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Can we safely target the WNT pathway?

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Abstract

WNT- β -catenin signalling is involved in a multitude of developmental processes and the maintenance of adult tissue homeostasis by regulating cell proliferation, differentiation, migration, genetic stability and apoptosis, as well as by maintaining adult stem cells in a pluripotent state. Not surprisingly, aberrant regulation of this pathway is therefore associated with a variety of diseases, including cancer, fibrosis and neurodegeneration. Despite this knowledge, therapeutic agents specifically targeting the WNT pathway have only recently entered clinical trials and none has yet been approved. This Review examines the problems and potential solutions to this vexing situation and attempts to bring them into perspective.

The evolutionarily conserved WNT- β -catenin pathway initiates a signalling cascade that is crucial during both normal embryonic development and throughout the life of the organism in virtually every tissue and organ system. It is an enormously complex and ancient pathway that dates back to the first anaerobic metazoans. The WNT- β -catenin network was first identified in 1982 with the discovery of the proto-oncogene *Int1* (now known as *Wnt1*) in mice¹. Five years later, the segment polarity gene *wingless*, the *Drosophila melanogaster* homolog of *Int1*, was cloned and shown to be required for proper wing formation². Injection of INT1 caused axis duplication in *Xenopus laevis* embryos, thereby demonstrating the highly conserved nature of the pathway³. Since then, the crucial role of WNT- β -catenin signalling in development has been demonstrated in an array of organ systems, including the brain, eye, spinal cord, bone, cartilage, skin, lung, teeth, mammary gland, gut, heart, liver, kidney, pancreas and the haematopoietic and reproductive systems^{4,5}. Moreover, WNT signalling has crucial roles in adulthood: in the daily processes of tissue homeostasis and regeneration in the hair and skin⁶, in the maintenance of intestinal homeostasis⁷ and in haematopoiesis^{8,9}. Furthermore, WNT- β -catenin signalling is involved in liver and lung repair after injury^{10–12} and in adult neurogenesis¹³. WNT signalling also has important roles in cell migration^{14,15}, genetic stability and instability^{16–18}, and apoptosis^{19,20}.

Given this vast array of functions, the WNT signalling cascade must therefore be tightly regulated to maintain proper tissue homeostasis, and aberrant WNT signalling has been

associated with many types of cancer^{21–23} as well as with other diseases including fibrosis^{24,25}, metabolic disease²⁶ and neurodegenerative disorders^{27–29}.

The significant role of aberrant WNT signalling in disease has engendered substantial efforts into the development of therapeutic approaches to target this pathway. However, a number of factors have thwarted progress in this field. First, the WNT signalling cascade is bewilderingly complex; there are 19 WNT ligands and more than 15 receptors and co-receptors distributed over seven protein families in mammals³⁰. Yet, this represents only the tip of the iceberg with regard to the difficulty in attempting to develop safe and effective specific therapeutics targeting the WNT pathway. For example, in addition to classical canonical WNT-induced activation of β -catenin–TCF (T-cell factor) transcriptional complexes, WNT proteins can elicit a variety of alternative responses, often grouped together as non-canonical WNT signalling³¹. Crosstalk from various non-WNT factors has also been reported to modulate nuclear β -catenin accumulation, as discussed in more detail below.

The ability to target the WNT signalling pathway offers enormous promise; however, like the sword of Damocles, substantial risks and concerns are ever present with regard to the targeting of such a crucial pathway in stem cell maintenance and tissue homeostasis. With this in mind, here we review recent efforts to modulate the WNT signalling cascade and critically analyse therapeutic approaches that are at various stages of development (FIG. 1).

Overview of the WNT signalling cascade

The WNT– β -catenin pathway initiates a signalling cascade that is crucial in both normal development and throughout life. Below, we discuss key aspects of the signalling cascade, and refer to several excellent recent reviews for more details^{30,32,33}.

WNTs are secreted, cysteine-rich glycoproteins that act as short-range ligands to locally activate receptor-mediated signalling pathways³⁰. The hallmark of this pathway is that it activates the transcriptional activity of the multifunctional armadillo repeat-containing protein β -catenin, which is the key mediator of WNT signalling. β -catenin dynamically localizes to multiple subcellular locations, including adherens junctions where it contributes to cell–cell contacts, the cytoplasm where its levels are tightly controlled, and the nucleus where it is involved in transcriptional regulation and chromatin modifications^{34,35}.

WNT morphogens are the central extracellular regulators of β -catenin dynamics; however, several other signalling cascades can also influence β -catenin dynamics, including hepatocyte growth factor (HGF; also known as scatter factor), prostaglandins, protein kinase A (PKA), E-cadherin, hypoxia, and so on^{36–38} (BOX 1). The cytoplasmic pool of β -catenin is tightly regulated via phosphorylation by the ‘destruction complex’, which includes glycogen synthase kinase 3β (GSK 3β), casein kinase 1α (CK 1α), the scaffold protein AXIN and the tumour suppressor adenomatous polyposis coli (APC), among others³⁹. In the absence of WNT signalling, phosphorylation marks cytoplasmic β -catenin for ubiquitylation and proteasomal degradation⁴⁰. A key step in the activation of WNT target genes is the formation of a complex between β -catenin and members of the TCF/LEF (lymphoid enhancer factor) family of transcription factors^{41,42}. To generate a transcriptionally active

complex, β -catenin–TCF recruits the transcriptional co-activator CREB-binding protein (CBP) or its closely related homologue E1A-associated protein p300, as well as other components of the basal transcription machinery^{43,44}.

Box 1

Alternative signalling cascades affecting β -catenin dynamics

The nuclear entry of β -catenin and the subsequent transcriptional processes affected by β -catenin are classically controlled by the so-called canonical WNT signalling cascade. However, in reality there are a number of alternative signalling pathways that can induce the nuclear translocation of β -catenin and its subsequent participation in transcription. The process of epithelial-to-mesenchymal transition (EMT) involves the downregulation of E-cadherin, which normally binds to cytoplasmic β -catenin in a complex with α -catenin that stabilizes epithelial architecture²⁶⁴; therefore, EMT is associated with the nuclear translocation of β -catenin²⁶⁵. EMT is a hallmark of metastasis²⁶⁶ and has also been linked to the generation of cancer stem cells and tumour-initiating cells²⁶⁷. A variety of receptor tyrosine kinases, including the receptors MET, FER, FYN and RON²⁶⁸, and non-receptor tyrosine kinases, including SRC²⁶⁹ and ABL²⁷⁰, can also disrupt the interaction of E-cadherin with β -catenin, thereby enhancing T cell factor (TCF)– β catenin transcription. Various G protein coupled receptor signal transduction pathways can activate TCF– β -catenin signalling, including prostaglandin E₂ (PGE₂) via the PGE₂ receptor EP2 and EP4 subtypes²⁷¹, PGF_{2 α} via the PGF receptor²⁷², leukotriene D₄ via the cysteinyl leukotriene receptors, as well as the activation of an array of G proteins such as G α , G α_{12} and G α_{13} (REFS 273,274). Additionally, environmental conditions such as hypoxia²⁷⁵ and high glucose levels²⁷⁶ can activate TCF– β -catenin signalling. Therefore, numerous signalling molecules and cascades also influence β -catenin dynamics and TCF– β -catenin transcription^{36–38}.

It is now clear that in addition to WNT– β -catenin-dependent WNT signalling (often referred to as ‘canonical’ signalling) (FIG. 1), there are β -catenin-independent outcomes (often referred to as ‘non-canonical’ signalling) within the WNT signalling cascade. It is worth noting that although designating WNT signalling pathways as either canonical or non-canonical may simplify their discussion, the reality is that crosstalk between these two ‘arms’ of the pathway, as well as other players, stimulates complex nonlinear networks (BOX 2); however, a detailed discussion of this is beyond the scope of this Review. For instance, HGF induces β -catenin stabilization in colorectal cancer cells via the MET-dependent inhibitory phosphorylation of Ser9 of GSK3 β ⁴⁵. Tyrosine phosphorylation of β -catenin by BCR–ABL in chronic myeloid leukaemia also leads to its stabilization and nuclear signalling activity by decreasing its binding affinity to E-cadherin⁴⁶.

Box 2

Non-canonical WNT signalling

The ‘canonical’ or β -catenin-dependent WNT signalling pathway has crucial roles in the regulation of diverse cell behaviours, including cell fate, proliferation and survival.

However, a second non-canonical pathway exists, whose major effects seem to be β -catenin-independent in that there is no apparent stabilization of cytoplasmic β -catenin. The non-canonical pathway is more associated with differentiation, cell polarity and migration²⁷⁷. Pathways affected by non-canonical signalling include calcium-dependent and small GTPase-dependent signalling networks and the planar cell polarity (PCP) signalling pathway^{278,279}, a pathway by which cells receive positional identity. Non-canonical signalling can be initiated by WNT–Frizzled receptor interactions; alternatively, RYK and ROR receptor tyrosine kinases can also act as WNT receptors to activate β -catenin-independent signalling³². β -catenin-independent signalling can also regulate small GTPases, such as RHOA, RAC and cell division control protein 42 (CDC42), in a Dishevelled-dependent manner²⁸⁰. Non-canonical WNT-activated calcium flux results in the activation of various kinase cascades, including protein kinase C (PKC), calcium/calmodulin-dependent protein kinase II (CaMKII) and JUN N-terminal kinase (JNK), and activates both AP1- and nuclear factor of activated T cells (NFAT) dependent transcription. Although dissection of the pathway into canonical and non-canonical components may be convenient for discussion purposes, the reality is that these are interacting and intersecting pathways that can coordinately regulate and orchestrate complex processes such as embryonic development, stem cell maintenance, tissue homeostasis and wound healing; moreover, when aberrantly regulated, they can be associated with tumorigenesis, metastasis and other diseases.

β -catenin is a member of the large armadillo protein family, which consists of three subfamilies: the p120 subfamily, the beta subfamily (comprising β -catenin and junction plakoglobin (also known as γ -catenin)) and the more distant alpha subfamily. Junction plakoglobin is involved in WNT–catenin signalling, even in the absence of β -catenin^{47–49}. Further adding to the complexity, β -catenin can bind to a broad spectrum of transcription factors other than TCF/LEF, thereby modulating a plethora of downstream biological processes including pluripotency, epithelial-to-mesenchymal transition, oxidative stress and melanocyte development⁵⁰.

The strict definition of canonical WNT signalling — using the TOP-Flash reporter assay as a read-out of β -catenin–TCF activity — might not capture all the important transcriptional changes caused by activated WNT signalling. A broader view of the nuclear role of β -catenin as a partner for many transcription factors (for example, forkhead box proteins (FOXOs), nuclear receptors, SOX, SMAD, and so on), gives a more complete picture⁵⁰. The broader role of WNT– β -catenin transcription must be taken into account when considering therapeutic strategies.

Role of the WNT– β -catenin pathway in disease

Given the crucial role of WNT signalling in virtually every organ system in normal homeostasis and repair after injury, it is not surprising that aberrant regulation of this signalling cascade is associated with an array of diseases. Beyond having an unequivocal role in multiple malignancies^{25,51}, aberrant WNT signalling apparently plays an important part in various other diseases, including neurological diseases, inflammatory and fibrotic

disease, and disorders of endocrine function and bone metabolism in adults^{52–54}. However, the ubiquitous nature of WNT signalling and its pleiotropic effects also raise considerable concern over the use of pathway agonists or antagonists to treat any particular indication, particularly in a chronic setting. The involvement of WNT signalling in various diseases is outlined below.

Cancer

Aberrant regulation of WNT signalling has emerged as a recurrent theme in cancer biology^{23,51}. Constituents of WNT signalling can basically be characterized as either positively or negatively acting components, where the negatively acting components that principally act to suppress tumorigenesis are found mutated or in a loss-of-function status in cancer, whereas the positive components are activated⁵¹. The discovery in 1991 that mutations in the tumour suppressor APC were associated with the vast majority of sporadic colorectal cancers via aberrant activation of WNT signalling provided considerable impetus to attempt to therapeutically target this pathway^{55,56}. Germline defects in APC cause familial adenomatous polyposis, in which affected individuals develop hundreds of polyps in the large intestine at an early age and ultimately progress to colorectal cancer with 100% penetrance⁵⁷. Loss of function in both alleles of APC is required for tumorigenesis and is linked to the protein's ability to regulate β -catenin protein stability⁵⁸ as well as chromosomal stability⁵⁹. APC is now noted as the most frequently mutated gene overall in human cancers^{60,61}. Mutations affecting the WNT pathway are not limited to colon cancer. For example, loss-of-function mutations in AXIN have been found in hepatocellular carcinomas, and oncogenic β -catenin mutations that were first described in colon cancer and melanoma⁶² were subsequently found to occur in a variety of solid tumours⁵³, including hepatocellular carcinomas⁶³, thyroid tumours⁶⁴ and ovarian endometrioid adenocarcinomas⁶⁵.

Epigenetic silencing is also frequently observed to alter levels of expression of negative regulators of the WNT– β -catenin pathway. For example, methylation of genes that encode putative extracellular WNT antagonists, such as the secreted Frizzled-related proteins (SFRPs), has been described in colon, breast, prostate, lung and other cancers^{66–70}. Increased expression of WNT ligands^{71–73} or effector proteins, including Dishevelled (DVL), has also been described^{74–76}. Tumour formation and progression has also been associated with aberrant β -catenin-independent WNT signalling⁷⁷.

However, even the simple notion that targeting aberrantly high WNT– β -catenin signalling in cancer would be universally beneficial has been called into question. For instance, although several studies have correlated increased WNT– β -catenin signalling in tumours with worse prognosis for patients with colorectal cancer^{78–80}, elevated WNT signalling does not correlate with reduced patient survival in all types of cancer. As a case in point, in patients with melanoma, active WNT– β -catenin signalling — as judged by nuclear β -catenin in tumours — is associated with a lower proliferative index and correlates with a more favourable prognosis^{81–83}. Moon and colleagues²³ have presented further evidence that WNT signalling can either promote or inhibit tumour initiation, growth, metastases and drug resistance in a cancer-stage-specific and a cancer-type-specific manner. For example, in a

mouse model of melanoma, overexpression of WNT3A results in a less aggressive disease phenotype with increased expression of melanocyte differentiation markers⁸⁴. Genetic evidence also supports a crucial role for the levels of β -catenin signalling in dictating tissue-specific predisposition to APC-driven tumorigenesis⁸⁵. Together, these studies further add to the challenges and complexity of developing safe and effective therapeutic agents targeting this complex cascade.

Neurological diseases

The importance of WNT signalling during embryonic development of the central nervous system is well established⁸⁶. The WNT pathway also regulates nervous system patterning and the regulation of neural plasticity. WNTs also have a role in axon guidance as well as in influencing synapse formation⁸⁷. Therefore, it is not surprising that aberrations in WNT signalling have been observed in neurological diseases in adulthood. For example, a Scottish family with a high incidence of schizophrenia, depression and bipolar disorder was found to carry a balanced chromosomal translocation involving the gene *DISC1* (disrupted in schizophrenia 1)⁸⁸. Subsequently, the protein product of *DISC1* was found to have an important role in neural development and neural progenitor proliferation⁸⁹. *DISC1* directly interacts with and inhibits GSK3 β activity, thereby enhancing β -catenin-mediated transcription⁹⁰.

Neuroanatomical observations and functional magnetic resonance imaging (MRI) have indicated that a major pathological hallmark in autistic individuals may be a premature overgrowth of the cerebral cortex, hippocampus, amygdala and cerebellum^{91–94}. Interestingly, transgenic mice expressing a constitutively active form of β -catenin in neuronal precursor cells developed a grossly enlarged cerebral cortex, hippocampus and amygdala^{95,96}. Importantly, microdeletion and microduplication copy number variations of genes involved in the canonical WNT signalling pathway (for example, Frizzled 9 (*FZD9*), B cell lymphoma 9 (*BCL9*) or cadherin 8 (*CDH8*)) are found in patients with autism spectrum disorder. Association studies investigating *WNT2*, *DISC1*, *MET*, dedicator of cytokinesis protein 4 (*DOCK4*) or Abelson helper integration site 1 (*AH11*; also known as joubertin) have provided additional evidence that the canonical WNT pathway might be affected in autism⁹⁷.

The WNT signalling cascade has also been implicated in Alzheimer's disease. Presenilin proteins, which have been associated with early-onset Alzheimer's disease, are negative regulators of canonical WNT signalling⁹⁸. Variant alleles in the WNT receptor LRP6 (low-density lipoprotein receptor-related protein 6) have been associated with Alzheimer's disease in population-based linkage analyses⁹⁹. This suggests that multiple mechanisms leading to aberrant WNT-mediated regulation of adult neurogenesis may be associated with Alzheimer's disease^{100–102}. As the underlying cause (or causes) of Alzheimer's disease have not been clearly elucidated, the mechanisms whereby aberrant WNT regulation may have a role in Alzheimer's disease are also not known. WNT signalling is involved in brain vascularization and blood–brain barrier formation¹⁰³, in synaptogenesis¹⁰⁴, in amyloid- β -induced neuroinflammation and neurotoxicity¹⁰⁵, as well as in neuronal degeneration^{106,107}.

Aberrant regulation of any or all of these processes could contribute to disease initiation and progression.

WNTs have also been implicated in neural tube defects. *Dvl2*-mutant mice are born with thoracic spina bifida and mice with mutations in both *Dvl1* and *Dvl2* have more severe neural tube defects¹⁰⁸. Mutations in AXIN can result in incomplete closure of the neural tube or malformation of the head folds¹⁰⁹. Aberrant LRP6 signalling (hypoactivity, hyperactivity or missense mutations in *LRP6*) can also cause neural tube defects^{110–112}. Interestingly, it has been demonstrated that a mutation in *LRP6* causes the crooked tail mutation in mice that exhibit neural tube defects, which can be ameliorated by dietary intake of folic acid¹¹⁰. The benefits of folic acid in preventing neural tube defects are well documented in human development, so understanding the role of WNT signalling in neurulation has important clinical implications.

