'homme B, et al. Phylogenetic analysis of the Wnt gene family. Insights from lophotrochozoan members. *Curr Biol* 2002; 12 (16): 1395.
18. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol* 2004; 20: 781-810.
19. Kadowaki T, et al. The segment polarity gene porcupine encodes a putative multitransmembrane protein involved in Wingless processing. *Genes Dev* 1996; 10 (24): 3116-28.
20. Cadigan KM, et al. Wingless repression of Drosophila frizzled 2 expression shapes the Wingless morphogen gradient in the wing. *Cell* 1998; 93 (5): 767-77.
21. Strigini M, Cohen SM. A Hedgehog activity gradient contributes to AP axial patterning of the Drosophila wing. *Development* 1997; 124 (22): 4697-705.
22. Greco V, Hannus M, Eaton S. Argosomes: a potential vehicle for the spread of morphogens through epithelia. *Cell* 2001; 106 (5): 633-45.
23. Hsiung F, et al. Dependence of Drosophila wing imaginal disc cytonemes on Decapentaplegic. *Nature* 2005; 437 (7058): 560-3.
24. Lin X, Perrimon N. Role of heparan sulfate proteoglycans in cell-cell signaling in Drosophila. *Matrix Biol* 2000; 19 (4): 303-7.
25. Tsuda M, et al. The cell-surface proteoglycan Dally regulates Wingless signalling in Drosophila. *Nature* 1999; 400 (6741): 276-80.
26. Lin X, Perrimon N. Dally cooperates with Drosophila Frizzled 2 to transduce Wingless signalling. *Nature* 1999; 400 (6741): 281-4.
27. Alexander CM, et al. Syndecan-1 is required for Wnt-1-induced mammary tumorigenesis in mice. *Nat Genet* 2000; 25 (3): 329-32.
28. Christian JL. BMP, Wnt and Hedgehog signals: how far can they go? *Curr Opin Cell Biol* 2000; 12 (2): 244-9.
29. Rattner A, et al. A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. *Proc Natl Acad Sci USA* 1997; 94 (7): 2859-63.
30. Hoang B, et al. Primary structure and tissue distribution of FRZB, a novel protein related to Drosophila frizzled, suggest a role in skeletal morphogenesis. *J Biol Chem* 1996; 271 (42): 26131-7.
31. Hsieh JC, et al. A new secreted protein that binds to Wnt proteins and inhibits their activities. *Nature* 1999; 398 (6726): 431-6.
32. Bafico A, et al. Interaction of frizzled related protein (FRP) with Wnt ligands and the frizzled receptor suggests alternative mechanisms for FRP inhibition of Wnt signaling. *J Biol Chem* 1999 274 (23): 16180-7.
33. Dennis S, et al. A secreted frizzled related protein, FrzA, selectively associates with Wnt-1 protein and regulates wnt-1 signaling. *J Cell Sci* 1999; 112 (Pt 21): 3815-20.
34. Leyns L, et al. Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. *Cell* 1997; 88 (6): 747-56.
35. Finch PW, et al. Purification and molecular cloning of a secreted, Frizzled-related antagonist of Wnt action. *Proc Natl Acad Sci USA* 1997; 94 (13): 6770-5.
36. Uren A, et al. Secreted frizzled-related protein-1 binds directly to Wingless and is a biphasic modulator of Wnt signaling. *J Biol Chem* 2000; 275 (6): 4374-82.
37. Piccolo S, et al. The head inducer Cerberus is a multifunctional antagonist of Nodal, BMP and Wnt signals. *Nature* 1999; 397 (6721): 707-10.
38. Bell E, et al. Cell fate specification and competence by Coco, a maternal BMP, TGFbeta and Wnt inhibitor. *Development* 2003; 130 (7): 1381-9.
39. Bhanot P, et al. A new member of the frizzled family from Drosophila functions as a Wingless receptor. *Nature* 1996; 382 (6588): 225-30.

40. Wang YK, et al. A novel human homologue of the Drosophila frizzled wnt receptor gene binds wingless protein and is in the Williams syndrome deletion at 7q11.23. *Hum Mol Genet* 1997; 6 (3): 465-72.
