TIMELINE

Wnt signalling and its impact on development and cancer

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Abstract | The Wnt signalling pathway is an ancient system that has been highly conserved during evolution. It has a crucial role in the embryonic development of all animal species, in the regeneration of tissues in adult organisms and in many other processes. Mutations or deregulated expression of components of the Wnt pathway can induce disease, most importantly cancer. The first gene to be identified that encodes a Wnt signalling component, *Int1* (integration 1), was molecularly characterized from mouse tumour cells 25 years ago. In parallel, the homologous gene *Wingless* in *Drosophila melanogaster*, which produces developmental defects in embryos, was characterized. Since then, further components of the Wnt pathway have been identified and their epistatic relationships have been defined. This article is a Timeline of crucial discoveries about the components and functions of this essential pathway.

We know today that Wnt and a handful of other signalling systems (Notch, Hedgehog, TGFβ (transforming growth factor-β)–BMP (bone morphogenetic protein) and receptor tyrosine kinases) are major molecular mechanisms that control embryonic development. These signalling systems operate beyond cell and tissue boundaries, but function as morphogens that are secreted from one cell or tissue type to activate surface receptors, signal transduction components and transcription factors in neighbouring cells or tissues, regulating processes such as cell proliferation, survival or differentiation. During development, the activity of such signalling systems is tightly regulated, whereas in cancer and other diseases they can escape this control. For example, a signalling component that functions transiently during development might become an oncogene when it undergoes a gain-of-function mutation. Alternatively, an inhibitor might suffer a loss-of-function mutation, lose its ability to regulate signalling and lose its functions as a tumour suppressor. Both types of change in Wnt signalling have been linked to cancer. Therefore, a great deal of effort is being invested worldwide in developing therapeutic agents that function by fine-tuning the Wnt pathway.

This article describes major milestones that have substantially contributed to our understanding of the Wnt signalling system. The history of Wnt research reads like a survey of disciplines and benchmarks in modern research, drawing together developmental genetics, cell biology, cancer research, biochemistry and immunology. In addition, Wnt research has covered the spectrum of model organisms, including worms, flies, frogs, mice and humans (Supplementary information S1 (table)), and therefore serves as an example of successful interdisciplinary research.