Finally, WNTs have also been implicated in Williams syndrome, which is caused by a heterozygous microdeletion of approximately 20 genes on chromosome 7 (REF. 113). The *FZD9* gene is within this chromosomal deletion in many patients. This neurodevelopmental disorder is characterized by an effusive personality, enhanced language ability, preserved social function, a high incidence of seizures and impaired spatial cognition¹¹⁴. Interestingly, *Fzd9*^{-/-} mice have neurodevelopmental defects in the dentate gyrus, visuospatial memory defects and a lowered seizure threshold¹¹⁵. *Fzd9*^{+/-} mice, which have the same genotype for this gene as patients with Williams syndrome, have an intermediate form of this dysfunction¹¹⁵. Therefore, it is quite likely that loss of FZD9 is responsible for some of the cognitive symptoms observed in patients with Williams syndrome.

Osteoporosis

The WNT pathway is also crucial for bone formation in adults. For example, the WNT co-receptor LRP5 has been linked to osteoporosis pseudoglioma syndrome¹¹⁶, an autosomal recessive disorder in which individuals have low bone mass and abnormal eye vasculature, and are predisposed to developing skeletal fractures. By contrast, a gain-of-function point mutation in *LRP5*, G171V, has been associated with increased muscle mass and skeletal strength in humans¹¹⁷. A direct role for WNT- β -catenin signalling has been demonstrated in osteoblasts at multiple stages, including in the promotion of osteoblast differentiation from progenitors and in promoting osteoblast and osteocyte survival *in vitro*^{118,119}. In addition, β -catenin was found to regulate the expression of osteoprotegerin, an inhibitor of osteoclast differentiation in mature osteoblasts^{120,121}.

Patients with sclerosteosis, who have high bone mass, were found to have homozygous mutations in the gene that encodes sclerostin (*SOST*), which is a circulating inhibitor of the WNT signalling pathway that acts to inhibit LRP5 function^{122,123}. Antibodies targeting sclerostin have been shown to increase the rate of bone formation and bone density in rats¹²⁴. A clear link to bone metabolism was first established by the discovery that *LRP5* mutations are associated with osteoporosis pseudoglioma syndrome, as mentioned above. Subsequently, patients with distinct types of hereditary diseases characterized by high bone mass were found to carry mutations in the *LRP5* extracellular domain^{117,125}, which rendered them resistant to inhibition by sclerostin^{126,127}.

Modulation of WNT signalling has emerged as a promising strategy for increasing bone density. Small-molecule inhibitors of GSK3 β increase bone mass, lower adiposity and reduce fracture risk in preclinical models. Neutralizing antibodies targeting the secreted WNT inhibitors Dickkopf 1 (DKK1), SFRP1 and sclerostin produce similar outcomes in animal models¹²⁸. A sclerostin-specific monoclonal antibody, romosozumab (also known as AMG 785), is currently in Phase III trials for post-menopausal osteoporosis.

Although homozygous loss of *SOST* has not been associated with an increased risk of cancer in patients, additional follow-up studies will be required to determine the long-term effects of increasing WNT signalling, in particular relating to cancer risk. In this regard, a recent study of 170 long-term users of the GSK3 inhibitor lithium (where the mean duration of lithium exposure was 21.4 years) demonstrated a significantly higher standardized incidence ratio of renal cancer compared with the general population: 7.51 (95% confidence interval (CI); 1.51–21.95) and 13.69 (95% CI; 3.68–35.06) in men and women, respectively¹²⁹. Whether this is WNT-related or not is unclear, as lithium has additional pharmacological effects beside WNT signalling activation via GSK3 inhibition. Furthermore, as discussed below in the section on ageing, several murine models of ageing and accelerated ageing have demonstrated enhanced WNT signalling.

Fibrosis

Fibrosis is characterized by an excessive accumulation of extracellular matrix components, which disrupts the physiological tissue architecture, leading to the dysfunction of the affected organ¹³⁰. Fibrosis in general has been suggested to account for approximately 45% of deaths in industrialized countries, thereby highlighting the great medical need for effective antifibrotic therapies²⁴. Activated WNT– β -catenin signalling has been implicated in fibrosis in a number of organ systems, including the lungs, which indicates that this developmental pathway can be reactivated in adult tissues following injury^{25,131–133}. Specific small-molecule WNT modulation in several murine models of fibrosis (such as lung and kidney models) has proven to be extremely effective. Specific inhibition of the CBP– β -catenin interaction was shown to not only ameliorate but also to reverse late-stage fibrotic injury in murine models of both lung and kidney fibrosis^{134,135}. However, again the role of WNT signalling in fibrosis is not completely straightforward. Several studies using WNT reporter mice provided evidence that activation of β -catenin signalling is an early event in the lung epithelium during the development of experimental fibrosis^{136,137}, and four published studies — including our own — have provided evidence that inhibition of WNT– β -catenin signalling can attenuate fibrosis^{134,138,139}. However, a recent publication demonstrated that Cre recombinase-mediated depletion of β -catenin from surfactant protein C-expressing alveolar epithelial type 2 (AT2) cells did not protect — but instead promoted — fibrosis in the same model¹⁴⁰.

The fate of ‘good’ versus ‘bad’ β -catenin signalling in lung epithelial cells is most probably dictated by which cofactor β -catenin binds to: CBP or p300. This model predicts that inhibition strategies will need to regulate WNT– β -catenin signalling to a level necessary for alveolar repair without promoting the fibroproliferation associated with fibrosis¹⁴¹.

Regenerative medicine: myocardial infarction and epithelial repair

The adult mammalian heart has a limited capability for self-repair after myocardial infarction; thus, therapeutic strategies that improve post-infarct cardiac function are crucially needed. WNT signalling is required to direct crucial biological processes during cardiac development and is important in both the proliferation and differentiation of various stem/progenitor cell populations¹⁴². Indeed, the renewal and differentiation of Isl1⁺ cardiovascular progenitors are both controlled by the WNT- β -catenin pathway¹⁴³.

Modulation of the WNT signalling pathway provides a pharmacological target for regenerative signalling in damaged myocardial tissue. In this regard, small-molecule intervention in the WNT signalling pathway with either pyrvinium or ICG-001 (FIG. 1; TABLE 1) have proven to be beneficial for the treatment of myocardial infarction in rodent models^{144,145}.

There are also other potential therapeutic benefits from WNT agonists in regenerative medicine, including WNTs and R-spondins — secreted proteins that potentiate WNT signalling through their interaction with their leucine-rich repeat-containing G protein-coupled receptors (LGRs) and the transmembrane E3 ubiquitin ligases RING finger protein 43 (RNF43) and zinc/RING finger protein 3 (ZNRF3)¹⁴⁶ (FIG. 2). These proteins are potent stimulators of adult stem cell proliferation both *in vitro* and *in vivo*. Consequently, in principle they could both potentially prove to be valuable therapeutic agents for regenerative medicine. Just as WNT signalling is important in many aspects of embryonic development, the WNT-enhancing ability of R-spondins, together with their dynamic expression patterns in embryonic tissues, predicts pleiotropic roles for R-spondins during embryogenesis and also in adult stem cell homeostasis¹⁴⁷. The *in vivo* therapeutic potential of R-spondins was initially described in a mouse model of inflammatory bowel disease¹⁴⁸ and subsequently for the amelioration of gastrointestinal syndrome induced by radiation¹⁴⁹ or intensive chemoradiotherapy¹⁵⁰. Although acute *in vivo* use of WNT signalling activators — WNTs, R-spondins or small molecules — to enhance stem cell proliferation may prove to be therapeutically beneficial, substantial concerns regarding their chronic *in vivo* use, as described previously in the section on osteoporosis, will need to be addressed before these approaches are likely to be clinically validated.

Autoimmune diseases: rheumatoid arthritis and colitis

WNT signalling has been implicated in the development of the immune system for quite some time, and TCF1 (also known as HNF1 α) and β -catenin are known to regulate T cell development and function¹⁵¹. However, the contributions of WNT signalling to adult immunity are only beginning to be realized. Studies in adult T cells have shown that the WNT effector TCF1 is required for the functional generation of memory CD8⁺ T cells^{152–155}. WNT signalling can also affect the differentiation of naive CD4⁺ T lymphocytes¹⁵⁶. WNT- β -catenin signalling has also been implicated in the development of T regulatory (T_{Reg}) cells that are involved in autoimmune disease, as well as in cancer and infectious diseases¹⁵⁷.

Interestingly, patients with rheumatoid arthritis exhibit higher expression of *WNT* and *FZD* genes in synovial membranes of affected joints compared with healthy individuals without rheumatoid arthritis¹⁵⁸. Increased expression of *WNT7B* in human synovial cells has been shown to induce inflammatory cytokines such as interleukin-1 β (IL-1 β), IL-6 and tumour necrosis factor (TNF) in patients with rheumatoid arthritis¹⁵⁹. β -catenin-independent *WNT5A* signalling has also been implicated in the development of rheumatoid arthritis¹⁵⁹.

Recently, elevated levels of β -catenin have been shown to prevent the differentiation of T_{Reg} cells and enhance T helper 17 (T_H17) commitment, which is potentially linked to both the pro-inflammatory state in colitis and to an increased risk of colon cancer development¹⁶⁰. β -catenin is also upregulated in pro-inflammatory monocytes and in antigen-presenting cells¹⁶¹. *WNT*- β -catenin signalling may be a crucially important pathway associated with the pro-inflammatory tumour microenvironment in inflammation-driven malignancies¹⁶².

Disorders of endocrine function

WNT signalling also has crucial roles in various endocrine functions and has therefore been implicated in several endocrine disorders¹⁶³. Although a detailed discussion of these roles is beyond the scope of this Review, key observations are discussed below.

WNT signalling is important in the regulation of insulin sensitivity and its dysregulation is implicated in the development of diabetes. In particular, *WNT10B* increases insulin sensitivity in skeletal muscle cells¹⁶⁴. Overexpression of *WNT5B* induces adipogenesis. Decreased expression of β -catenin-independent *WNT5B*, which has been demonstrated in patients with type 2 diabetes, may increase susceptibility to type 2 diabetes¹⁶⁵. Additionally, polymorphisms in the transcription factor *TCF7L2* (also known as *TCF4*), are linked to increased susceptibility to type 2 diabetes¹⁶⁶. Individuals with at-risk alleles of *TCF7L2* exhibit impaired insulin secretion, and *TCF7L2* in pancreatic β -cells appears to have a crucial role in glucose metabolism through the regulation of pancreatic β -cell mass¹⁶⁷.

Recently, researchers treated cadaver-derived intact human islets with conditioned medium from L-cells that constitutively produced *WNT3A*, R-spondin 3 and Noggin, to which inhibitors of RHO-associated protein kinase (ROCK) and RHOA were added to augment cell survival. This led to an approximately 20-fold increase in β -cell proliferation compared with glucose alone¹⁶⁸. Importantly, treatment with this conditioned medium did not impair glucose-stimulated insulin secretion or decrease the insulin content of the cells. In transcriptome-wide gene expression profiling and follow-up signalling studies, the researchers showed that the conditioned media treatment specifically promoted *WNT* signalling¹⁶⁸. However, translation of this to an *in vivo* therapeutic strategy to expand human pancreatic β -cells could prove to be extremely difficult owing to the potential need for chronic administration and effects on non-target tissues.

Ageing

Although perhaps not a disease per se, ageing and an ageing population in general are creating major health and economic issues. It is clear that with age there is a decline in both the fidelity and efficiency of the homeostatic and repair processes in the body. This could be

due to a decline in tissue stem cell populations (such as haematopoietic stem cells (HSCs) as well as stem cells in the skin). However, recent data indicate that there is a decrease in the 'effectiveness' of these stem cells to serve as a regenerative pool during homeostasis, and therefore repair processes decrease with age¹⁶⁹. Interestingly, several mouse models of premature ageing and decreased effectiveness of repair after injury (that is, increased fibrosis) have demonstrated an increase in WNT signalling^{170–172}. Epidemiological data demonstrate that the risk of developing cancer, fibrosis or neurodegeneration increases significantly with age, starting at approximately the age of 50. As previously discussed, these diseases have all been associated with aberrant WNT signalling. Again, the potential to ameliorate the ageing process by safely modulating WNT signalling, particularly within the stem/progenitor population, could have a major impact not only on health and disease but also on medical economics.

Challenges in drugging the WNT pathway

There are a number of issues that must be considered in the development of therapeutic strategies to modulate the WNT pathway. Therapeutic agents that target crucial developmental signal transduction pathways (that is, the WNT, Notch, Hedgehog and bone morphogenetic protein (BMP) pathways) can have devastating effects on embryonic patterning, as evidenced by the chemically induced onset of cyclopia in lambs by the Hedgehog inhibitor cyclopamine and also in children with severely shortened limbs owing to the effects of thalidomide. Furthermore, somatic stem cell homeostatic and regenerative processes after injury also utilize these same pathways for the generation of new tissue and the maintenance of the somatic stem cell niche.

WNT signalling, specifically involving WNT4, is also involved in the development of the female reproductive system. During mammalian embryogenesis, WNT4 is expressed in the gonads of both sexes before sex determination events take place and is subsequently downregulated in the male gonad. Inactivation of WNT4 in mice has revealed that it is important at several steps of female reproductive development¹⁷³.

Nevertheless, despite the teratogenic effects of Hedgehog inhibition and the potential risks associated with targeting a crucial pathway involved in stem cell regulation, on 30 January 2012 vismodegib (Erivedge; Curis/Genentech) — a Smoothed antagonist that disrupts Hedgehog signalling — became the first drug to be approved by the FDA for the treatment of locally advanced and metastatic basal cell carcinoma. As a class, the Hedgehog inhibitors have multiple adverse effects, including muscle spasms and cramps, alopecia, dysgeusia, fatigue, weight loss and diarrhoea¹⁷⁴. However, these adverse effects are largely reversible after treatment is stopped. Serious concerns about the use of Hedgehog inhibitors during pregnancy and early childhood development still exist. Given the crucial role of WNT signalling in homeostasis in multiple organ systems (the intestine, haematopoietic system, skin and hair, and so on), similar side effects, as well as others (for example, bone loss or breakage) may be anticipated. Therefore when considering the targeting of crucial developmental pathways that are utilized by both cancer stem cells as well as normal somatic stem cells, the 'Jekyll and Hyde'-like potential behaviour of this class of therapeutic agents remains an ever-present issue¹⁷⁵.

Agonists or antagonists: is that the question?

It was originally believed that the choice of agonist versus antagonist, in particular for the treatment of oncologic malignancies, was relatively straightforward. However, the notion that universally antagonizing WNT- β -catenin signalling in cancer would be beneficial has been called into question²³, as elevated WNT signalling does not correlate with reduced patient survival in all types of cancer.

WNT signalling appears to have an important role in neuroprotection and neural regeneration after brain injury, and enhanced WNT- β -catenin signalling is present in newly derived glial progenitors and astrocytes in a model of traumatic brain injury¹⁷⁶. However, *AXIN2*, a WNT target gene that negatively regulates the pathway by promoting β -catenin degradation, was found to be expressed in immature oligodendrocyte progenitor cells in white matter lesions of human newborns with neonatal hypoxic-ischaemic and gliotic brain damage, as well as in active multiple sclerosis lesions in adults¹⁷⁷. *AXIN2* is essential for normal remyelination, and the small molecule XAV939, which inhibits tankyrase and thereby inhibits WNT signalling (FIG. 1; TABLE 1), stabilized *AXIN2* levels in oligodendrocyte progenitor cells from the brain and spinal cord and accelerated their differentiation and remyelination after hypoxic and demyelinating injury¹⁷⁷.

We are therefore left with a conundrum: what do we need to do to treat these various indications in which WNT signalling apparently has an important role? Do we need to increase or decrease WNT signalling to achieve a beneficial therapeutic effect? Or is it that aberrations in WNT signalling cannot be targeted using a single universal strategy, but rather that the clinical decisions will need to be made on a personalized basis? Why is there so much debate and controversy surrounding this topic?

Briefly, we believe that the problem is partly due to the following three reasons. The first is the multiple ways in which WNT signalling is read out (such as nuclear β -catenin levels, reporter assays and endogenous gene expression), which are highly cell-type dependent. The second is that the role of transcriptionally active nuclear β -catenin is highly underestimated by some of these readouts (for example, TOP-Flash), as β -catenin binds to a broad spectrum of transcription factors other than TCF/LEF and can modulate a plethora of downstream biological processes⁵⁰. Last, and most importantly, WNT signalling is temporal and so during the processes of development, normal homeostasis or regeneration and repair, both the activation and subsequent diminution of WNT signalling are required, as highlighted by the essential roles of negative feedback loops (for example, *AXIN2* expression) in the normal developmental and reparative programmes.

Our view, which is now beginning to be borne out in clinical trials with the specific CBP- β -catenin antagonist PRI-724, is that fixing aberrant WNT signalling must take into account the disparate effects of this pathway. In its simplest form, the roles of WNT in cell proliferation versus differentiation — symmetric versus asymmetric differentiation in stem/progenitor populations — must be balanced (BOX 3; FIG. 3). Thus for the optimum therapeutic effect, a fine balance between these two dichotomous arms of the WNT signalling pathway needs to be maintained.