41. Hsieh JC, et al. Biochemical characterization of Wnt-frizzled interactions using a soluble, biologically active vertebrate Wnt protein. *Proc Natl Acad Sci USA* 1999; 96 (7): 3546-51.
42. Wehrli M, et al. Arrow encodes an LDL-receptor-related protein essential for Wingless signalling. *Nature* 2000; 407 (6803): 527-30.
43. Strutt D. Frizzled signalling and cell polarisation in Drosophila and vertebrates. *Development* 2003; 130 (19): 4501-13.
44. Veeman MT, Axelrod JD, Moon RT. A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. *Dev Cell* 2003; 5 (3): 367-77.
45. Willert K, Nusse R. Beta-catenin: a key mediator of Wnt signaling. *Curr Opin Genet Dev* 1998; 8 (1): 95-102.
46. Giles RH, van Es JH, Clevers H. Caught up in a Wnt storm: Wnt signaling in cancer. *Biochim Biophys Acta* 2003; 1653 (1): 1-24.
47. Yanagawa S, et al. The dishevelled protein is modified by wingless signaling in Drosophila. *Genes Dev* 1995; 9 (9): 1087-97.
48. Sun TQ, et al. PAR-1 is a Dishevelled-associated kinase and a positive regulator of Wnt signalling. *Nat Cell Biol* 2001; 3 (7): 628-36.
49. Liu T, et al. G protein signaling from activated rat frizzled-1 to the beta-catenin-Lef-Tcf pathway. *Science* 2001; 292 (5522): 1718-22.
50. Yost C, et al. GBP, an inhibitor of GSK-3, is implicated in Xenopus development and oncogenesis. *Cell* 1998; 93 (6): 1031-41.
51. Chen W, et al. Dishevelled 2 recruits beta-arrestin 2 to mediate Wnt5A-stimulated endocytosis of Frizzled 4. Science 2003; 301 (5638): 1391-4.
52. Li L, et al. Axin and Frat1 interact with dvl and GSK, bridging Dvl to GSK in Wnt-mediated regulation of LEF-1. *Embo J* 1999; 18 (15): 4233-40.
53. Fagotto F, Gluck U, Gumbiner BM. Nuclear localization signal-independent and importin/karyopherin-independent nuclear import of beta-catenin. *Curr Biol* 1998; 8 (4): 181-90.
54. Rosin-Arbesfeld R, Townsley F, Bienz M. The APC tumour suppressor has a nuclear export function. *Nature* 2000; 406 (6799): 1009-12.
55. Neufeld KL, et al. Adenomatous polyposis coli protein contains two nuclear export signals and shuttles between the nucleus and cytoplasm. *Proc Natl Acad Sci USA* 2000; 97 (22): 12085-90.
56. Neufeld KL, et al. APC-mediated downregulation of beta-catenin activity involves nuclear sequestration and nuclear export. *EMBO Rep* 2000; 1 (6): 519-23.
57. Townsley FM, Cliffe A, Bienz M. Pygopus and Legless target Armadillo/beta-catenin to the nucleus to enable its transcriptional co-activator function. *Nat Cell Biol* 2004; 6 (7): 626-33.
58. Waterman ML. Lymphoid enhancer factor/T cell factor expression in colorectal cancer. *Cancer Metastasis Rev* 2004; 23 (1-2): 41-52.
59. Giese K, Cox J, Grosschedl R. The HMG domain of lymphoid enhancer factor 1 bends DNA and facilitates assembly of functional nucleoprotein structures. *Cell* 1992; 69 (1): 185-95.
60. Baeg GH, et al. Heparan sulfate proteoglycans are critical for the organization of the extracellular distribution of Wingless. *Development* 2001; 128 (1): 87-94.
61. Glise B, Jones DL, Ingham PW. Notch and Wingless modulate the response of cells to Hedgehog signalling in the Drosophila wing. *Dev Biol* 2002; 248 (1): 93-106.
62. Hayward P, et al. Notch modulates Wnt signalling by associating with Armadillo/beta-catenin and regulating its transcriptional activity. *Development* 2005; 132 (8): 1819-30.