Early discoveries

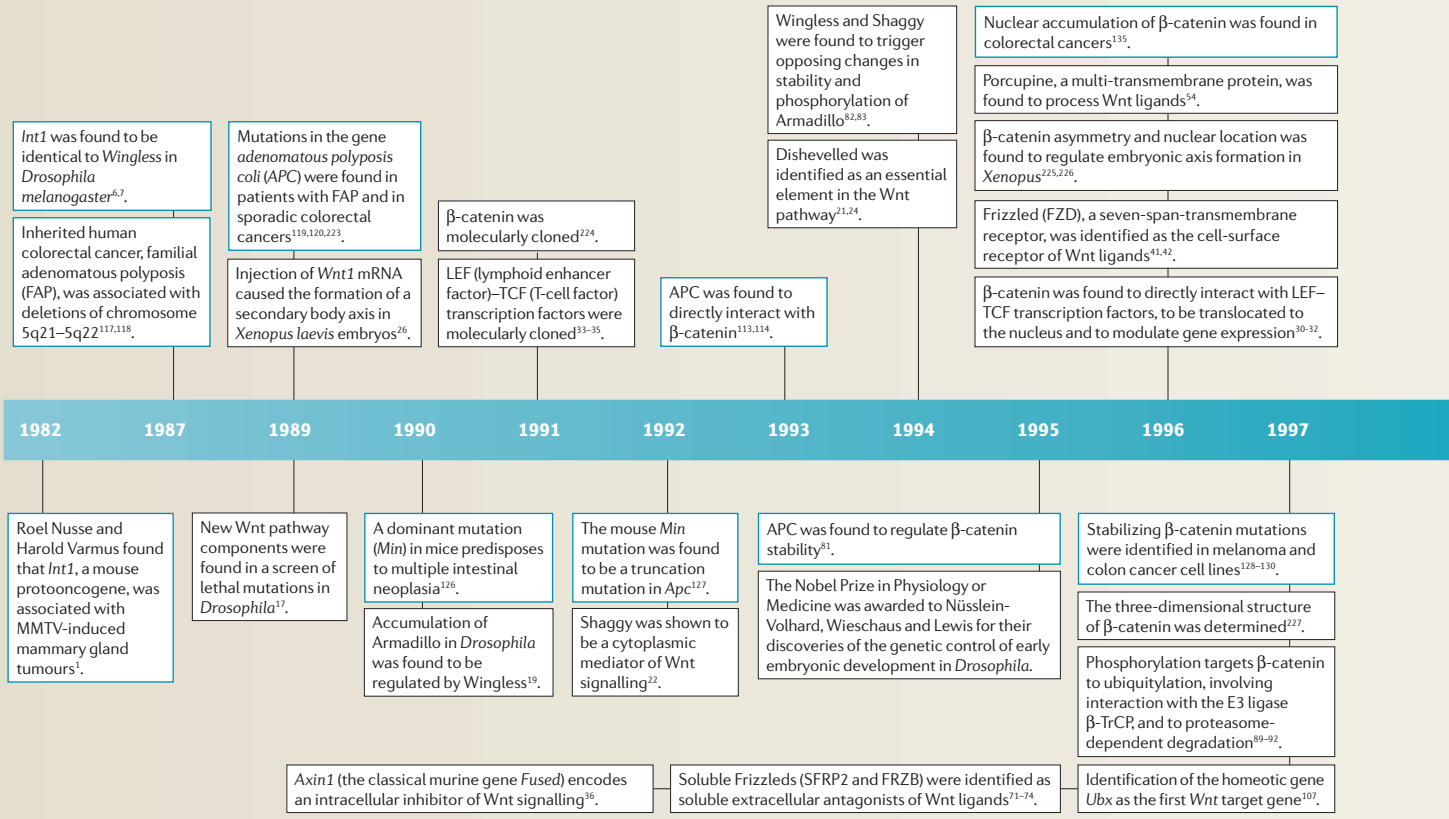
In 1982, Roel Nusse and Harold Varmus, then working at the University of California, San Francisco, USA, reported that a tumour virus (mouse mammary tumour virus, MMTV) induced mammary gland tumours in mice by activating the expression of a hitherto unknown gene that they named *Int1* (integration 1)[1] (TIMELINE). A spontaneous loss-of-function mutation in the mouse, *swaying*, that lacked the anterior cerebellum (and was first described in 1967 (REF. 2)) was shown to be a mutant allele of *Int1* (REFS 3,4). A *Drosophila melanogaster* mutant lacking wings, *Wingless* (*Wg*), was described in 1973 (REF. 5), and this fly gene turned out to be the homologue of mammalian *Int1* (REFS 6,7). The *Wg* mutation also caused segmentation defects in *Drosophila* embryos, and a number of segment polarity gene mutations in *Drosophila* had already been produced and extensively characterized by Nüsslein-Volhard and Wieschaus[8,9]. Subsequently, the developmental phenotypes were traced to mutations in components of the Wnt signalling system and the work on these *Drosophila* mutants was awarded the Nobel Prize in Physiology or Medicine in 1995 (TIMELINE). Today, the term Wnt is therefore an amalgam of *Wg* and *Int*[10].

There had been some earlier work on Wnt signalling, in 'precloning' times, when the underlying pathways and mechanisms had not been identified. In the 1930s, viral insertion was discovered to promote mammary tumours in laboratory mice (see REF. 11 for an example). Even earlier, in a famous experiment conducted in 1924, Mangold and Spemann grafted dorsal lips of the blastopore from developing newt embryos onto the opposing side of the embryo, inducing a second body axis — a twin-headed embryo[12]. The cause was the activity of the protein later termed Wnt in the transplanted tissue fragment[13]. This work was awarded the Nobel Prize in 1935. Moreover, in an experiment performed in 1902 by Morgan, the simple salt lithium chloride also induced double axes in frog embryos by activating the pathway later termed the Wnt signalling pathway[14,15].