Box 3**WNT signalling in stem cells**

WNT glycoproteins, in collaboration with the integration of signals from various other key signal transduction networks (for example, the Notch, Hedgehog, JAK (Janus kinase)–STAT (signal transducer and activator of transcription), bone morphogenetic protein (BMP), Hippo and fibroblast growth factor (FGF)–MAPK (mitogen-activated protein kinase) signalling pathways), have essential roles in balancing the self-renewal and differentiation of adult stem cells (FIG. 2). However there is no consensus as to whether WNT signalling is important for the proliferation and maintenance of potency (pluri- or multipotency)^{281–283} or the differentiation of stem/progenitor cells^{142,284}. WNT– β -catenin signalling has been demonstrated to maintain pluripotency in embryonic stem cells²⁸² and is crucial for the expansion of neural progenitors, thereby increasing brain size⁹⁵. Recently, a crucial role for β -catenin has been demonstrated in the maintenance of the expression of the key pluripotency transcription factor octamer-binding protein 4 (OCT4; also known as POU5F1) in a T cell factor (TCF)-independent fashion²⁸⁵. However, WNT– β -catenin signalling is also required for the neural differentiation of embryonic stem cells²⁸⁶ and in fate-decision in neural crest stem cells²⁸⁷; moreover, WNT3A has been reported to promote differentiation into the neural and astrocytic lineages by inhibiting neural stem cell maintenance²⁸⁸. Clearly, WNT– β -catenin signalling also has a crucial role in the lineage decision and commitment of stem cells. These dramatically different outcomes upon activation of the WNT signalling cascade have fuelled enormous controversy concerning the role of WNT signalling in the maintenance of potency and induction of differentiation.

WNT and cancer stem cells

The similarities between normal adult somatic stem cells and cancer stem cells (CSCs) suggest that the same signalling pathways that are involved in regulating somatic stem cell maintenance are also involved in the regulation of CSCs^{289–291}. CSCs exist in an array of tumour types, including leukaemias as well as brain, breast, prostate and colon cancers, and are associated with disease recurrence, multidrug resistance and metastasis²⁹². CSCs express similar markers and exhibit cellular behaviours that are highly reminiscent of somatic stem cells. In addition to WNT signalling, a number of crucial pathways are involved in maintenance or loss of potency in stem cells (that is, the Notch, Hedgehog, transforming growth factor- β (TGF β)–BMP, JAK–STAT, FGF–MAPK–PI3K (phosphoinositide 3-kinase) and Hippo pathways). Aberrant regulation of these same pathways leads to neoplastic proliferation in the same tissues. One conclusion that can be drawn is that there are multiple points of intersection and crosstalk, including feedback and feedforward loops, connecting the various signalling cascades that modulate ‘stemness’. One major fundamental intrinsic difference between CSCs and long-lived normal somatic stem cells may be in their decision to divide asymmetrically or symmetrically^{255,293}. FIGURE 3 shows both asymmetric and symmetric modes of stem cell division. This, in principle, could provide a unique opportunity to therapeutically target CSCs without damaging the normal endogenous stem cell populations²⁵⁵. Clearly, successful therapeutic manipulation of endogenous somatic ‘stemness’ (normal or

cancerous) will require precision to affect the desired transformation without having deleterious effects — particularly depletion — on the normal stem cell populations²⁹⁴.

Crosstalk with the WNT pathway

“No man is an island”, and similarly no pathway can be modulated without affecting others. For example, a number of growth factor pathways can activate β -catenin signalling independently of WNTs, via activation of both their receptor and non-receptor tyrosine kinases (BOX 1).

HGF, via activation of its receptor tyrosine kinase MET, mediates the phosphorylation of Tyr654 and Tyr670 of β -catenin, thereby inducing its dissociation from E-cadherin¹⁷⁸. Similarly, Tyr142 and Tyr654 phosphorylation by FMS-like tyrosine kinase 3 (FLT3) leads to the dissociation of β -catenin from its complex with E-cadherin¹⁷⁹. Phosphorylation of Tyr654 also enhances the binding of β -catenin to TCF4, further synergizing to enhance WNT- β -catenin signalling¹⁸⁰. Not surprisingly, therefore, both the tyrosine kinase inhibitor imatinib (Gleevec; Novartis) and the small molecule PHA665752, which inhibits MET-mediated phosphorylation, have been shown to reduce WNT- β -catenin signalling^{181,182}. In addition, as previously alluded to, WNTs collaborate with various other key developmental pathways^{183,184}, in particular in stem and progenitor populations (FIG. 2). Consistent with this extensive interwoven network, various small-molecule inhibitors of these pathways, including inhibitors of Notch and the mitogen-activated protein kinase (MAPK) pathway^{185,186}, indirectly affect WNT signalling. The WNT and RAS signalling cascades have been observed to synergistically contribute to tumour initiation and development in a number of transgenic mouse tumour models, including colon¹⁸⁷, intestine¹⁸⁸ and liver¹⁸⁹ tumours. The convergence of these signalling pathways upregulates the expression of various genes (that is, cyclooxygenase 2 (*COX2*), *MYC* and *IL8*) that promote tumorigenesis^{190–192}. Interestingly, monoallelic expression of an activating KRAS mutation in the mouse intestine has no phenotype but can promote tumorigenesis in APC-deficient mice¹⁸⁸. Crosstalk between the WNT and Notch pathways has been implicated in an array of developmental processes, including intestinal homeostasis¹⁹³, vascular remodelling¹⁹⁴ and somitogenesis¹⁹⁵.

We have also demonstrated that small-molecule antagonists of phosphatases and kinases can maintain mouse and human embryonic stem cell pluripotency in a WNT-dependent fashion^{196,197}. Inhibition of the protein phosphatase 2A (PP2A) regulatory subunits PR72 and PR130 using the small molecule IQ-1 maintained pluripotency in murine embryonic stem cells in a WNT-dependent manner¹⁹⁶. Similarly, the small molecule ID-8, an inhibitor of dual specificity YAK1-related kinases (DYRKs), allows for long-term WNT-mediated maintenance of human embryonic stem cells¹⁹⁷. Salinomycin, an antibiotic potassium ionophore, was reported to act as a selective inhibitor of epithelial-to-mesenchymal transition and induce cytotoxicity in breast cancer stem cells¹⁹⁸. Subsequently, it was shown that salinomycin, as well as another potassium ionophore, nigericin, blocked the phosphorylation of the WNT co-receptor LRP6 and induced its degradation, thereby downregulating WNT signalling¹⁹⁹.

Nonspecific WNT pathway modulators

Therapeutic agents that specifically target the WNT pathway have only recently entered clinical trials, although a few FDA-approved drugs do affect WNT signalling, albeit nonspecifically. Lithium salts have been used clinically for decades to treat psychiatric disorders such as manic depression. Lithium, among its multiple pharmacological actions, inhibits the constitutively active kinase GSK3, thereby stabilizing β -catenin and activating WNT signalling²⁰⁰. Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and sulindac, as well as the selective COX2 inhibitor celecoxib (Celebrex; Pfizer), inhibit the activity of COX, a key enzyme in the arachidonic acid cascade (TABLE 1). Various experimental and epidemiological studies in humans have suggested that aspirin and other NSAIDs (for example, sulindac; see TABLE 1) have chemopreventive effects mainly in colon cancer^{201–207}. Increased COX-generated prostaglandin E₂ (PGE₂) suppresses β -catenin degradation, and so inhibition of the WNT– β -catenin signalling pathway is one of the potential mechanisms of action of NSAIDs^{208,209}. NSAIDs have been demonstrated to reduce polyp formation in patients with familial adenomatous polyposis, which is associated with autosomal dominant mutations in the *APC* gene and the activation of WNT– β -catenin signalling^{201,210}.

Vitamins, notably retinoids, which are synthesized from vitamin A in the body, are used in some forms of cancer therapy (notably acute promyelocytic leukaemia) and also in chemoprevention. The active form of vitamin D, 1 α ,25-dihydroxyvitamin D₃, and its synthetic derivatives have shown chemopreventive effects in animal models of colorectal and breast cancers. Although the mechanism by which vitamins inhibit the WNT– β -catenin signalling pathway is not fully elaborated, it has been suggested that activated nuclear receptors for vitamins interact with β -catenin and compete with TCFs for the transcriptional co-activators p300 and CBP^{211,212} (FIG. 1). Recently, it has also been suggested that both vitamin A and vitamin D might induce inhibitory proteins of the WNT– β -catenin signalling pathway; for example, retinoids may induce Disabled homolog 2 (DAB2), whereas vitamin D may induce and DKK1 and DKK4 (REF. 213).

Polyphenols are a group of chemicals that are found in plants and are characterized by the presence of more than one phenol unit or building block per molecule. Several polyphenols, such as quercetin, epigallocatechin-3-gallate (EGCG), curcumin and resveratrol (TABLE 1), have been implicated as inhibitors of the WNT– β -catenin signalling pathway, but the mechanisms of action of these agents are not clear owing to their inherent lack of specificity and inhibitory effects on multiple pathways^{214–218}. The redox capacities of this class of molecules may have a role in their ability to inhibit WNT signalling, as redox regulation of transcription — including the WNT signalling cascade²¹⁹ — is relatively ubiquitous²²⁰.

Other classes of drugs that can inhibit WNT signalling have been identified by screening libraries of FDA-approved drugs. For example, the anti-helminthic drug pyrvinium was identified as an agent that could potentiate the activity of CK1 α , leading to enhanced degradation of β -catenin and the co-activator Pygopus as well as a reduction in WNT– β -catenin signalling²²¹ (FIG. 1; TABLE 1). In a mouse model of myocardial infarction, pyrvinium demonstrated improvements in cardiac remodelling¹⁴⁴.

Molecularly targeted agents

In addition to the existing nonspecific modulators of WNT signalling, several molecularly targeted agents are being developed. Those reported to date can be classified into five classes: β -catenin–TCF antagonists; drugs that bind the PDZ domain of DVL; other mechanism-based inhibitors that principally target enzymes (for example, Porcupine, tankyrase and kinase inhibitors); biologics; and drugs that target WNT co-activators.

β -catenin–TCF antagonists

Effectively inhibiting the protein–protein interaction between TCF and β -catenin via a small molecule is not a trivial task, as the binding affinity between these two large protein molecules is apparently quite high (~20 nM). However, to date a number of very different approaches have been successful in identifying small-molecule inhibitors of this interaction.

In one of the earliest examples, a high-throughput enzyme-linked immunosorbent assay (ELISA) screen for the detection of the β -catenin–TCF interaction was utilized²²². The primary screen of approximately 7,000 natural products and 45,000 synthetic compounds yielded eight compounds that dose-dependently inhibited complex formation (half-maximal inhibitory concentration (IC₅₀) <10 μ M). Secondary assays generally demonstrated efficacy for the inhibition of WNT-specific reporter gene activity, colon cancer cell proliferation and β -catenin-induced axis duplication in *Xenopus laevis* embryos. Two structurally related compounds, PKF115-584 and CGP049090, (TABLE 1) proved to be the most effective. However, the molecular mechanism by which these compounds interfere with the WNT signalling pathway remains unclear. Interestingly, both PKF115-584 and CGP049090 also disrupt the β -catenin–APC interaction, implicating potential direct binding to β -catenin²²².

Subsequently, a cell-based high-throughput assay in *D. melanogaster* cells with a WNT-responsive luciferase reporter was used to screen 14,977 compounds, including known bioactive molecules, fungal extracts and members of a synthetic library. The primary screen yielded 34 hit compounds, three of which — iCRT3, iCRT5 and iCRT14 (FIG. 1; TABLE 1) — also disrupted the β -catenin–TCF interaction *in vitro*, inhibited the expression of WNT target genes and were cytotoxic to colorectal cancer cells²²³. Unfortunately, TOP-Flash inhibition in the same colorectal cancer cell lines, which would be a hallmark of a β -catenin–TCF interaction inhibitor, was not demonstrated. In another high-throughput screen of a large compound library, 2,4-diamino-quinazoline (TABLE 1) was identified as an inhibitor of the β -catenin–TCF4 pathway; this compound is currently in the lead optimization stage of development²²⁴. Although the actual molecular target for this class of inhibitors was not detailed, based on its molecular structure it is highly probable that it targets a kinase or kinases in the WNT– β -catenin pathway.

Trosset and colleagues²²⁵ identified the synthetic compound PNU-74654 using structure-based virtual ligand (*in silico*) screening for a β -catenin–TCF antagonist. However, the biological activity of PNU-74654 was not reported (TABLE 1). Another virtual screen, utilizing the 1990 small-molecule diversity set of the US National Cancer Institute to identify compounds that would bind to the armadillo repeat structure extracted from the X-ray structure of the β -catenin–TCF4 complex, identified a large set of compounds, among

which the top-ranked compound was the organo-copper complex BC21 (TABLE 1). Although a thorough validation of the mode of action of BC21 was not performed, it did reduce β -catenin–TCF reporter activity, the expression of WNT target genes and the viability of a colon cancer cell line ($IC_{50} = 15\mu M$)²²⁶. Despite the fact that these high-throughput screens and docking studies have yielded a number of hits, in general they are limited by relatively broad activity profiles and/or unclear mechanisms of action²²⁷.

The α -helix is a common secondary structural motif mediating crucial interactions between protein–protein binding partners, including the β -catenin–BCL-9 and the β -catenin–TCF4 interfaces. Utilizing a stapled peptide approach, both the β -catenin–BCL9 (REF. 228) and the β -catenin–TCF4 interfaces have been targeted, yielding potent *in vitro* inhibitors ($IC_{50} \sim 10$ – 20 nM), which displayed low micromolar cell-based inhibition²²⁹. The clinical utility of the stapled peptide class of molecules has yet to be established.

PDZ domain of DVL binders

Another potential mechanism for decreasing WNT signalling is to target the PDZ domain of DVL. DVL is an essential protein in the WNT signalling cascade that transduces extracellular WNT signals to downstream components. DVL utilizes its PDZ domain, a common protein–protein interaction motif that recognizes short peptides, to bind to the carboxy-terminal region of the Frizzled receptors. Three compounds (NSC 668036, FJ9 and 3289–8625) (FIG. 1; TABLE 1), which have the ability to block WNT signalling *in vivo*, were identified through *in silico* screening and nuclear magnetic resonance (NMR) spectroscopy approaches^{230–232}. These compounds generally inhibit the Frizzled–PDZ interaction in the micromolar range *in vitro*.

Porcupine and tankyrase inhibitors

In 2009, two reports were published describing WNT– β -catenin inhibitors that act on alternative molecular targets. Chen and colleagues²³³ described several small-molecule IWRs (inhibitors of WNT response), such as IWR-1 ($IC_{50} = 180$ nM) (FIG. 1; TABLE 1), that stabilize the protein AXIN, and another group of compounds termed IWP (inhibitors of WNT production), at least one of which (IWP-2; $IC_{50} = 27$ nM) inhibits the acyltransferase Porcupine, which is an essential protein for WNT secretion (FIG. 1; TABLE 1). Subsequently, Huang *et al.* described the WNT inhibitor XAV939, which induces the stabilization of AXIN by inhibiting the poly(ADP)-ribosylating enzymes tankyrase 1 and tankyrase 2 (REF. 234) (FIG. 1). Tankyrases have been shown to interact with a highly conserved domain of AXIN and promote its ubiquitylation and degradation²³⁴. It was determined that IWR-1 also inhibits tankyrase²³⁴. Tankyrase inhibitors successfully curtail WNT-mediated cellular responses, which demonstrates that stabilization of AXIN is a potentially viable strategy for inhibiting the WNT– β -catenin signalling cascade. More recently, various additional potent and selective tankyrase and Porcupine inhibitors have been described²³⁵.

Tankyrase 1 and tankyrase 2 are part of the larger family of poly(ADP-ribose) polymerase (PARP) enzymes. The development of more selective second-generation tankyrase inhibitors²³⁵ is in progress. However, the full anti-tumour potential of even these analogues

may not be realized owing to the intestinal toxicity associated with on-target inhibition of WNT- β -catenin signalling and cell proliferation in intestinal crypts, which was observed in preclinical models²³⁶.

Loss-of-function mutations in the liver kinase B1 (*LKB1*; also known as *STK11*) tumour suppressor gene restrain the activity of WNT receptors (such as Frizzled), and Porcupine inhibitors counter deviant pathway activities driven by *LKB1* loss of function *in vitro*. *RNF43* is a gene encoding a transmembrane ubiquitin ligase. RNF43 antagonizes WNT signalling in adult stem cells by ubiquitylating Frizzled receptors, which leads to endocytosis of the WNT receptor. Conversely, binding of ZNRF3 or RNF43 to the receptors LGR4, LGR5 or LGR6, and simultaneously to R-spondin, blocks Frizzled ubiquitylation and enhances WNT signalling^{146,147,237–239}. RNF43 has been suggested as a negative regulator of WNT signalling, and mutations in RNF43 have been identified in various tumours, including cystic pancreatic tumours. Interestingly, the Porcupine inhibitor LGK974 inhibited proliferation and induced differentiation in RNF43-mutant but not wild-type pancreatic cancer cells. The presence of *RNF43* mutations may possibly be used as a predictive biomarker for patient selection, supporting the clinical development of Porcupine inhibitors in certain subtypes of cancer²⁴⁰.

Biological agents

Another approach that has received significant attention is the targeting of specific WNTs or WNT receptors that are aberrantly overexpressed in tumours. For example, intraperitoneal injections of WNT3A-neutralizing antibodies decrease proliferation and induce apoptosis in a mouse model of prostate cancer²⁴¹. Antibodies targeting Frizzled receptors have also been shown to be effective in various preclinical models, including those of breast, colon and liver cancer, and appear to preferentially spare normal tissue^{242,243} (FIG. 1). The monoclonal antibody OMP-18R5 (FIG. 1; TABLE 1), which was initially identified by binding to FZD7, in fact interacts with five Frizzled receptors through a conserved epitope within the extracellular domain and blocks canonical WNT signalling induced by multiple WNT family members²⁴⁴. In patient-derived mouse xenograft models, OMP-18R5 inhibited the growth of a range of tumour types, reduced the fraction of cells that could seed new tumours and exhibited synergy with standard-of-care chemotherapeutic agents²⁴⁴.

WNT co-activator modulators

To generate a transcriptionally active complex, β -catenin recruits the transcriptional co-activator CBP or its closely related homologue p300, as well as other components of the basal transcriptional machinery, leading to the expression of a host of downstream target genes⁴. These two co-activators have long been considered as having largely redundant roles. Recent work has documented that CBP and p300 interact with hundreds of proteins in their roles as master orchestrators of transcription and, despite their high degree of homology, accumulating evidence indicates that CBP and p300 are not redundant but have definitive and unique roles both *in vitro* and *in vivo*^{245–247}.