63. Hu H, et al. Sequential roles of Hedgehog and Wnt signaling in osteoblast development. *Development* 2005; 132 (1): 49-60.
64. Huang HC, Klein PS. Interactions between BMP and Wnt signaling pathways in mammalian cancers. *Cancer Biol Ther* 2004; 3 (7): 676-8.
65. Taipale J, Beachy PA. The Hedgehog and Wnt signalling pathways in cancer. *Nature* 2001; 411 (6835): 349-54.
66. Watt FM. Unexpected Hedgehog-Wnt interactions in epithelial differentiation. *Trends Mol Med* 2004; 10 (12): 577-80.
67. Zhou S, Eid K, Glowacki J. Cooperation between TGF-beta and Wnt pathways during chondrocyte and adipocyte differentiation of human marrow stromal cells. *J Bone Miner Res* 2004; 19 (3): 463-70.
68. Peifer M, et al. A model system for cell adhesion and signal transduction in Drosophila. *Dev Suppl* 1993: 163-76.
69. Clifford RL, Deacon K, Knox AJ. Novel regulation of vascular endothelial growth factor-A (VEGF-A) by transforming growth factor (beta)1: requirement for Smads, (beta)-catenin, and GSK3(beta). *J Biol Chem* 2008; 283 (51): 35337-53.
70. Nelson WJ, Nusse R. Convergence of Wnt, beta-catenin, and cadherin pathways. *Science* 2004; 303 (5663): 1483-7.
71. Reya T, Clevers H. Wnt signalling in stem cells and cancer. *Nature* 2005; 434 (7035): 843-50.
72. Austin TW, et al. A role for the Wnt gene family in hematopoiesis: expansion of multilineage progenitor cells. *Blood* 1997; 89 (10): 3624-35.
73. Staal FJ, Clevers HC. WNT signalling and haematopoiesis: a WNT-WNT situation. *Nat Rev Immunol* 2005; 5 (1): 21-30.
74. Reya T, et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* 2003; 423 (6938): 409-14.
75. Reya T, et al. Wnt signaling regulates B lymphocyte proliferation through a LEF-1 dependent mechanism. *Immunity* 2000; 13 (1): 15-24.
76. Xu Y, et al. Deletion of beta-catenin impairs T cell development. *Nat Immunol* 2003; 4 (12): 1177-82.

77. Cobas M, et al. Beta-catenin is dispensable for hematopoiesis and lymphopoiesis. *J Exp Med* 2004; 199 (2): 221-9.
78. Nakamura Y, et al. Mutations of the adenomatous polyposis coli gene in familial polyposis coli patients and sporadic colorectal tumors. *Princess Takamatsu Symp* 1991; 22: 285-92.
79. Boyden LM, et al. High bone density due to a mutation in LDL-receptor-related protein 5. *N Engl J Med* 2002; 346 (20): 1513-21.
80. Toomes C, et al. Mutations in LRP5 or FZD4 underlie the common familial exudative vitreoretinopathy locus on chromosome 11q. *Am J Hum Genet* 2004; 74 (4): 721-30.
81. Anderton BH. Alzheimer's disease: clues from flies and worms. *Curr Biol* 1999; 9 (3): R106-9.
82. van Gijn ME, et al. The wnt-frizzled cascade in cardiovascular disease. *Cardiovasc Res* 2002; 55 (1): 16-24.
83. Moon RT, et al. WNT and beta-catenin signalling: diseases and therapies. *Nat Rev Genet* 2004; 5 (9): 691-701.
84. Zhou L, et al. Tyrosine kinase inhibitor STI-571/Gleevec down-regulates the beta-catenin signaling activity. *Cancer Lett* 2003; 193 (2): 161-70.
85. Hanai J, et al. Endostatin is a potential inhibitor of Wnt signaling. *J Cell Biol* 2002; 158 (3): 529-39.
86. Dihlmann S, von Knebel Doeberitz M. Wnt/beta-catenin-pathway as a molecular target for future anti-cancer therapeutics. *Int J Cancer* 2005; 113 (4): 515-24.
87. Sinner D, et al. Sox17 and beta-catenin cooperate to regulate the transcription of endodermal genes. *Development* 2004; 131 (13): 3069-80.

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