The canonical Wnt signalling pathway

Following the discovery of *Int1*, for almost 10 years most successful research into the Wnt pathway was in the developmental field, before the link to human cancer was realized (see next section). Many of the genes in the Wnt pathway, which were first discovered to function transiently in development, turned out to act as oncogenes and tumour suppressors when deregulated in human cancer. Thus, several of the *Drosophila* mutants identified by Nüsslein-Volhard and Wieschaus's genetic screen in the late 1970s and early 1980s[8,9], and in other screens (for example, that of Perrimon and collaborators[16,17]), displayed defects in embryonic segmentation — that is, segment polarity defects — similar to *Wingless* mutants. These were caused by mutations in *armadillo* (β-catenin in vertebrates), *dishevelled* or *porcupine* genes, as shown by the groups of Wieschaus, Nusse, Perrimon and collaborators[8,9,17–22]. Whereas wild-type embryos contained segments with alternating rows of spikes and naked belts, segments in mutants contained only spikes. By contrast, mutations such as *zeste white 3* (also known as *shaggy*; encodes glycogen synthase kinase 3β) caused opposite phenotypes; that is, completely naked segments. Epistatic analysis of double mutants in the early 1990s demonstrated that these segment polarity genes function as components of a newly discovered signal transduction pathway, the canonical Wnt pathway[19–25] (TIMELINE).

Timeline | **Milestones in the 25 years since the discovery of the first Wnt gene**