Using the TOP-Flash reporter gene system in SW480 colon carcinoma cells, we identified ICG-001 as an inhibitor of β -catenin-TCF transcriptional activity from a library of 5,000

secondary-structure mimetics. ICG-001 (FIG. 1; TABLE 1) had an IC_{50} value of 3 μ M in this assay. Using an affinity chromatography approach, we identified and subsequently validated — using a gain-of-function and loss-of-function strategy — that ICG-001 binds specifically and with high affinity (\sim 1 nM) to the co-activator CBP. Importantly, ICG-001 does not bind to the closely related homologue p300, despite the fact that these two co-activators are up to 93% identical with even higher homology at the amino acid level^{248,249}. We further demonstrated that selectively blocking the interaction between CBP and β -catenin with ICG-001 led to the initiation of a differentiation programme.

This led to the development of our model of differential co-activator usage, which highlights the distinct roles of the co-activators CBP and p300 in the WNT- β -catenin signalling cascade¹⁰¹. The crucial feature of the model is that the utilization of CBP induces a transcriptional programme that promotes proliferation and the maintenance of potency, whereas utilization of p300 leads to a transcriptional programme that initiates differentiation. Subsequently, we identified several small molecules (IQ-1 and ID-8) that selectively block the p300- β -catenin interaction, thereby increasing the CBP- β -catenin interaction, which maintains potency (pluri- or multipotency) in a variety of mouse and human stem cell populations^{196,197,250,251}. The therapeutic potential of the selective CBP- β -catenin antagonist ICG-001 has been examined in several preclinical tumour models, where it has demonstrated the ability to safely eliminate drug-resistant tumour-initiating cells^{252–254}.

Interestingly, CBP- β -catenin antagonists have also demonstrated efficacy in various injury models, including models of pulmonary and renal fibrosis^{134,135} as well as myocardial infarction¹⁴⁵, which suggests that these antagonists can induce therapeutic differentiation without the subsequent cell elimination observed in cancer stem cell models. It appears that the differential effects of CBP- β -catenin antagonists on cancer stem cells versus normal somatic stem cells are cell intrinsic and not due to a differential ability of the drug to enter these two cell types. We proposed that CBP- β -catenin antagonists take advantage of the intrinsic propensity of cancer stem cells to increase their number of symmetric divisions at the expense of asymmetric divisions as a result of various mutations (for example, mutations in genes encoding the tumour suppressor p53 and phosphatase and tensin homolog (PTEN)). Normal endogenous long-term repopulating stem cells preferentially divide asymmetrically, with one daughter cell remaining in the niche and the other developing into a transient amplifying cell required for generating the new tissue involved in repair processes²⁵⁵.

In principle, substantial concerns about specificity could be raised with regard to the use of small-molecule inhibitors that target a co-activator protein with perhaps as many as 500 molecular partners, including an array of transcription factors. However, these concerns have not been borne out either preclinically or clinically to date (see below). This is perhaps surprising at first. A full discussion of the many advantages of a small-molecule therapeutic that selectively targets the amino-terminus of CBP is beyond the scope of this Review²⁵⁵. However, we would like to point out a few salient features that all have a role: the extremely high biochemical selectivity of ICG-001 and PRI-724 for their molecular target; the disruption of only a small subset of CBP interactions; and the unique properties of the two KAT3 co-activators, CBP and p300, which evolutionarily diverged more than 500 million

years ago. That being said, as ICG-001 is a CBP–catenin antagonist, by virtue of its binding to the N-terminus of CBP, its inhibitory effects go beyond the classical TCF– β -catenin antagonism for which it was originally screened in colorectal cancer cells²⁵⁵.

From bench to bedside: agents in the clinic

Targeting the WNT signalling pathway in the clinic using small-molecule therapeutics or even biologics is still in its infancy. Despite intensive investigation of the pathway and the unveiling of a multitude of potential therapeutic points of intervention in the pathway, as well as the identification of reagents that interfere with some of these targets, it is still unclear which approach will provide both clinical efficacy and safety.

Porcupine is a member of a 16-gene family of *O*-acyltransferases and it specifically palmitoylates WNTs, thereby enabling WNT secretion^{53,256}. Importantly, at a dose of 3 mg per kg per day for 14 days, which was sufficient to cause regression of WNT-driven tumours in rats, the Porcupine inhibitor LGK974 was well tolerated without abnormal histopathological findings in other WNT-dependent tissues (for example, the intestine). LGK974 exhibited a 63% delay in tumour growth when administered at a dose of 3 mg per kg per day. At a dose of 20 mg per kg per day for 14 days, however, loss of intestinal epithelium was observed, which was consistent with the anticipated effects of disrupted WNT signalling in the intestine. From a clinical therapeutic standpoint, this study demonstrated a potential therapeutic window for the use of this class of inhibitors²⁵⁷.

Recently, Novartis initiated a Phase I trial of LGK974 ($IC_{50} = 400$ nM) (FIG. 1; TABLE 1) in a variety of malignancies (melanoma, breast cancer and pancreatic adenocarcinoma) that are associated with aberrant WNT signalling, with the primary objective of obtaining a maximum tolerated dose. This trial has not been completed and there is no publicly available information on the results. Again, concerns about on-target toxicity (that is, WNT inhibition and effects on intestinal stem cells, bone turnover and haematopoiesis) as well as off-target toxicity (that is, inhibition of other members of this class of *O*-acyltransferases or similar enzymes) will need to be carefully evaluated.

Two WNT-targeting biologics developed by OncoMed Pharmaceuticals have entered clinical trials within the past 2 years. OMP18R5 is a fully humanized monoclonal antibody that binds to FZD1, FZD2, FZD5, FZD7 and FZD8. This open-label Phase Ia study for solid tumours was completed very recently, and the results were reported at the American Society of Clinical Oncology (ASCO) annual meeting in June 2013 (REF. 258). A total of 18 patients were treated in five dose escalation cohorts (0.5 and 1 mg per kg weekly; 0.5 mg per kg every 2 weeks; and 1 and 2.5 mg per kg every 3 weeks). The most common drug-related adverse events included grade 1 and 2 fatigue, vomiting, abdominal pain, constipation, diarrhoea and nausea. The only potentially drug-related adverse events greater than or equal to grade 3 were dose-limiting toxicities of grade 3 diarrhoea and vomiting in one patient receiving 1 mg per kg every week, and one patient receiving 0.5 mg per kg every week had a bone fracture on day 110. There were three cases of prolonged stable disease in patients with neuroendocrine tumours.

The second OncoMed agent, OMP-54F28, is being co-developed with Bayer and is an Fc fusion protein with FZD8, which binds all WNT ligands (FIG. 1; TABLE 1). This clinical trial in solid tumours was initiated last year with the primary end point again being safety, and is currently still enrolling participants. Concerns about the potential deleterious effects of these agents on bone formation and turnover have been prospectively addressed in the trial design, with all participants receiving vitamin D₃ and calcium carbonate for 30 days after discontinuing OMP-54F28. Individuals will also be monitored during the study for the effects of the agent on bone density and turnover.

JW Pharmaceutical initiated a clinical study of the small molecule CWP232291, a prodrug form of a compound that is reported to target SRC-associated in mitosis 68 kDa protein (SAM68; also known as KHDRBS1) (TABLE 1). SAM68 binds to the armadillo repeat domain of APC via its tyrosine-rich β -domain and regulates alternative splicing of TCF1 (REF. 259). Accumulation of the TCF1E splice variant strongly transactivates WNT target genes, thus SAM68-mediated pre-mRNA splicing may potentially modulate WNT signalling²⁵⁹. However, owing to the multimodular structure of SAM68, the apoptosis induced by this compound has been attributed to multiple effects, including the activation of nuclear factor- κ B (NF- κ B), alternative splicing of BCL-2 and WNT-mediated down-regulation of the anti-apoptotic protein BIRC5 (baculoviral IAP repeat-containing protein 5; also known as survivin). A Phase Ia study to determine the maximum tolerated dose in patients with acute myeloid leukaemia was initiated in July 2011 and is still ongoing. No results related to this study have been publicly disclosed.

PRI-724 (FIG. 1; TABLE 1), a specific CBP and β -catenin antagonist developed by Prism Pharma and partnered with Eisai Pharmaceuticals, entered an open-label Phase Ia safety study in individuals with solid tumours, where the expression of the biomarker BIRC5 was measured by immunomagnetic real-time PCR in circulating tumour cells. We have previously shown that inhibition of the β -catenin–CBP interaction with ICG-001 potently inhibits *BIRC5* gene transcription and expression. Furthermore, a chromatin immunoprecipitation assay confirmed that CBP is the crucial co-activator for TCF– β -catenin-mediated *BIRC5* transcription²⁶⁰. The results of this trial were reported at the ASCO meeting in June 2013 (REF. 261). Overall, 18 patients were treated (dose escalation from 40 to 1,280 mg per m² per day) via continuous infusion for 7 days. PRI-724 had an acceptable toxicity profile, with only one dose-limiting toxicity of grade 3 reversible hyperbilirubinaemia. Downregulation of BIRC5 in circulating tumour cells correlated with increasing plasma drug concentrations²⁶¹. Additional trials with PRI-724 are underway, including combination trials with a modified FOLFOX6 regimen for patients with refractory colorectal cancer, a Phase Ib trial for patients with refractory pancreatic cancer in combination with gemcitabine, and a Phase I/II trial for patients with haematological malignancies.

How can WNT antagonists and modulators most effectively be used therapeutically to treat cancer? Clinical experience with these agents is still quite limited at present; however, a few features that will, to some extent, determine the potential efficacy of an agent in a particular disease, are becoming apparent. These features include knowledge of where in the pathway the therapeutic agent acts — upstream (for example, LGK974, OMP18R5 or OMP-54F28)

or downstream (for example, PRI-724) of β -catenin-induced transcription — and which mutations are present in the particular disease being targeted (for example, APC in colorectal cancer). For instance, upstream targeting agents are not likely to be efficacious in most colorectal cancers, as the APC mutations that are present in >80% of patients cause constitutive nuclear β -catenin translocation. Furthermore, targeting a particular point in the pathway may effectively target only a subset of patients. For example, in a battery of pancreatic cancer cell lines, LGK974 was efficacious only in cells carrying mutations in RNF43. This suggests that RNF43 mutations could be used as a predictive biomarker for patient selection, at least for this class of molecules in pancreatic cancer²⁴⁰. Preliminary results also indicate that combination therapy will be required to effectively treat most malignancies, at least in the majority of cases. This is consistent with the earlier discussion about WNT crosstalk with other pathways, such as the HGF–MET pathway. Several Phase Ib/IIa trials are underway in which a WNT antagonist or modulator is being used in combination with cytotoxic agents (for example, FOLFOX, gemcitabine, cytarabidine or a taxane derivative) or a targeted agent (for example, dasatinib).

The potential for WNT inhibitors and modulators to eliminate drug-resistant cancer stem cells and tumour-initiating cells via forced differentiation, thereby sensitizing resistant tumours to conventional therapy, is a particularly appealing strategy. Such an approach is currently being tested clinically in chronic myeloid leukaemia (both minimal residual disease and blast crisis) with PRI-724 in combination with the kinase inhibitor dasatinib. There are obvious concerns about combining cytotoxic agents with WNT inhibitors, which will have to be carefully monitored in the clinic. Interestingly, WNT modulation using CBP–catenin antagonists in preclinical models partially ameliorated the toxicity associated with chemotherapy²⁵⁵.

Future directions

More than 30 years after the ground-breaking discovery of WNT signalling and extensive investigation into this fundamental and highly evolutionarily conserved pathway, it is still unclear whether we will be able to successfully target the WNT signalling cascade for therapeutic purposes. Within the past several years, a number of agents, both small molecules and biologics, have entered clinical trials. Despite exciting preclinical data in various tumour and other disease models, it is still too early to know whether any of these therapeutic agents that specifically target the WNT pathway will be efficacious with an acceptable safety profile. It is already clear that successfully targeting aberrant WNT signalling in cancer, as well as in more chronic situations, will require a fine balancing act, wherein the ‘dark side’ of WNT signalling in disease can be abrogated without interfering with the crucial role of WNT signalling in tissue homeostasis (for example, in the intestinal epithelium, bone, and so on) and repair. Despite all of the potential safety concerns regarding the therapeutic targeting of the WNT signalling pathway, there is also tremendous excitement as our knowledge of this pathway — including its role in both normal physiology and patho-physiology and the discovery of potential new therapeutic points of intervention^{262,263} — continues to increase. The next several years should further clarify the answer to the question: “Can we safely target the WNT pathway?”.

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References

1. Nusse R, Varmus H. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. *Cell*. 1982; 31:99–109. [PubMed: 6297757]
2. Baker NE. Molecular cloning of sequences from wingless, a segment polarity gene in *Drosophila*: the spatial distribution of a transcript in embryos. *EMBO J*. 1987; 6:1765–1773. [PubMed: 16453776]
3. McMahon AP, Moon RT. Ectopic expression of the proto-oncogene int-1 in *Xenopus* embryos leads to duplication of the embryonic axis. *Cell*. 1989; 58:1075–1084. [PubMed: 2673541]
4. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol*. 2004; 20:781–810. [PubMed: 15473860]
5. van Amerongen R, Nusse R. Towards an integrated view of Wnt signaling in development. *Development*. 2009; 136:3205–3214. [PubMed: 19736321]
6. Alonso L, Fuchs E. Stem cells in the skin: waste not, Wnt not. *Genes Dev*. 2003; 17:1189–1200. [PubMed: 12756224]
7. Pinto D, Clevers H. Wnt control of stem cells and differentiation in the intestinal epithelium. *Exp Cell Res*. 2005; 306:357–363. [PubMed: 15925592]
8. Nemeth MJ, Mak KK, Yang Y, Bodine DM. β -catenin expression in the bone marrow microenvironment is required for long-term maintenance of primitive hematopoietic cells. *Stem Cells*. 2009; 27:1109–1119. [PubMed: 19415781]
9. Malhotra S, Kincade PW. Wnt-related molecules and signaling pathway equilibrium in hematopoiesis. *Cell Stem Cell*. 2009; 4:27–36. [PubMed: 19128790]
10. Monga SP. Role of Wnt/ β -catenin signaling in liver metabolism and cancer. *Int J Biochem Cell Biol*. 2011; 43:1021–1029. [PubMed: 19747566]
11. Beers MF, Morrisey EE. The three R's of lung health and disease: repair, remodeling, and regeneration. *J Clin Invest*. 2011; 121:2065–2073. [PubMed: 21633173]
12. Whyte JL, Smith AA, Helms JA. Wnt signaling and injury repair. *Cold Spring Harb Perspect Biol*. 2012; 4:a008078. [PubMed: 22723493]
13. Inestrosa NC, Arenas E. Emerging roles of Wnts in the adult nervous system. *Nature Rev Neurosci*. 2010; 11:77–86. [PubMed: 20010950]
14. Cisternas P, Henriquez JP, Brandan E, Inestrosa NC. Wnt signaling in skeletal muscle dynamics: myogenesis, neuromuscular synapse and fibrosis. *Mol Neurobiol*. 2013; 49:574–589. [PubMed: 24014138]
15. Webster MR, Weeraratna ATA. Wnt-er migration: the confusing role of β -catenin in melanoma metastasis. *Sci Signal*. 2013; 6:e11.
16. Hoffmeyer K, et al. Wnt/ β -catenin signaling regulates telomerase in stem cells and cancer cells. *Science*. 2012; 336:1549–1554. [PubMed: 22723415]
17. Rawson JB, et al. Promoter methylation of *Wnt5a* is associated with microsatellite instability and BRAF V600E mutation in two large populations of colorectal cancer patients. *Br J Cancer*. 2011; 104:1906–1912. [PubMed: 21587258]
18. Alberici P, Fodde R. The role of the APC tumor suppressor in chromosomal instability. *Genome Dyn*. 2006; 1:149–170. [PubMed: 18724059]
19. Frisch SM, Schaller M, Cieply B. Mechanisms that link the oncogenic epithelial-mesenchymal transition to suppression of anoikis. *J Cell Sci*. 2013; 126:21–29. [PubMed: 23516327]
20. Pecinas-Slaus N. Wnt signal transduction pathway and apoptosis: a review. *Cancer Cell Int*. 2010; 10:22. [PubMed: 20591184]