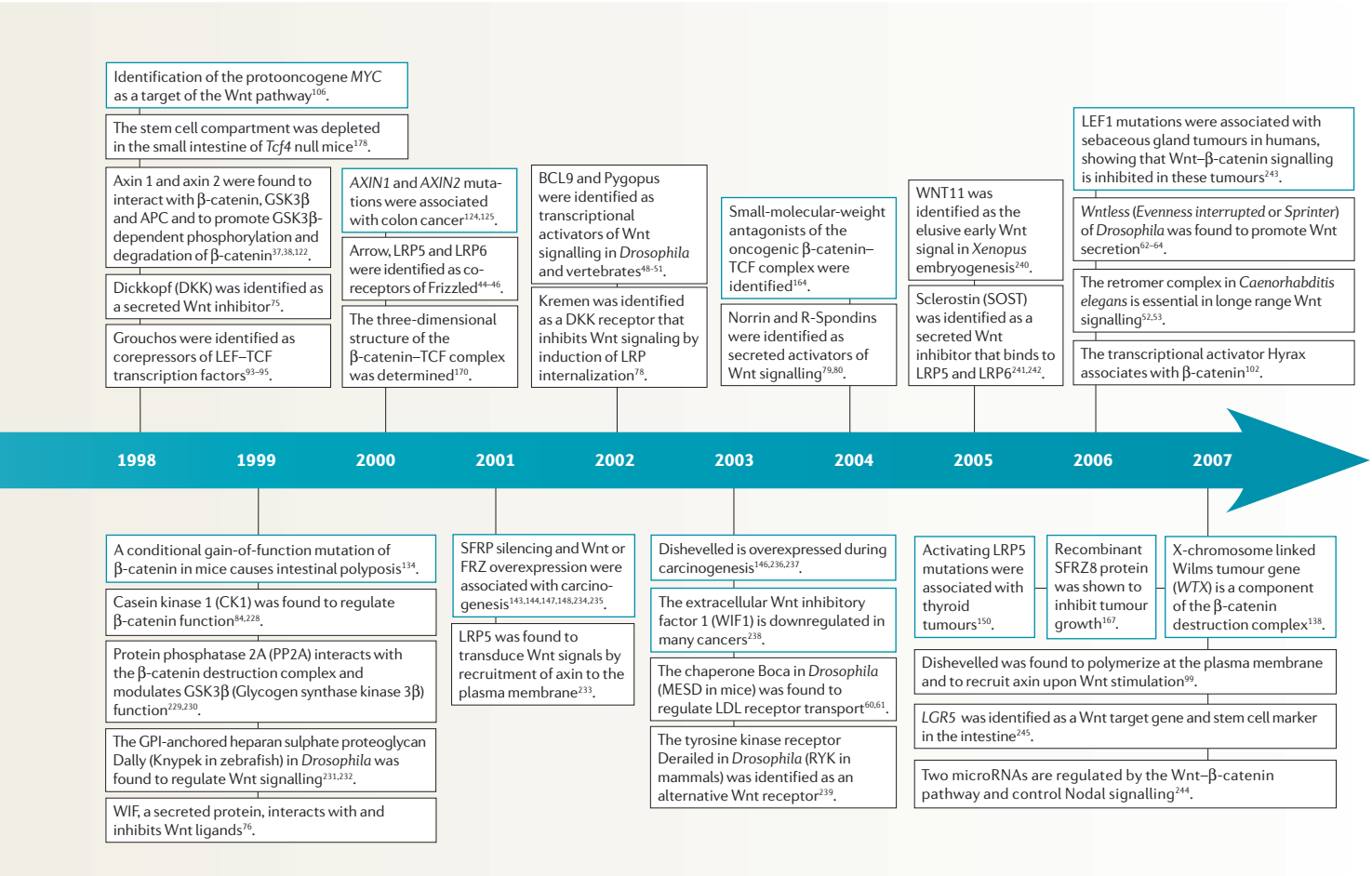


Blue boxes indicate discoveries of Wnt pathway components in cancer

Axis duplication in frog embryos (the experimental model system of *Xenopus laevis* is used) turned out to be an easy assay to define and characterize components of the Wnt pathway. Injection of vertebrate *WNT1* mRNA into early *Xenopus* embryos resulted in duplications of the body axis and twin-headed embryos, as shown by McMahon and Moon[26] (TIMELINE). Axis duplication was also induced by injections of mRNA for dishevelled, β-catenin, dominant-negative *gsk3*β or *lef1* (lymphoid enhancer factor 1)[27–30] (TIMELINE). In addition, biochemical analyses by us and others demonstrated direct interactions between these components, and in particular demonstrated that β-catenin — a molecule that is already known to interact with the cell-adhesion molecule E-cadherin — translocates to the nucleus where it binds the transcription factors LEF1 and TCF (T-cell factor), thereby converting LEF1 into a transcriptional activator[30–32]. This finding was crucial in understanding the mechanism by which cytoplasmic Wnt signals confer changes in gene expression in the nucleus. A further assay for determining Wnt–TCF transcriptional activity, the TOPflash reporter assay, was developed by Clevers and collaborators and is now used worldwide[32].

The history of TCF and LEF is interesting because the two factors had already been molecularly cloned by Grosschedl, Jones, Clevers and collaborators in 1991 before their connection to Wnt signalling was recognized. This again illustrates how different areas of research have merged[33–35]. By contrast, negative regulators of the Wnt pathway, obtained by injections of *axin1* and *axin2* mRNAs into *Xenopus* embryos, produced a complete loss of the body axis[36–38] (TIMELINE). Genetic analyses in mice confirmed that *Axin1* or β-catenin control axis formation[36,39]. We should also mention that the *Axin1* mutation analysed by Costanini and collaborators[36] was the classical mouse mutant *Fused*, described in 1949 (REF. 40), which was subsequently recognized as a part of the Wnt pathway. The identification and characterization of another negative regulator of the Wnt pathway, the tumour suppressor gene *Apc* (adenomatosis polyposis coli), is discussed in the next section. In summary, these findings demonstrated that the basic mechanism and the components of Wnt signalling are conserved between invertebrates and vertebrates (Supplementary information S1 (table)). The conservation of the members of the Wnt pathway and their similar interactions in flies, frogs and mice also suggested that this pathway is relevant to human development and may have a role in human disease.