21. Porfiri E, et al. Induction of a β -catenin-LEF-1 complex by wnt-1 and transforming mutants of β -catenin. *Oncogene*. 1997; 15:2833–2839. [PubMed: 9419974]
22. de La Coste A, et al. Somatic mutations of the β -catenin gene are frequent in mouse and human hepatocellular carcinomas. *Proc Natl Acad Sci USA*. 1998; 95:8847–8851. [PubMed: 9671767]
23. Anastas JN, Moon RT. WNT signalling pathways as therapeutic targets in cancer. *Nature Rev Cancer*. 2013; 13:11–26. [PubMed: 23258168]
24. Dees C, Distler JH. Canonical Wnt signaling as a key-regulator of fibrogenesis — implications for targeted therapies? *Exp Dermatol*. 2013; 22:710–713. [PubMed: 24118232]
25. Chilosi M, et al. Aberrant Wnt/ β -catenin pathway activation in idiopathic pulmonary fibrosis. *Am J Pathol*. 2003; 162:1495–1502. [PubMed: 12707032]
26. Schinner S. Wnt-signalling and the metabolic syndrome. *Horm Metab Res*. 2009; 41:159–163. [PubMed: 19214925]
27. Inestrosa NC, Montecinos-Oliva C, Fuenzalida M. Wnt signaling: role in Alzheimer disease and schizophrenia. *J Neuroimmune Pharmacol*. 2012; 7:788–807. [PubMed: 23160851]
28. Berwick DC, Harvey K. The importance of Wnt signalling for neurodegeneration in Parkinson's disease. *Biochem Soc Trans*. 2012; 40:1123–1128. [PubMed: 22988876]
29. Okerlund ND, Cheyette BN. Synaptic Wnt signaling — a contributor to major psychiatric disorders? *J Neurodev Disord*. 2011; 3:162–174. [PubMed: 21533542]
30. Niehrs C. The complex world of WNT receptor signalling. *Nature Rev Mol Cell Biol*. 2012; 13:767–779. [PubMed: 23151663]
31. Veeman MT, Axelrod J, Moon RT. A second canon. Functions and mechanisms of β -catenin-independent Wnt signaling. *Dev Cell*. 2003; 5:367–377. [PubMed: 12967557]
32. Angers S, Moon RT. Proximal events in Wnt signal transduction. *Nature Rev Mol Cell Biol*. 2009; 10:468–477. [PubMed: 19536106]
33. Nusse R, Varmus H. Three decades of Wnts: a personal perspective on how a scientific field developed. *EMBO J*. 2012; 31:2670–2684. [PubMed: 22617420]
34. Fagotto F. Looking beyond the Wnt pathway for the deep nature of β -catenin. *EMBO Rep*. 2013; 14:422–433. [PubMed: 23598517]
35. Miller RK, Hong JY, Muñoz WA, McCrea PD. β -catenin versus the other armadillo catenins: assessing our current view of canonical Wnt signaling. *Prog Mol Biol Transl Sci*. 2013; 116:387–407. [PubMed: 23481204]
36. Brembeck FH, Rosário M, Birchmeier W. Balancing cell adhesion and Wnt signaling, the key role of β -catenin. *Curr Opin Genet Dev*. 2006; 16:51–59. [PubMed: 16377174]
37. van Veelen W, et al. β -catenin tyrosine 654 phosphorylation increases Wnt signalling and intestinal tumorigenesis. *Gut*. 2011; 60:1204–1212. [PubMed: 21307168]
38. Kawabata A. Prostaglandin E2 and pain — an update. *Biol Pharm Bull*. 2011; 34:1170–1173. [PubMed: 21804201]
39. Gumbiner BM. Carcinogenesis: a balance between β -catenin and APC. *Curr Biol*. 1997; 7:R444–R446.
40. Rubinfeld B, et al. Binding of GSK3 β to the APC- β -catenin complex and regulation of complex assembly. *Science*. 1996; 272:1023–1026. [PubMed: 8638126]
41. Brunner E, Peter O, Schweizer L, Basler K. *Pangolin* encodes a Lef-1 homologue that acts downstream of Armadillo to transduce the Wingless signal in *Drosophila*. *Nature*. 1997; 385:829–833. [PubMed: 9039917]
42. van de Wetering M, et al. Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene dTCF. *Cell*. 1997; 88:789–799. [PubMed: 9118222]
43. Takemaru KI, Moon RT. The transcriptional coactivator CBP interacts with β -catenin to activate gene expression. *J Cell Biol*. 2000; 149:249–254. [PubMed: 10769018]
44. Hecht A, Vlemminckx K, Stemmler MP, van Roy F, Kemler R. The p300/CBP acetyltransferases function as transcriptional coactivators of β -catenin in vertebrates. *EMBO J*. 2000; 19:1839–1850. [PubMed: 10775268]

45. Rasola A, et al. A positive feedback loop between hepatocyte growth factor receptor and β -catenin sustains colorectal cancer cell invasive growth. *Oncogene*. 2007; 26:1078–1087. [PubMed: 16953230]
46. Coluccia AM, et al. Bcr-Abl stabilizes β -catenin in chronic myeloid leukemia through its tyrosine phosphorylation. *EMBO J*. 2007; 26:1456–1466. [PubMed: 17318191]
47. Kim YM, et al. The γ catenin/CBP complex maintains survivin transcription in β -catenin deficient/depleted cancer cells. *Curr Cancer Drug Targets*. 2011; 11:213–225. [PubMed: 21158719]
48. Maeda O, et al. Plakoglobin (γ -catenin) has TCF/LEF family-dependent transcriptional activity in β -catenin-deficient cell line. *Oncogene*. 2004; 23:964–972. [PubMed: 14661054]
49. Jeannot G, et al. Long-term, multilineage hematopoiesis occurs in the combined absence of β -catenin and γ -catenin. *Blood*. 2008; 111:142–149. [PubMed: 17906078]
50. Le NH, Franken P, Fodde R. Tumour-stroma interactions in colorectal cancer: converging on β -catenin activation and cancer stemness. *Br J Cancer*. 2008; 98:1886–1893. [PubMed: 18506144]
51. Polakis P. Drugging Wnt signalling in cancer. *EMBO J*. 2012; 31:2737–2746. erratum 31, 3375 (2012). [PubMed: 22617421]
52. Zimmerman ZF, Moon RT, Chien AJ. Targeting Wnt pathways in disease. *Cold Spring Harb Perspect Biol*. 2012; 4:a008086. [PubMed: 23001988]
53. Herr P, Hausmann G, Basler K. WNT secretion and signalling in human disease. *Trends Mol Med*. 2012; 18:483–493. [PubMed: 22796206]
54. Clevers H, Nusse R. Wnt/ β -catenin signaling and disease. *Cell*. 2012; 149:1192–1205. [PubMed: 22682243]
55. Groden J, et al. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell*. 1991; 66:589–600. [PubMed: 1651174]
56. Kinzler KW, et al. Identification of FAP locus genes from chromosome 5q21. *Science*. 1991; 253:661–665. [PubMed: 1651562]
57. Bodmer WF. Cancer genetics: colorectal cancer as a model. *J Hum Genet*. 2006; 51:391–396. [PubMed: 16596323]
58. Polakis P. The many ways of Wnt in cancer. *Curr Opin Genet Dev*. 2007; 17:45–51. [PubMed: 17208432]
59. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. *Nature*. 1998; 396:643–649. [PubMed: 9872311]
60. Clements WM, Lowy AM, Groden J. Adenomatous polyposis coli/ β -catenin interaction and downstream targets: altered gene expression in gastrointestinal tumors. *Clin Colorectal Cancer*. 2003; 3:113–120. [PubMed: 12952568]
61. Drier Y, et al. Somatic rearrangements across cancer reveal classes of samples with distinct patterns of DNA breakage and rearrangement-induced hypermutability. *Genome Res*. 2013; 23:228–235. [PubMed: 23124520]
62. Rubinfeld B, et al. Stabilization of β -catenin by genetic defects in melanoma cell lines. *Science*. 1997; 275:1790–1792. [PubMed: 9065403]
63. Han ZG. Functional genomic studies: insights into the pathogenesis of liver cancer. *Annu Rev Genom Hum Genet*. 2012; 13:171–205.
64. Sastre-Perona A, Santisteban P. Role of the wnt pathway in thyroid cancer. *Front Endocrinol*. 2012; 3:31.
65. Gatcliffe TA, Monk BJ, Planutis K, Holcombe RF. Wnt signaling in ovarian tumorigenesis. *Int J Gynecol Cancer*. 2008; 18:954–962. [PubMed: 17986238]
66. Caldwell GM, et al. The Wnt antagonist sFRP1 in colorectal tumorigenesis. *Cancer Res*. 2004; 64:883–888. [PubMed: 14871816]
67. Lee AY, et al. Expression of the secreted frizzled-related protein gene family is downregulated in human mesothelioma. *Oncogene*. 2004; 23:6672–6676. [PubMed: 15221014]
68. Suzuki H, et al. Epigenetic inactivation of *SFRP* genes allows constitutive WNT signaling in colorectal cancer. *Nature Genet*. 2004; 36:417–422. This study describes the discovery of a causal link between epigenetic inactivation of WNT antagonists (SFRP) and constitutive WNT signalling activity in colon cancers. [PubMed: 15034581]

69. Fukui T, et al. Transcriptional silencing of secreted frizzled related protein 1 (SFRP 1) by promoter hypermethylation in non-small-cell lung cancer. *Oncogene*. 2005; 24:6323–6327. [PubMed: 16007200]
70. Zou H, et al. Aberrant methylation of secreted frizzled-related protein genes in esophageal adenocarcinoma and Barrett's esophagus. *Int J Cancer*. 2005; 116:584–591. [PubMed: 15825175]
71. Rhee C, et al. Wnt and frizzled receptors as potential targets for immunotherapy in head and neck squamous cell carcinomas. *Oncogene*. 2002; 21:6598–6605. [PubMed: 12242657]
72. Wong SC, et al. Expression of frizzled-related protein and Wnt-signalling molecules in invasive human breast tumours. *J Pathol*. 2002; 196:145–153. [PubMed: 11793365]
73. Milovanovic T, et al. Expression of Wnt genes and frizzled 1 and 2 receptors in normal breast epithelium and infiltrating breast carcinoma. *Int J Oncol*. 2004; 25:1337–1342. [PubMed: 15492823]
74. Okino K, et al. Up-regulation and overproduction of DVL-1, the human counterpart of the *Drosophila* dishevelled gene, in cervical squamous cell carcinoma. *Oncol Rep*. 2003; 10:1219–1223. [PubMed: 12883684]
75. Uematsu K, et al. Wnt pathway activation in mesothelioma: evidence of Dishevelled overexpression and transcriptional activity of β -catenin. *Cancer Res*. 2003; 63:4547–4551. [PubMed: 12907630]
76. Uematsu K, et al. Activation of the Wnt pathway in non small cell lung cancer: evidence of dishevelled overexpression. *Oncogene*. 2003; 22:7218–7221. [PubMed: 14562050]
77. Kikuchi A, Yamamoto H. Tumor formation due to abnormalities in the β -catenin-independent pathway of Wnt signaling. *Cancer Sci*. 2008; 99:202–208. [PubMed: 18271916]
78. Cao X, Eu KW, Seow-Choen F, Zao Y, Cheah PY. APC mutation and phenotypic spectrum of Singapore familial adenomatous polyposis patients. *Eur J Hum Genet*. 2000; 8:42–48. [PubMed: 10713886]
79. Takada T, et al. Methylation-associated silencing of the Wnt antagonist *SFRP1* gene in human ovarian cancers. *Cancer Sci*. 2004; 95:741–744. [PubMed: 15471560]
80. Lugli A, et al. Prognostic significance of the wnt signalling pathway molecules APC, β -catenin and E-cadherin in colorectal cancer: a tissue microarray-based analysis. *Histopathology*. 2007; 50:453–464. [PubMed: 17448021]
81. Kageshita T, et al. Loss of β -catenin expression associated with disease progression in malignant melanoma. *Br J Dermatol*. 2001; 145:210–216. [PubMed: 11531781]
82. Maelandsmo GM, Holm R, Nesland JM, Fodstad Ø, Flørenes VA. Reduced β -catenin expression in the cytoplasm of advanced-stage superficial spreading malignant melanoma. *Clin Cancer Res*. 2003; 9:3383–3388. [PubMed: 12960126]
83. Bachmann IM, Straume O, Puntervoll HE, Kalvenes MB, Akslen LA. Importance of P-cadherin, β -catenin, and Wnt5a/frizzled for progression of melanocytic tumors and prognosis in cutaneous melanoma. *Clin Cancer Res*. 2005; 11:8606–8614. [PubMed: 16361544]
84. Chien AJ, et al. Activated Wnt/ β -catenin signaling in melanoma is associated with decreased proliferation in patient tumors and a murine melanoma model. *Proc Natl Acad Sci USA*. 2009; 106:1193–1198. [PubMed: 19144919]
85. Bakker ER, et al. β -catenin signaling dosage dictates tissue-specific tumor predisposition in *Apc*-driven cancer. *Oncogene*. 2013; 32:4579–4585. [PubMed: 23045279]
86. Salinas PC, Zou Y. Wnt signaling in neural circuit assembly. *Annu Rev Neurosci*. 2008; 31:339–354. [PubMed: 18558859]
87. Freese JL, Pino D, Pleasure SJ. Wnt signaling in development and disease. *Neurobiol Dis*. 2010; 38:148–153. [PubMed: 19765659]
88. Millar JK, Christie S, Semple CA, Porteous DJ. Chromosomal location and genomic structure of the human translin-associated factor X gene (*TRAX*; *TSNAX*) revealed by intergenic splicing to *DISC1*, a gene disrupted by a translocation segregating with schizophrenia. *Genomics*. 2000; 67:69–77. [PubMed: 10945471]
89. Blackwood DH, et al. Schizophrenia and affective disorders — cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. *Am J Hum Genet*. 2001; 69:428–433. [PubMed: 11443544]

90. Mao Y, et al. Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3 β / β -catenin signaling. *Cell*. 2009; 136:1017–1031. [PubMed: 19303846]
91. Courchesne E, et al. Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology*. 2001; 57:245–254. [PubMed: 11468308]
92. Courchesne E, Carper R, Akshoomoff N. Evidence of brain overgrowth in the first year of life in autism. *J Am Med Assoc*. 2003; 290:337–344.
93. Sparks BF, et al. Brain structural abnormalities in young children with autism spectrum disorder. *Neurology*. 2002; 59:184–192. [PubMed: 12136055]
94. Schumann CM, et al. The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. *J Neurosci*. 2004; 24:6392–6401. [PubMed: 15254095]
95. Chenn A, Walsh CA. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. *Science*. 2002; 297:365–369. This article highlights the importance of β -catenin in neuronal stem cells and neuronal development. [PubMed: 12130776]
96. Chenn A, Walsh CA. Increased neuronal production, enlarged forebrains and cytoarchitectural distortions in β -catenin overexpressing transgenic mice. *Cereb Cortex*. 2003; 13:599–606. [PubMed: 12764034]
97. Kalkman HO. A review of the evidence for the canonical Wnt pathway in autism spectrum disorders. *Mol Autism*. 2012; 3:10. [PubMed: 23083465]
98. Kang DE, et al. Presenilin couples the paired phosphorylation of β -catenin independent of axin: implications for β -catenin activation in tumorigenesis. *Cell*. 2002; 110:751–762. [PubMed: 12297048]
99. De Ferrari GV, et al. Common genetic variation within the low-density lipoprotein receptor-related protein 6 and late-onset Alzheimer's disease. *Proc Natl Acad Sci USA*. 2007; 104:9434–9439. [PubMed: 17517621]
100. Wang R, Dineley KT, Sweatt JD, Zheng H. Presenilin 1 familial Alzheimer's disease mutation leads to defective associative learning and impaired adult neurogenesis. *Neuroscience*. 2004; 126:305–312. [PubMed: 15207348]
101. Teo JL, Ma H, Nguyen C, Lam C, Kahn M. Specific inhibition of CBP/ β -catenin interaction rescues defects in neuronal differentiation caused by a presenilin-1 mutation. *Proc Natl Acad Sci USA*. 2005; 102:12171–12176. This is the first publication that describes the model for differential usage of coactivators in WNT- β -catenin signalling to explain the divergent outcomes — that is, proliferation versus differentiation via activation of the WNT signalling cascade. [PubMed: 16093313]
102. Crews L, Patrick C, Adame A, Rockenstein E, Masliah E. Modulation of aberrant CDK5 signaling rescues impaired neurogenesis in models of Alzheimer's disease. *Cell Death Dis*. 2011; 2:e120. [PubMed: 21368891]
103. Liebner S, Plate KH. Differentiation of the brain vasculature: the answer came blowing by the Wnt. *J Angiogenes Res*. 2010; 2:1. [PubMed: 20150991]
104. Dickins EM, Salinas PC. Wnts in action: from synapse formation to synaptic maintenance. *Front Cell Neurosci*. 2013; 7:162. [PubMed: 24223536]
105. Li B, et al. WNT5A signaling contributes to A β -induced neuroinflammation and neurotoxicity. *PLoS ONE*. 2011; 6:e22920. [PubMed: 21857966]
106. Hackam AS. The Wnt signaling pathway in retinal degenerations. *IUBMB Life*. 2005; 57:381–388. [PubMed: 16012046]
107. L'episcopo F, et al. A Wnt1 regulated Frizzled-1/ β -catenin signaling pathway as a candidate regulatory circuit controlling mesencephalic dopaminergic neuron-astrocyte crosstalk: therapeutic relevance for neuron survival and neuroprotection. *Mol Neurodegener*. 2011; 6:49. [PubMed: 21752258]
108. Hamblet NS, et al. Dishevelled 2 is essential for cardiac outflow tract development, somite segmentation and neural tube closure. *Development*. 2002; 129:5827–5838. [PubMed: 12421720]
109. Zeng L, et al. The mouse Fused locus encodes Axin, an inhibitor of the Wnt signaling pathway that regulates embryonic axis formation. *Cell*. 1997; 90:181–192. [PubMed: 9230313]