Since the mid 1990s, many more components of the Wnt signalling pathway have been discovered, in particular the Wnt cell surface receptors Frizzled[41–43], LRP5 (LDL-receptor related protein 5) and LRP6 (known as Arrow in *Drosophila*)[44–46]. Once again, the combination of genetic analyses in *Drosophila* and biochemical work in *Xenopus* was the main contributor in identifying these two types of Wnt receptor (Supplementary information S1 (table)). The nuclear components Legless (known in vertebrates as B-cell lymphoma 9 (BCL9)) and Pygopus were identified by the laboratories of Basler, Bienz, Cadigan and others[47–51] (TIMELINE). Legless and Pygopus are co-activators of β-catenin–TCF signalling in *Drosophila*; their role in vertebrates is still incompletely understood.



Particular progress has also been made in the identification of components of the secretory branch of the Wnt pathway that function in or are secreted from neighbouring cells. These include the action of the retromer complex in Wnt secretion[52,53], Porcupine in Wnt processing[54], palmitoylation and glycosylation of Wnt ligands[54–56], lipoprotein particles[57,58] and chaperones[59–61], the transmembrane protein Wntless (also known as Evenness interrupted or Sprinter)[62–64] and the secreted heparan sulphate proteoglycans[65,66] (reviewed in REFS 67–69). Palmitoylation of Wnt ligands is essential for their graded action in development[58,70] (reviewed in REF. 68).

The known components of the Wnt signalling pathway can be assembled as follows (FIG. 1): secreted Wnt proteins (there are 19 Wnt genes in the human genome) bind to Frizzled receptors and LRP5–LRP6 co-receptors in the plasma membrane. Several inhibitors of this interaction were identified at the end of the 1990s, including secreted Frizzled-related proteins (SFRPs), Dickkopfs (DKKs) and Wnt inhibitory factor 1 (WIF1), by the laboratories of deRobertis, Niehrs and Nathans[71–76] (TIMELINE). For instance, DKK1 antagonizes Wnt signalling during head formation in mice[77]. An additional DKK-receptor, Kremen, was shown to inhibit the Wnt signalling pathway by internalization of LRP[78]. Moreover, secreted proteins such as Norrin and R-Spondin were shown to be activators of the canonical Wnt pathway owing to their interaction with Frizzled–LRP receptors[79,80]. In the mid 1990s, it was shown by the groups of Polakis, Nusse, Wieschaus and collaborators[81–83] that the control of β-catenin stability is crucial in Wnt signalling (TIMELINE). Therefore, in the absence of Wnt ligands (FIG. 1a), cytoplasmic β-catenin is recruited into a destruction complex, in which it interacts with APC and the axins, and is N-terminally phosphorylated by casein kinase 1α (CK1α) and GSK3β[84–88] (TIMELINE). Following phosphorylation, β-catenin is targeted for proteasome-dependent degradation involving interaction with β-TrCP (β-transducin repeat-containing protein), a component of the E3 ubiquitin ligase complex[89–92] (TIMELINE). Therefore, in the non-activated state, cytoplasmic β-catenin levels remain low and LEF and TCF in the nucleus interact with Grouchos to repress Wnt-specific target genes[93–95] (TIMELINE). Mutations in genes that control β-catenin stability, such as those that encode members of the destruction complex (APC or axins), or β-catenin itself, have been associated with cancer progression (see next section).

In the presence of canonical Wnt ligands (FIG. 1b), LRP5–LRP6 is phosphorylated by CK1γ and GSK3β[96,97] (and possibly other protein kinases yet to be identified), and Dishevelled is recruited to the plasma membrane, where it interacts with Frizzled receptors and polymerizes with other Dishevelled molecules[98,99]. Phosphorylation of LRP5 or LRP6 and the formation of the Dishevelled polymer, as well as internalization with caveolin[100], serve as mediators for the translocation of axin to the plasma membrane and inactivation of the destruction complex. The inactivation of the destruction complex allows the cytoplasmic stabilization and translocation of β-catenin to the nucleus. Many aspects of this current model for inactivation of the destruction

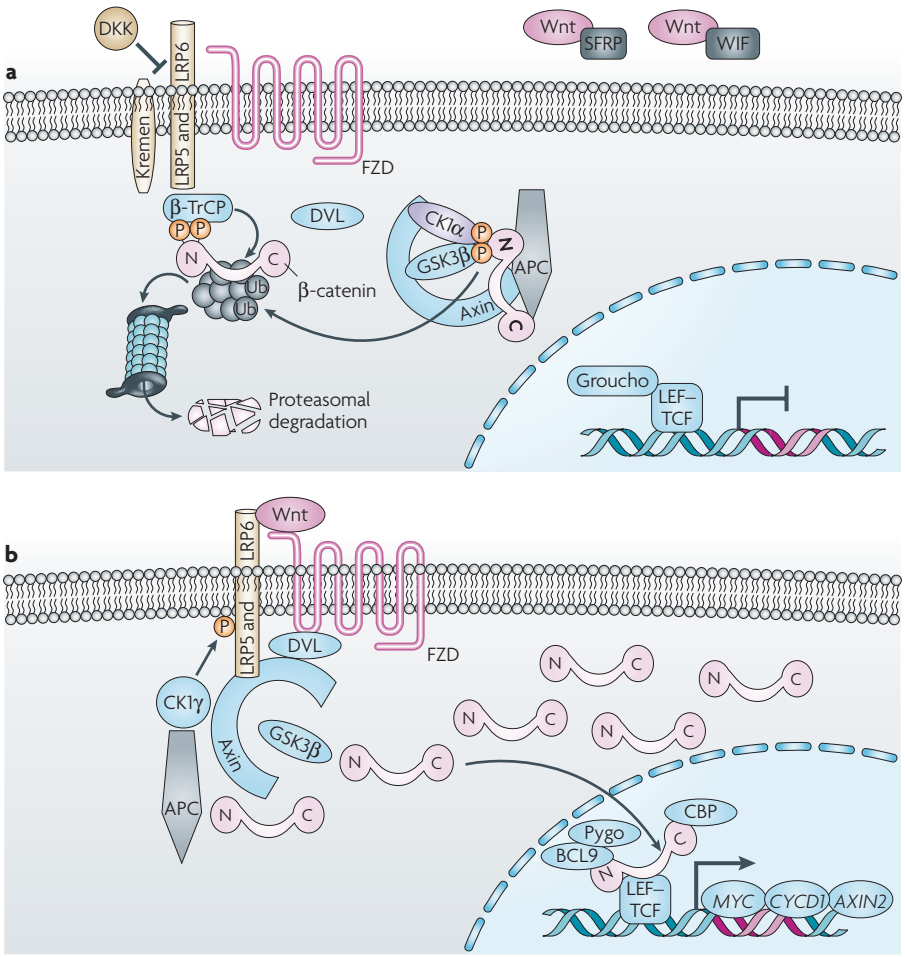


Figure 1 | **The canonical Wnt–β-catenin pathway. a** | In the absence of Wnt ligands, β-catenin is recruited into the destruction complex with APC (adenomatous polyposis coli) and the axins. Following N-terminal phosphorylation of β-catenin by the kinases CK1α (casein kinase 1α) and GSK3β (glycogen synthase kinase 3β), and subsequent ubiquitylation by β-TrCP (β-transducin repeat-containing protein, an E3 ubiquitin ligase), β-catenin is proteasomally degraded. Low cytoplasmic levels of β-catenin ensure transcriptional repression of Wnt target genes by recruitment of the corepressor Groucho to LEF (lymphoid enhancer factor)–TCF (T-cell factor) transcription factors. **b** | In the presence of Wnt ligands, LRP5 (LDL-related receptor protein 5) and LRP6 are phosphorylated by CK1γ and GSK3β, and Dishevelled (DVL) molecules are recruited to the plasma membrane to interact with Frizzled (FZD) receptors and other Dishevelled molecules. Interaction of axin with phosphorylated LRP5, LRP6 and the Dishevelled polymer leads to the inactivation of the destruction complex and subsequently to the stabilization of β-catenin and its translocation to the nucleus. In the nucleus, β-catenin forms a transcriptionally active complex with LEF and TCF transcription factors by displacing Grouchos and interacting with co-activators such as BCL9 (B-cell lymphoma 9), Pygopus (Pygo) and CBP (CREB binding protein). *CYCD1*, cyclin D1; DKK, Dickkopf; SFRP, secreted Frizzled-related protein; P, phosphorylation; Ub, ubiquitylation; WIF, Wnt inhibitory factor 1.

complex by the action of Wnt ligands need to be clarified further. In the nucleus, β-catenin forms a transcriptionally active complex with LEF and TCF transcription factors[30–32] by displacing Grouchos and interacting with other co-activators such as BCL9, Pygopus, CBP (CREB-binding protein) or Hyrax[47–51,101–105] (TIMELINE). CBP and Hyrax control gene expression through chromatin remodelling and by influencing RNA polymerase II.