110. Carter M, et al. Crooked tail (Cd) model of human folate-responsive neural tube defects is mutated in Wnt coreceptor lipoprotein receptor-related protein 6. *Proc Natl Acad Sci USA*. 2005; 102:12843–12848. [PubMed: 16126904]
111. Kokubu C, et al. Skeletal defects in *ringelschwanz* mutant mice reveal that Lrp6 is required for proper somitogenesis and osteogenesis. *Development*. 2004; 131:5469–5480. [PubMed: 15469977]
112. Pinson KI, Brennan J, Monkley S, Avery BJ, Skarnes WC. An LDL-receptor-related protein mediates Wnt signalling in mice. *Nature*. 2000; 407:535–538. [PubMed: 11029008]
113. Bellugi U, Lichtenberger L, Mills D, Galaburda A, Korenberg JR. Bridging cognition, the brain and molecular genetics: evidence from Williams syndrome. *Trends Neurosci*. 1999; 22:197–207. [PubMed: 10322491]
114. Doyle TF, Bellugi U, Korenberg JR, Graham J. Everybody in the world is my friend” hypersociability in young children with Williams syndrome. *Am J Med Genet A*. 2004; 124A: 263–273. [PubMed: 14708099]
115. Zhao C, et al. Hippocampal and visuospatial learning defects in mice with a deletion of frizzled 9, a gene in the Williams syndrome deletion interval. *Development*. 2005; 132:2917–2927. [PubMed: 15930120]
116. Gong Y, et al. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell*. 2001; 107:513–523. In conjunction with reference 125, this paper demonstrates the importance of WNT- β -catenin signalling in bone development and the therapeutic possibility that WNT signalling can be manipulated safely to increase bone density. [PubMed: 11719191]
117. Boyden LM, et al. High bone density due to a mutation in LDL-receptor-related protein 5. *N Engl J Med*. 2002; 346:1513–1521. [PubMed: 12015390]
118. Day TF, Guo X, Garrett-Beal L, Yang Y. Wnt/ β -catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. *Dev Cell*. 2005; 8:739–750. [PubMed: 15866164]
119. Rodda SJ, McMahon AP. Distinct roles for Hedgehog and canonical Wnt signaling in specification, differentiation and maintenance of osteoblast progenitors. *Development*. 2006; 133:3231–3244. [PubMed: 16854976]
120. Glass DA, et al. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev Cell*. 2005; 8:751–764. [PubMed: 15866165]
121. Holmen SL, et al. Essential role of β -catenin in postnatal bone acquisition. *J Biol Chem*. 2005; 280:21162–21168. [PubMed: 15802266]
122. Li X, et al. Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. *J Biol Chem*. 2005; 280:19883–19887. [PubMed: 15778503]
123. Semenov M, Tamai K, He X. SOST is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor. *J Biol Chem*. 2005; 280:26770–26775. [PubMed: 15908424]
124. Li X, et al. Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis. *J Bone Miner Res*. 2009; 24:578–588. [PubMed: 19049336]
125. Little RD, Recker RR, Johnson ML. High bone density due to a mutation in LDL-receptor-related protein 5. *N Engl J Med*. 2002; 347:943–944. In conjunction with reference 116, this paper demonstrates the importance of WNT- β -catenin signalling in bone development and the therapeutic possibility that WNT signalling can be manipulated safely to increase bone density. [PubMed: 12239268]
126. Ellies DL, et al. Bone density ligand, sclerostin, directly interacts with LRP5 but not LRP5G171V to modulate Wnt activity. *J Bone Miner Res*. 2006; 21:1738–1749. [PubMed: 17002572]
127. Semenov M, He X. LRP5 mutations linked to high bone mass diseases cause reduced LRP5 binding and inhibition by SOST. *J Biol Chem*. 2006; 281:38276–38284. [PubMed: 17052975]
128. Hoepfner LH, Secreto FJ, Westendorf JJ. Wnt signaling as a therapeutic target for bone diseases. *Expert Opin Ther Targets*. 2009; 13:485–496. [PubMed: 19335070]
129. Zaidan, M., et al. Increased risk of solid renal tumors in lithium-treated patients. *Kidney Int*. 2014. <http://dx.doi.org/10.1038/ki.2014.2>

130. Gabrielli A, Avvedimento EV, Krieg T. Scleroderma. *N Engl J Med*. 2009; 360:1989–2003. [PubMed: 19420368]
131. Surendran K, Schiavi S, Hruska KA. Wnt-dependent β -catenin signaling is activated after unilateral ureteral obstruction, and recombinant secreted frizzled-related protein 4 alters the progression of renal fibrosis. *J Am Soc Nephrol*. 2005; 16:2373–2384. [PubMed: 15944336]
132. Myung SJ, et al. Wnt signaling enhances the activation and survival of human hepatic stellate cells. *FEBS Lett*. 2007; 581:2954–2958. [PubMed: 17544413]
133. Königshoff M, et al. Functional Wnt signaling is increased in idiopathic pulmonary fibrosis. *PLoS ONE*. 2008; 3:e2142. [PubMed: 18478089]
134. Henderson WR Jr, et al. Inhibition of Wnt/ β -catenin/CREB binding protein (CBP) signaling reverses pulmonary fibrosis. *Proc Natl Acad Sci USA*. 2010; 107:14309–14314. This study first demonstrates that idiopathic pulmonary fibrosis may be pharmacologically reversible via modulation of WNT- β -catenin signalling. [PubMed: 20660310]
135. Hao S, et al. Targeted inhibition of β -catenin/CBP signaling ameliorates renal interstitial fibrosis. *J Am Soc Nephrol*. 2011; 22:1642–1653. [PubMed: 21816937]
136. Königshoff M, et al. WNT1-inducible signaling protein-1 mediates pulmonary fibrosis in mice and is upregulated in humans with idiopathic pulmonary fibrosis. *J Clin Invest*. 2009; 119:772–787. [PubMed: 19287097]
137. Flozak AS, et al. β -catenin/T-cell factor signaling is activated during lung injury and promotes the survival and migration of alveolar epithelial cells. *J Biol Chem*. 2010; 285:3157–3167. [PubMed: 19933277]
138. Ulsamer A, et al. Axin pathway activity regulates *in vivo* pY654- β -catenin accumulation and pulmonary fibrosis. *J Biol Chem*. 2012; 287:5164–5172. [PubMed: 22203675]
139. Akhmetshina A, et al. Activation of canonical Wnt signalling is required for TGF- β mediated fibrosis. *Nature Commun*. 2012; 3:735. [PubMed: 22415826]
140. Tanjore H, et al. β -catenin in the alveolar epithelium protects from lung fibrosis after intratracheal bleomycin. *Am J Respir Crit Care Med*. 2013; 187:630–639. [PubMed: 23306543]
141. Gottardi CJ, Königshoff M. Considerations for targeting β -catenin signaling in fibrosis. *Am J Respir Crit Care Med*. 2013; 187:566–568. [PubMed: 23504360]
142. Dravid G, et al. Defining the role of Wnt/ β -catenin signaling in the survival, proliferation, and self-renewal of human embryonic stem cells. *Stem Cells*. 2005; 23:1489–1501. [PubMed: 16002782]
143. Qyang Y, et al. The renewal and differentiation of *Isl1*⁺ cardiovascular progenitors are controlled by a Wnt/ β -catenin pathway. *Cell Stem Cell*. 2007; 1:165–179. [PubMed: 18371348]
144. Saraswati S, et al. Pyrvinium, a potent small molecule Wnt inhibitor, promotes wound repair and post-MI cardiac remodeling. *PLoS ONE*. 2010; 5:e15521. [PubMed: 21170416]
145. Sasaki T, Hwang H, Nguyen C, Kloner RA, Kahn M. The small molecule Wnt signaling modulator ICG-001 improves contractile function in chronically infarcted rat myocardium. *PLoS ONE*. 2013; 8:e75010. [PubMed: 24069374]
146. de Lau W, Peng WC, Gros P, Clevers H. The R-spondin/Lgr5/Rnf43 module: regulator of Wnt signal strength. *Genes Dev*. 2014; 28:305–316. [PubMed: 24532711]
147. de Lau WBM, Snel B, Clevers HC. The R-spondin protein family. *Genome Biol*. 2012; 13:242. [PubMed: 22439850]
148. Zhao J, et al. R-spondin1, a novel intestinotrophic mitogen, ameliorates experimental colitis in mice. *Gastroenterology*. 2007; 132:1331–1343. [PubMed: 17408649]
149. Bhanja P, et al. Protective role of R-spondin1, an intestinal stem cell growth factor, against radiation-induced gastrointestinal syndrome in mice. *PLoS ONE*. 2009; 4:e8014. [PubMed: 19956666]
150. Zhou WJ, Geng ZH, Spence JR, Geng JG. Induction of intestinal stem cells by R-spondin 1 and Slit2 augments chemoradioprotection. *Nature*. 2013; 50:107–111. [PubMed: 23903657]
151. Yu Q, Sharma A, Sen JM. TCF1 and β -catenin regulate T cell development and function. *Immunol Res*. 2010; 47:45–55. [PubMed: 20082155]

152. Gattinoni L, et al. Wnt signaling arrests effector T cell differentiation and generates CD8⁺ memory stem cells. *Nature Med.* 2009; 15:808–813. [PubMed: 19525962]
153. Jeannot G, et al. Essential role of the Wnt pathway effector Tcf-1 for the establishment of functional CD8 T cell memory. *Proc Natl Acad Sci USA.* 2010; 107:9777–9782. [PubMed: 20457902]
154. Zhao DM, et al. Constitutive activation of Wnt signaling favors generation of memory CD8 T cells. *J Immunol.* 2010; 184:1191–11989. [PubMed: 20026746]
155. Zhou X, et al. Differentiation and persistence of memory CD8⁺ T cells depend on T cell factor 1. *Immunity.* 2010; 33:229–240. [PubMed: 20727791]
156. Notani D, et al. Global regulator SATB1 recruits β -catenin and regulates T_H2 differentiation in Wnt-dependent manner. *PLoS Biol.* 2010; 8:e1000296. [PubMed: 20126258]
157. Ding Y, Shen S, Lino AC, Curotto de Lafaille MA, Lafaille JJ. β -catenin stabilization extends regulatory T cell survival and induces anergy in nonregulatory T cells. *Nature Med.* 2008; 14:162–169. [PubMed: 18246080]
158. Sen M, et al. Expression and function of wingless and frizzled homologs in rheumatoid arthritis. *Proc Natl Acad Sci USA.* 2000; 97:2791–2796. [PubMed: 10688908]
159. Nakamura Y, Nawata M, Wakitani S. Expression profiles and functional analyses of Wnt-related genes in human joint disorders. *Am J Pathol.* 2005; 167:97–105. [PubMed: 15972956]
160. Keerthivasan S, et al. β -catenin promotes colitis and colon cancer through imprinting of proinflammatory properties in T Cells. *Sci Transl Med.* 2014; 6:225ra28.
161. Otero K, et al. Macrophage colony-stimulating factor induces the proliferation and survival of macrophages via a pathway involving DAPI2 and β -catenin. *Nature Immunol.* 2009; 10:734–743. [PubMed: 19503107]
162. Wood LD, et al. The genomic landscapes of human breast and colorectal cancers. *Science.* 2007; 318:1108–1113. [PubMed: 17932254]
163. García-Jiménez C. Wnt and incretin connections. *Vitam Horm.* 2010; 84:355–387. [PubMed: 21094908]
164. Abiola M, et al. Activation of Wnt/ β -catenin signaling increases insulin sensitivity through a reciprocal regulation of Wnt10b and SREBP-1c in skeletal muscle cells. *PLoS ONE.* 2009; 4:e8509. [PubMed: 20041157]
165. Welters HJ, Kulkarni RN. Wnt signaling: relevance to β -cell biology and diabetes. *Trends Endocrinol Metab.* 2008; 19:349–355. [PubMed: 18926717]
166. Grant SF, et al. Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. *Nature Genet.* 2006; 38:320–323. This study shows that *TCF7L2* (also known as *TCF4*) human polymorphisms are associated with an increased risk of type 2 diabetes. This has been subsequently validated in a number of studies in diverse populations. [PubMed: 16415884]
167. Takamoto I, et al. *TCF7L2* in mouse pancreatic β cells plays a crucial role in glucose homeostasis by regulating β cell mass. *Diabetologia.* 2014; 57:542–553. [PubMed: 24317852]
168. Aly H, et al. A novel strategy to increase the proliferative potential of adult human β -cells while maintaining their differentiated phenotype. *PLoS ONE.* 2013; 8:e66131. [PubMed: 23776620]
169. Rando TA. Stem cells, ageing and the quest for immortality. *Nature.* 2006; 441:1080–1086. [PubMed: 16810243]
170. Hernandez L, et al. Functional coupling between the extracellular matrix and nuclear lamina by Wnt signaling in progeria. *Dev Cell.* 2010; 19:413–425. [PubMed: 20833363]
171. Liu H, et al. Augmented Wnt signaling in a mammalian model of accelerated aging. *Science.* 2007; 317:803–806. [PubMed: 17690294]
172. Brack AS, et al. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science.* 2007; 317:807–810. References 170–172 demonstrate that increased WNT signalling is associated with ageing. [PubMed: 17690295]
173. Bernard P, Harley VR. Wnt4 action in gonadal development and sex determination. *Int J Biochem Cell Biol.* 2007; 39:31–43. [PubMed: 16905353]
174. Lear JT. Oral hedgehog-pathway inhibitors for basal-cell carcinoma. *N Engl J Med.* 2012; 366:2225–2226. [PubMed: 22670909]

175. Sakata T, Chen JK. Chemical 'Jekyll and Hyde's: small-molecule inhibitors of developmental signaling pathways. *Chem Soc Rev.* 2011; 40:4318–4331. [PubMed: 21505654]
176. White BD, et al. β -catenin signaling increases in proliferating NG2⁺ progenitors and astrocytes during post-traumatic gliogenesis in the adult brain. *Stem Cells.* 2010; 28:297–307. [PubMed: 19960516]
177. Fancy SP, et al. Axin2 as regulatory and therapeutic target in newborn brain injury and remyelination. *Nature Neurosci.* 2011; 14:1009–1016. [PubMed: 21706018]
178. Previdi S, et al. Interaction between human-breast cancer metastasis and bone microenvironment through activated hepatocyte growth factor/Met and β -catenin/Wnt pathways. *Eur J Cancer.* 2010; 46:1679–1691. [PubMed: 20350802]
179. Kajiguchi T, et al. FLT3 regulates β -catenin tyrosine phosphorylation, nuclear localization, and transcriptional activity in acute myeloid leukemia cells. *Leukemia.* 2007; 21:2476–2484. [PubMed: 17851558]
180. Kajiguchi T, et al. Y654 of β -catenin is essential for FLT3/ITD-related tyrosine phosphorylation and nuclear localization of β -catenin. *Eur J Haematol.* 2012; 88:314–320. [PubMed: 22126602]
181. Zhou L, et al. Tyrosine kinase inhibitor STI-571/Gleevec down-regulates the β -catenin signaling activity. *Cancer Lett.* 2003; 193:161–170. [PubMed: 12706873]
182. Christensen JG, et al. A selective small molecule inhibitor of c-Met kinase inhibits c-Met-dependent phenotypes *in vitro* and exhibits cytoreductive antitumor activity *in vivo*. *Cancer Res.* 2003; 63:7345–7355. [PubMed: 14612533]
183. Hayward P, et al. Notch modulates Wnt signalling by associating with Armadillo/ β -catenin and regulating its transcriptional activity. *Development.* 2005; 132:1819–1830. [PubMed: 15772135]
184. Bertrand FE, Angus CW, Partis WJ, Sigounas G. Developmental pathways in colon cancer: crosstalk between WNT, BMP, Hedgehog and Notch. *Cell Cycle.* 2012; 11:4344–4351. [PubMed: 23032367]
185. Lukaszewicz AI, McMillan MK, Kahn M. Small molecules and stem cells. Potency and lineage commitment: the new quest for the fountain of youth. *J Med Chem.* 2010; 53:3439–3453. [PubMed: 20047330]
186. Yao J, et al. Combination treatment of PD98059 and DAPT in gastric cancer through induction of apoptosis and downregulation of WNT/ β -catenin. *Cancer Biol Ther.* 2013; 14:833–839. [PubMed: 23792588]
187. Janssen KP, et al. APC and oncogenic KRAS are synergistic in enhancing Wnt signaling in intestinal tumor formation and progression. *Gastroenterology.* 2003; 131:1096–1109. [PubMed: 17030180]
188. Sansom OJ, et al. Loss of Apc allows phenotypic manifestation of the transforming properties of an endogenous K-ras oncogene *in vivo*. *Proc Natl Acad Sci USA.* 2006; 103:14122–14127. [PubMed: 16959882]
189. Harada N, et al. Hepatocarcinogenesis in mice with β -catenin and Ha-Ras gene mutations. *Cancer Res.* 2004; 64:48–54. [PubMed: 14729607]
190. Kerkhoff E, et al. Regulation of *c-myc* expression by Ras/Raf signalling. *Oncogene.* 1998; 16:211–216. [PubMed: 9464539]
191. Araki Y, et al. Regulation of cyclooxygenase-2 expression by the Wnt and Ras pathways. *Cancer Res.* 2003; 63:728–734. [PubMed: 12566320]
192. Masckauchán TN, Shawber CJ, Funahashi Y, Li CM, Kitajewski J. Wnt/ β -catenin signaling induces proliferation, survival and interleukin-8 in human endothelial cells. *Angiogenesis.* 2005; 8:43–51. [PubMed: 16132617]
193. Radtke F, Clevers H. Self-renewal and cancer of the gut: two sides of a coin. *Science.* 2005; 307:1904–1909. [PubMed: 15790842]
194. Corada M, et al. The Wnt/ β -catenin pathway modulates vascular remodeling and specification by upregulating Dll4/Notch signaling. *Dev Cell.* 2010; 18:938–949. [PubMed: 20627076]
195. Aulehla A, et al. Wnt3a plays a major role in the segmentation clock controlling somitogenesis. *Dev Cell.* 2003; 4:395–406. [PubMed: 12636920]
196. Miyabayashi T, et al. Wnt/ β -catenin/CBP signaling maintains long-term murine embryonic stem cell pluripotency. *Proc Natl Acad Sci USA.* 2007; 104:5668–5673. [PubMed: 17372190]

197. Hasegawa K, et al. Wnt signaling orchestration with a small molecule DYRK inhibitor provides long-term xeno-free human pluripotent cell expansion. *Stem Cells Transl Med.* 2012; 1:18–28. References 196 and 197 demonstrate that selective inhibition of WNT- β -catenin-p300 signalling maintains both mouse and human stem cell pluripotency. [PubMed: 23197636]
198. Gupta PB, et al. Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell.* 2009; 138:645–659. [PubMed: 19682730]
199. Lu D, et al. Salinomycin inhibits Wnt signaling and selectively induces apoptosis in chronic lymphocytic leukemia cells. *Proc Natl Acad Sci USA.* 2011; 108:13253–13257. [PubMed: 21788521]
200. Klein PS, Melton DA. A molecular mechanism for the effect of lithium on development. *Proc Natl Acad Sci USA.* 1996; 93:8455–8459. [PubMed: 8710892]
201. Giardiello FM, et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. *N Engl J Med.* 1993; 328:131–136. [PubMed: 8416424]
202. Schreinemachers DM, Everson RB. Aspirin use and lung, colon, and breast cancer incidence in a prospective study. *Epidemiology.* 1994; 5:138–146.
203. Dihlmann S, Siermann A, von Knebel Doeberitz M. The nonsteroidal anti-inflammatory drugs aspirin and indomethacin attenuate β -catenin/TCF-4 signaling. *Oncogene.* 2001; 20:645–653. [PubMed: 11313997]
204. Thun MJ, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. *J Natl Cancer Inst.* 2002; 94:252–266. [PubMed: 11854387]
205. Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. *N Engl J Med.* 2003; 348:883–890. [PubMed: 12621132]
206. Baron JA, et al. A randomized trial of aspirin to prevent colorectal adenomas. *N Engl J Med.* 2003; 348:891–899. [PubMed: 12621133]
207. Boon EM, et al. Sulindac targets nuclear β -catenin accumulation and Wnt signalling in adenomas of patients with familial adenomatous polyposis and in human colorectal cancer cell lines. *Br J Cancer.* 2004; 90:224–229. [PubMed: 14710233]
208. Grösch S, Tegeder I, Niederberger E, Bräutigam L, Geisslinger G. COX-2 independent induction of cell cycle arrest and apoptosis in colon cancer cells by the selective COX-2 inhibitor celecoxib. *FASEB J.* 2001; 15:2742–2744. [PubMed: 11606477]
209. Maier TJ, Janssen A, Schmidt R, Geisslinger G, Grösch S. Targeting the β -catenin/APC pathway: a novel mechanism to explain the cyclooxygenase-2-independent anticarcinogenic effects of celecoxib in human colon carcinoma cells. *FASEB J.* 2005; 19:1353–1355. [PubMed: 15946992]
210. Phillips RK, et al. A randomised, double blind, placebo controlled study of celecoxib, a selective cyclooxygenase 2 inhibitor, on duodenal polyposis in familial adenomatous polyposis. *Gut.* 2002; 50:857–860. [PubMed: 12010890]
211. Shah S, Hecht A, Pestell R, Byers SW. Trans-repression of β -catenin activity by nuclear receptors. *J Biol Chem.* 2003; 278:48137–48145. [PubMed: 12972427]
212. Pálmer HG, et al. Vitamin D₃ promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of β -catenin signaling. *J Cell Biol.* 2001; 154:369–387. [PubMed: 11470825]
213. Pendás-Franco N, et al. Vitamin D and Wnt/ β -catenin pathway in colon cancer: role and regulation of *DICKKOPF* genes. *Anticancer Res.* 2008; 28:2613–2623. [PubMed: 19035286]
214. Rao CV, et al. Chemoprevention of colon carcinogenesis by phenylethyl-3-methylcaffeate. *Cancer Res.* 1995; 55:2310–2315. [PubMed: 7757981]
215. Jaiswal AS, Marlow BP, Gupta N, Narayan S. β -catenin-mediated transactivation and cell-cell adhesion pathways are important in curcumin (diferuylmethane)-induced growth arrest and apoptosis in colon cancer cells. *Oncogene.* 2002; 21:8414–8427. [PubMed: 12466962]
216. Park CH, et al. Quercetin, a potent inhibitor against β -catenin/Tcf signaling in SW480 colon cancer cells. *Biochem Biophys Res Commun.* 2005; 328:227–234. [PubMed: 15670774]
217. Kim J, et al. Suppression of Wnt signaling by the green tea compound (–)-epigallocatechin 3-gallate (EGCG) in invasive breast cancer cells. Requirement of the transcriptional repressor HBP1. *J Biol Chem.* 2006; 281:10865–10875. [PubMed: 16495219]

218. Roccaro AM, et al. Resveratrol exerts antiproliferative activity and induces apoptosis in Waldenström's macroglobulinemia. *Clin Cancer Res.* 2008; 14:1849–1858. [PubMed: 18347188]
219. Funato Y, Michiue T, Asashima M, Miki H. The thioredoxin-related redox-regulating protein nucleoredoxin inhibits Wnt- β -catenin signalling through dishevelled. *Nature Cell Biol.* 2006; 8:501–508. [PubMed: 16604061]
220. Antelmann H, Helmann JD. Thiol-based redox switches and gene regulation. *Antioxid Redox Signal.* 2011; 14:1049–1063. [PubMed: 20626317]
221. Thorne CA, et al. Small-molecule inhibition of Wnt signaling through activation of casein kinase 1 α . *Nature Chem Biol.* 2010; 6:829–836. [PubMed: 20890287]
222. Lepourcelet M, et al. Small-molecule antagonists of the oncogenic Tcf/ β -catenin protein complex. *Cancer Cell.* 2004; 5:91–102. This publication reports the first identification of natural small-molecule inhibitors (although not truly selective) of TCF- β -catenin complexes using an ELISA-based high-throughput screen. [PubMed: 14749129]
223. Gonsalves FC, et al. An RNAi-based chemical genetic screen identifies three small-molecule inhibitors of the Wnt/*wingless* signaling pathway. *Proc Natl Acad Sci USA.* 2011; 108:5894–5863.
224. Chen Z, et al. 2,4-diamino-quinazolines as inhibitors of β -catenin/Tcf-4 pathway: potential treatment for colorectal cancer. *Bioorg Med Chem Lett.* 2009; 19:4980–4983. [PubMed: 19640711]
225. Trosset JY, et al. Inhibition of protein-protein interactions: the discovery of druglike β -catenin inhibitors by combining virtual and biophysical screening. *Proteins.* 2006; 64:60–67. [PubMed: 16568448]
226. Tian W, et al. Structure-based discovery of a novel inhibitor targeting the β -catenin/Tcf4 interaction. *Biochemistry.* 2012; 51:724–731. [PubMed: 22224445]
227. Hahne G, Grossmann TN. Direct targeting of β -catenin: inhibition of protein-protein interactions for the inactivation of Wnt signaling. *Bioorg Med Chem.* 2013; 21:4020–4026. [PubMed: 23566764]
228. Takada K, et al. Targeted disruption of the BCL9/ β -catenin complex inhibits oncogenic Wnt signaling. *Sci Transl Med.* 2012; 4:148ra117.
229. Grossmann TN, et al. Inhibition of oncogenic Wnt signaling through direct targeting of β -catenin. *Proc Natl Acad Sci USA.* 2012; 109:17942–17947. [PubMed: 23071338]
230. Shan J, Shi DL, Wang J, Zheng J. Identification of a specific inhibitor of the dishevelled PDZ domain. *Biochemistry.* 2005; 44:15495–15503. [PubMed: 16300398]
231. Fujii N, et al. An antagonist of dishevelled protein-protein interaction suppresses β -catenin-dependent tumor cell growth. *Cancer Res.* 2007; 67:573–579. [PubMed: 17234765]
232. Grandy D, et al. Discovery and characterization of a small molecule inhibitor of the PDZ domain of dishevelled. *J Biol Chem.* 2009; 284:16256–16263. [PubMed: 19383605]
233. Chen B, et al. Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. *Nature Chem Biol.* 2009; 5:100–107. [PubMed: 19125156]
234. Huang SM, et al. Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. *Nature.* 2009; 461:614–620. This publication provides evidence for the role of tankyrase in WNT signalling and a rationale for developing specific tankyrase inhibitors to target the WNT pathway. [PubMed: 19759537]
235. Lehtiö L, Chi NW, Krauss S. Tankyrases as drug targets. *FEBS J.* 2013; 280:3576–3593. [PubMed: 23648170]
236. Lau T, et al. A novel tankyrase small-molecule inhibitor suppresses APC mutation-driven colorectal tumor growth. *Cancer Res.* 2013; 73:3132–3144. [PubMed: 23539443]
237. Hao HX, et al. ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. *Nature.* 2012; 485:195–200. [PubMed: 22575959]
238. Jacob LS, et al. Genome-wide RNAi screen reveals disease-associated genes that are common to Hedgehog and Wnt signaling. *Sci Signal.* 2011; 4:ra4. [PubMed: 21266715]
239. Koo BK, et al. Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. *Nature.* 2012; 488:665–669. [PubMed: 22895187]

240. Jiang X, et al. Inactivating mutations of RNF43 confer Wnt dependency in pancreatic ductal adenocarcinoma. *Proc Natl Acad Sci USA*. 2013; 110:12649–12654. [PubMed: 23847203]
241. Li X, et al. Prostate tumor progression is mediated by a paracrine TGF- β /Wnt3a signaling axis. *Oncogene*. 2008; 27:7118–7130. [PubMed: 18724388]
242. Wei W, Chua MS, Grepper S, So SK. Soluble Frizzled-7 receptor inhibits Wnt signaling and sensitizes hepatocellular carcinoma cells towards doxorubicin. *Mol Cancer*. 2011; 10:16. [PubMed: 21314951]
243. Pode-Shakked N, et al. Resistance or sensitivity of Wilms' tumor to anti-FZD7 antibody highlights the Wnt pathway as a possible therapeutic target. *Oncogene*. 2011; 30:1664–1680. [PubMed: 21472018]
244. Gurney A, et al. Wnt pathway inhibition via the targeting of Frizzled receptors results in decreased growth and tumorigenicity of human tumors. *Proc Natl Acad Sci USA*. 2012; 109:11717–11722. This manuscript describes preclinical studies utilizing the monoclonal antibody OMP-18R5, which is currently in Phase I clinical trials. [PubMed: 22753465]
245. Kung AL, et al. Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP. *Genes Dev*. 2000; 14:272–277. [PubMed: 10673499]
246. Yamauchi T, et al. Increased insulin sensitivity despite lipodystrophy in *Crebbp* heterozygous mice. *Nature Genet*. 2002; 30:221–226. [PubMed: 11818964]
247. Roth JF, et al. Differential role of p300 and CBP acetyltransferase during myogenesis: 300 acts upstream of MyoD and Myf5. *EMBO J*. 2003; 22:5186–5196. [PubMed: 14517256]
248. McMillan M, Kahn M. Investigating Wnt signaling: a chemogenomic safari. *Drug Discov Today*. 2005; 10:1467–1474. [PubMed: 16243267]
249. Emami KH, et al. A small molecule inhibitor of β -catenin/CREB-binding protein transcription [corrected]. *Proc Natl Acad Sci USA*. 2004; 101:12682–12687. This study and subsequent studies utilizing specific small-molecule CBP antagonists (including those detailed in references 252 and 253) provided the rationale for the clinical development of the second-generation CBP–catenin antagonist PRI-724, which is currently in Phase I/II clinical trials. [PubMed: 15314234]
250. Marson A, et al. Wnt signaling promotes reprogramming of somatic cells to pluripotency. *Cell Stem Cell*. 2008; 3:132–135. [PubMed: 18682236]
251. Schenke-Layland K, et al. Recapitulation of the embryonic cardiovascular progenitor cell niche. *Biomaterials*. 2011; 2:2748–2756. [PubMed: 21257198]
252. Wend P, et al. Wnt/ β -catenin signalling induces MLL to create epigenetic changes in salivary gland tumours. *EMBO J*. 2013; 32:1977–1989. [PubMed: 23736260]
253. Gang EJ, et al. Small-molecule inhibition of CBP/catenin interactions eliminates drug-resistant clones in acute lymphoblastic leukemia. *Oncogene*. 2013; 33:2169–2178. [PubMed: 23728349]
254. He K, et al. Cancer cells acquire a drug resistant, highly tumorigenic, cancer stem-like phenotype through modulation of the PI3K/Akt/ β -catenin/CBP pathway. *Int J Cancer*. 2014; 134:43–54. [PubMed: 23784558]
255. Kahn M. Symmetric division versus asymmetric division: a tale of two coactivators. *Future Med Chem*. 2011; 3:1745–1763. This article outlines the mechanism whereby selective CBP–catenin antagonists can safely eliminate cancer stem cells via forced differentiation without having deleterious effects on the normal somatic stem cell populations. [PubMed: 22004083]
256. Takada R, et al. Monounsaturated fatty acid modification of Wnt protein: its role in Wnt secretion. *Dev Cell*. 2006; 11:791–801. [PubMed: 17141155]
257. Liu J, et al. Targeting Wnt-driven cancer through the inhibition of Porcupine by LGK974. *Proc Natl Acad Sci USA*. 2013; 110:20224–20229. This study describes the discovery of the small-molecule Porcupine inhibitor LGK974, which is currently in Phase I clinical trials. [PubMed: 24277854]
258. Smith DC, et al. First-in-human evaluation of the human monoclonal antibody vantictumab (OMP-18R5; anti-Frizzled) targeting the WNT pathway in a phase I study for patients with advanced solid tumors. *J Clin Oncol Abstr*. 2013; 31:2540.
259. Morishita EC, et al. Crystal structures of the armadillo repeat domain of adenomatous polyposis coli and its complex with the tyrosine-rich domain of Sam68. *Structure*. 2011; 19:1496–1508. [PubMed: 22000517]

260. Ma H, Nguyen C, Lee KS, Kahn M. Differential roles for the coactivators CBP and p300 on TCF/ β -catenin-mediated survivin gene expression. *Oncogene*. 2005; 24:3619–3631. [PubMed: 15782138]
261. el-Khoueiry A, et al. A phase I first-in-human study of PRI-724 in patients (pts) with advanced solid tumors. *J Clin Oncol Abstr*. 2013; 31:2501.
262. Mahmoudi T, et al. The kinase TNIK is an essential activator of Wnt target genes. *EMBO J*. 2009; 28:3329–3340. [PubMed: 19816403]
263. Shitashige M, et al. Traf2- and Nck-interacting kinase is essential for Wnt signaling and colorectal cancer growth. *Cancer Res*. 2010; 70:5024–5033. [PubMed: 20530691]
264. Onder TT, et al. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. *Cancer Res*. 2008; 68:3645–3654. [PubMed: 18483246]
265. Kim K, Daniels KJ, Hay ED. Tissue-specific expression of β -catenin in normal mesenchyme and uveal melanomas and its effect on invasiveness. *Exp Cell Res*. 1998; 245:79–90. [PubMed: 9828103]
266. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. *Cell*. 2011; 147:275–292. [PubMed: 22000009]
267. Mani SA, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell*. 2008; 133:704–715. [PubMed: 18485877]
268. Wagh PK, et al. β -catenin is required for Ron receptor-induced mammary tumorigenesis. *Oncogene*. 2011; 30:3694–3704. [PubMed: 21423209]
269. Coluccia AM, et al. SKI-606 decreases growth and motility of colorectal cancer cells by preventing pp60(c-Src)-dependent tyrosine phosphorylation of β -catenin and its nuclear signaling. *Cancer Res*. 2006; 66:2279–2286. [PubMed: 16489032]
270. Ress A, Moelling K. Bcr interferes with β -catenin-Tcf1 interaction. *FEBS Lett*. 2006; 580:1227–1230. [PubMed: 16442529]
271. Fujino H, West KA, Regan JW. Phosphorylation of glycogen synthase kinase-3 and stimulation of T-cell factor signaling following activation of EP2 and EP4 prostanoid receptors by prostaglandin E2. *J Biol Chem*. 2002; 277:2614–2619. [PubMed: 11706038]
272. Fujino H, Regan JW. FP prostanoid receptor activation of a T-cell factor/ β -catenin signaling pathway. *J Biol Chem*. 2001; 276:12489–12492. [PubMed: 11278257]
273. Liu X, Rubin JS, Kimmel AR. Rapid, Wnt-induced changes in GSK3 β associations that regulate β -catenin stabilization are mediated by G α proteins. *Curr Biol*. 2005; 15:1989–1997. [PubMed: 16303557]
274. Meigs TE, Fields TA, McKee DD, Casey PJ. Interaction of G α 12 and G α 13 with the cytoplasmic domain of cadherin provides a mechanism for β -catenin release. *Proc Natl Acad Sci USA*. 2001; 98:519–524. [PubMed: 11136230]
275. Mazumdar J, et al. O₂ regulates stem cells through Wnt/ β -catenin signalling. *Nature Cell Biol*. 2010; 12:1007–1013. [PubMed: 20852629]
276. Chocarro-Calvo A, García-Martínez JM, Ardila-González S, De la Vieja A, García-Jiménez C. Glucose-induced β -catenin acetylation enhances Wnt signaling in cancer. *Mol Cell*. 2013; 49:474–486. [PubMed: 23273980]
277. Gómez-Orte E, Sáenz-Narciso B, Moreno S, Cabello J. Multiple functions of the noncanonical Wnt pathway. *Trends Genet*. 2013; 29:545–553. [PubMed: 23846023]
278. Tada M, Kai M. Noncanonical Wnt/PCP signaling during vertebrate gastrulation. *Zebrafish*. 2009; 6:29–40. [PubMed: 19292674]
279. Wang Y. Wnt/planar cell polarity signaling: a new paradigm for cancer therapy. *Mol Cancer Ther*. 2009; 8:2103–2109. [PubMed: 19671746]
280. Lai SL, Chien AJ, Moon RT. Wnt/Fz signaling and the cytoskeleton: potential roles in tumorigenesis. *Cell Res*. 2009; 19:532–545. [PubMed: 19365405]
281. Ginis I, et al. Differences between human and mouse embryonic stem cells. *Dev Biol*. 2004; 269:360–380. [PubMed: 15110706]

282. Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nature Med.* 2004; 10:55–63. [PubMed: 14702635]
283. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature.* 2001; 414:105–111. [PubMed: 11689955]
284. Otero JJ, Fu W, Kan L, Cuadra AE, Kessler JA. β -catenin signaling is required for neural differentiation of embryonic stem cells. *Development.* 2004; 131:3545–3557. [PubMed: 15262888]
285. Faunes F, et al. A membrane-associated β -catenin/Oct4 complex correlates with ground-state pluripotency in mouse embryonic stem cells. *Development.* 2013; 140:1171–1183. [PubMed: 23444350]
286. Zechner D, et al. β -catenin signals regulate cell growth and the balance between progenitor cell expansion and differentiation in the nervous system. *Dev Biol.* 2003; 258:406–418. [PubMed: 12798297]
287. Hari L, et al. Lineage-specific requirements of β -catenin in neural crest development. *J Cell Biol.* 2002; 159:867–880. [PubMed: 12473692]
288. Muroyama Y, Kondoh H, Takada S. Wnt proteins promote neuronal differentiation in neural stem cell culture. *Biochem Biophys Res Commun.* 2004; 313:915–921. [PubMed: 14706629]
289. LaBarge M. The difficulty of targeting cancer stem cell niches. *Clin Cancer Res.* 2010; 16:3121–3129. [PubMed: 20530700]
290. Merchan AA, Matsui W. Targeting Hedgehog — a cancer stem cell pathway. *Clin Cancer Res.* 2010; 16:3130–3140. [PubMed: 20530699]
291. Pannuti A, et al. Targeting Notch to target cancer stem cells. *Clin Cancer Res.* 2010; 16:3141–3152. [PubMed: 20530696]
292. Holland JD, Klaus A, Garratt AN, Birchmeier W. Wnt signaling in stem and cancer stem cells. *Curr Opin Cell Biol.* 2013; 25:254–264. This is an excellent recent review on the role of WNT signalling in cancer stem cells. [PubMed: 23347562]
293. Cicalese A, et al. The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. *Cell.* 2009; 138:1083–1095. [PubMed: 19766563]
294. Takahashi-Yanaga F, Kahn M. Targeting Wnt signaling: can we safely eradicate cancer stem cells? *Clin Cancer Res.* 2010; 16:3153–3162. [PubMed: 20530697]

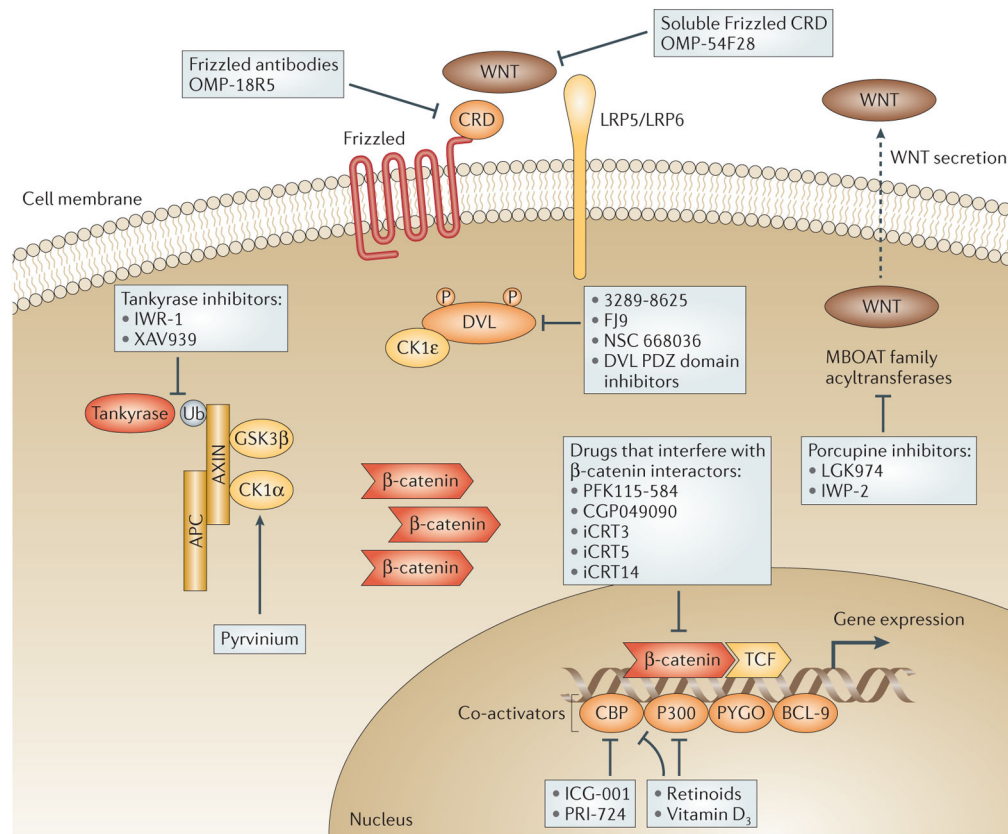


Figure 1. A simplified representation of the canonical WNT– β -catenin signalling cascade

The WNT– β -catenin signalling cascade has crucial roles in cell fate, proliferation, survival and migration. In the absence of extracellular WNT glycoproteins, a destruction complex — including the proteins adenomatous polyposis coli (APC), glycogen synthase kinase 3 β (GSK3 β) and AXIN — phosphorylates β -catenin, targeting it for ubiquitylation and proteasomal degradation. The binding of WNTs to Frizzled receptors and the co-receptors LRP5 (low-density lipoprotein receptor-related protein 5) and LRP6 relays a signal through Dishevelled (DVL), which results in inhibition of the destruction complex and nuclear entry of β -catenin. In the nucleus, β -catenin acts as a bridge between members of the T cell factor (TCF) family of transcription factors and the basal transcriptional apparatus via co-activators (CREB-binding protein (CBP), E1A-associated protein p300, the co-activator Pygopus (PYGO), B cell lymphoma 9 (BCL-9), and so on). Over the past decade, numerous studies have identified inhibitors at various points along the pathway, a few of which have recently entered clinical trials. These are discussed in detail within the main text. CK1 α , casein kinase 1 α ; CRD, cysteine-rich domain; MBOAT, membrane-bound *O*-acyltransferase.

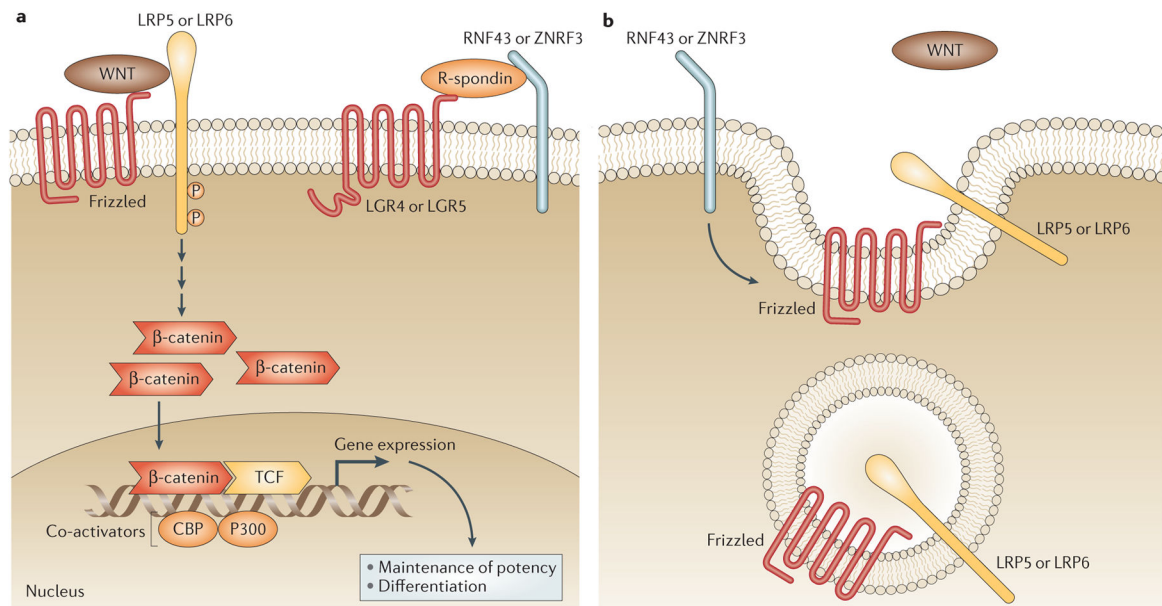


Figure 2. WNT signalling in stem cells

This figure shows the dichotomous roles of WNT signalling in the expansion and maintenance of potency in stem cells, as well as in the initiation of differentiation and lineage commitment. Frizzled and low-density lipoprotein receptor-related proteins (LRPs) on the surface of stem cells bind WNTs to regulate the nuclear translocation of β -catenin and activation of its transcriptional role in stem cell gene expression via recruitment of the kynurenine aminotransferase 3 (KAT3) co-activators CREB-binding protein (CBP) or E1A-associated protein p300 (part a). Enzymatic interaction of the transmembrane E3 ligases RING finger protein 43 (RNF43) and zinc/RING finger protein 3 (ZNRF3) can — through a negative feedback loop — lead to abrogation of WNT signalling via endocytosis of the Frizzled–LRP complex (part b). However, when R-spondin is present (as in part a) and recruited to leucine-rich repeat-containing G protein-coupled receptor 4 (LGR4) and LGR5, RNF43 and ZNRF3 interact with R-spondin, resulting in membrane clearance of the complex and the potentiation of WNT signalling. WNT signalling is important for proliferation and maintenance of potency as well as the differentiation of stem cells.

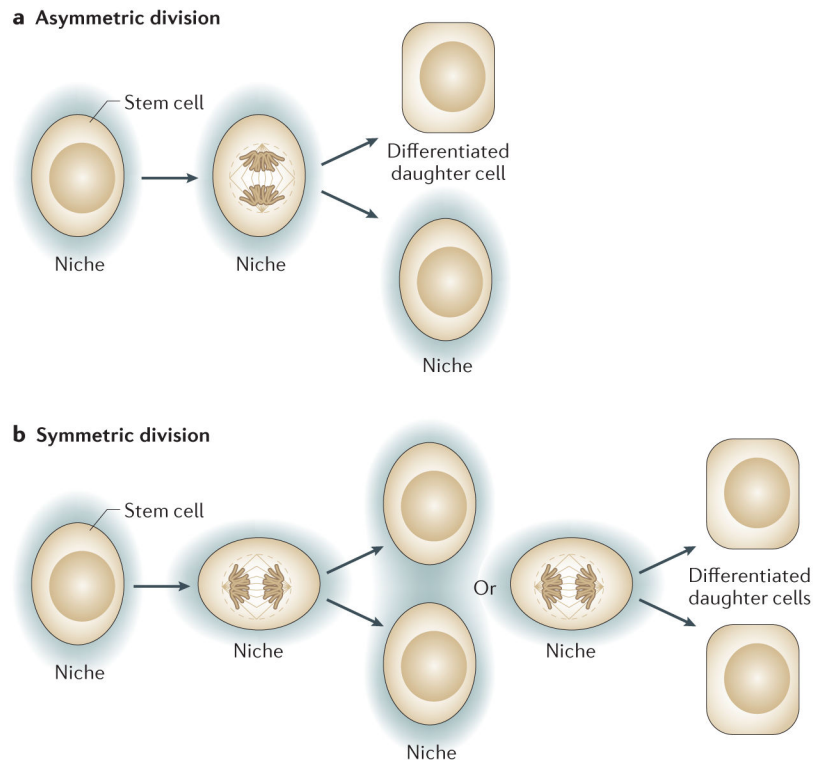
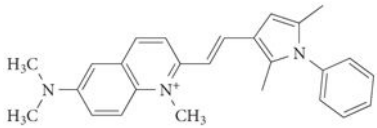
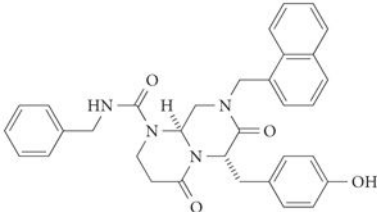
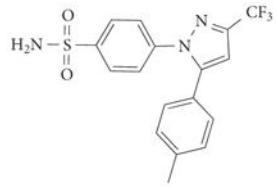
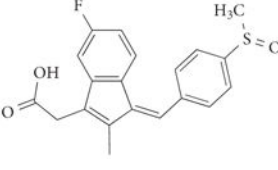
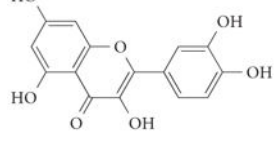
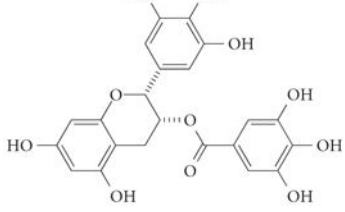


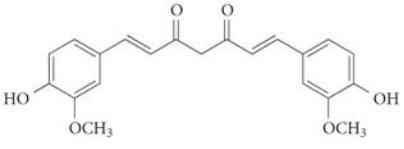
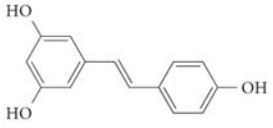
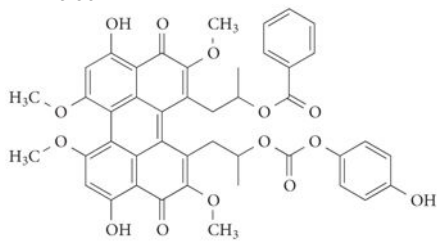
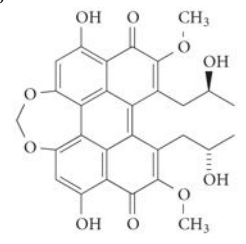
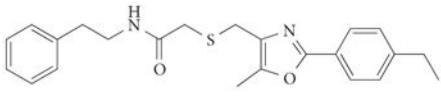
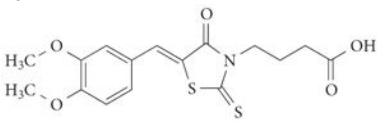
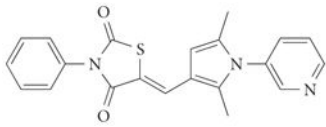
Figure 3. Modes of stem cell division

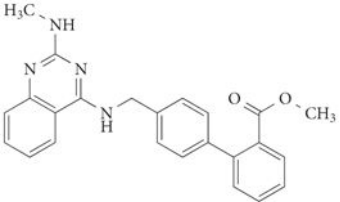
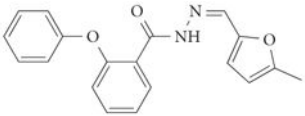
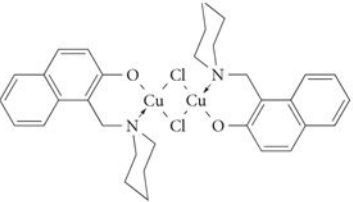
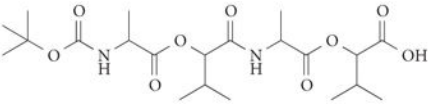
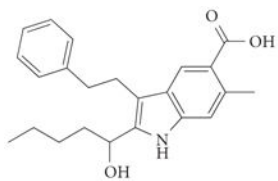
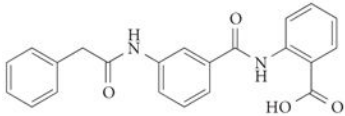
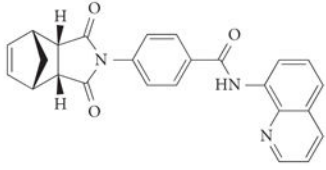
This figure depicts the modes of division — that is, asymmetric (part **a**) or symmetric (part **b**; either non-differentiative or differentiative) — in stem cells and illustrates how the orientation of the mitotic spindle in relation to the stem cell niche regulates the outcome of the division.

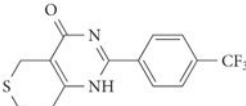
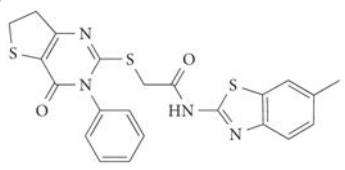
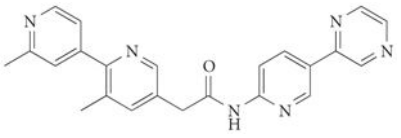
Table 1

Selected compounds that modulate the WNT pathway

Compound	Target	Development stage
Pyrvinium 	CK1 α	FDA-approved antihelminth
ICG-001 	CBP	Preclinical
Celecoxib 	COX2	FDA-approved (Pfizer)
Sulindac 	COX1, COX2	FDA-approved
Quercetin 	Antioxidant	-
EGCG 	Antioxidant	-

Compound	Target	Development stage
Curcumin 	Nonspecific antioxidant	-
Resveratrol 	Antioxidant, sirtuin activator	-
PKF115-584 	β -catenin-TCF	Preclinical
CGP049090 	β -catenin-TCF	Preclinical
iCRT3 	β -catenin-TCF	Preclinical
iCRT5 	β -catenin-TCF	Preclinical
iCRT14 	β -catenin-TCF	Preclinical

Compound	Target	Development stage
2,4-diamino-quinazoline series 	β -catenin–TCF; kinase inhibitor?	Preclinical
PNU-74654 	β -catenin–TCF	Preclinical
BC21 	β -catenin–TCF?	Preclinical
NSC 668036 	DVL	Preclinical
FJ9 	DVL	Preclinical
3289-8625 	DVL	Preclinical
IWR-1 	Tankyrase 1, tankyrase 2	Preclinical

Compound	Target	Development stage
XAV939 	Tankyrase 1, tankyrase 2	Preclinical
IWP2 	Porcupine	Preclinical
OMP-18R5 (mAb)	Frizzled	Phase I
LGK974 	Porcupine	Phase I
OMP-54F28 (FZD8–Fc fusion)	WNTs	Phase I
CWP232291 (structure not publicly available)	SAM68	Phase I
PRI-724 (structure not publicly available)	CBP	Phase I

CBP, CREB-binding protein; CK1 α , casein kinase 1 α ; COX, cyclooxygenase; DVL, Dishevelled; FDA, US Food and Drug Administration; FZD8, Frizzled 8; mAb, monoclonal antibody; SAM68, SRC-associated in mitosis 68 kDa protein; TCF, T cell factor.