The identification of the protooncogene *MYC* as a direct transcriptional target of Wnt–β-catenin signalling in 1998 shed light on the transforming activity of the Wnt pathway in cancer[106] (TIMELINE) (see next section). However, the first direct target gene of β-catenin–LEF — *Ultrabithorax* in *Drosophila* — was actually identified by Bienz[107] (TIMELINE). Further Wnt target genes have been discovered in the late 1990s and in the current decade. These include target genes that function in cell differentiation (siamois and brachyury), signalling (*VEGF* (vascular endothelial growth factor), *FGF4* (fibroblast growth factor 4) and *FGF18*), proliferation (cyclin D1 and *MYC*), adhesion (E-cadherin and *NRCAM* (neuronal cell-adhesion molecule)) and many further genes that are components of the Wnt pathway itself, demonstrating that Wnt signalling can autoregulate its activity in a positive and negative manner (for example, Frizzleds, DKKs, LRPs, Axin 2, β-TrCP and LEF–TCF). For a frequently updated overview on the Wnt pathway and its target genes see Roel Nusse's webpage.

Although the focus of this Timeline article is Wnt–β-catenin — that is, canonical Wnt signalling — it is important to note that some Wnt ligands and Frizzled receptors, and the Dishevelleds, are capable of activating a β-catenin-independent, non-canonical Wnt signalling cascade. The fact that Dishevelleds are involved in both canonical and non-canonical signalling was important for the realization that Frizzled relatives might be Wnt receptors[108]. Examples of non-canonical Wnt signalling are the planar cell polarity (PCP) pathway and the Ca^{2+}-dependent Wnt signalling pathway (for comprehensive reviews of non-canonical Wnt signalling see REFS 109,110). Many functions of non-canonical Wnt signalling have been described. For example, signalling by the PCP pathway in *Drosophila* and *Xenopus* embryos results in polarization of cells and directed cell motility, which is referred to as convergent extension movement. The non-canonical Wnt signalling pathway will not be discussed further here. Mutations in components of non-canonical Wnt signalling in human cancer, that is oncogenes or tumour suppressor genes, have not been described. However, it is interesting to note that the non-canonical *Wnt5a* has transforming capacity in cell culture[111,112].

Canonical Wnt signalling in cancer

Until the end of 1993 there was no overlap between research on Wnt signalling and human cancer. Then, Vogelstein, Kinzler and Polakis reported an important biochemical interaction between the tumour suppressor APC and the Wnt pathway component β-catenin[113,114] (FIG. 1; TIMELINE; Supplementary information S1 (table)). Two types of repeat in APC are essential for interaction with β-catenin — three 15-amino-acid and seven 20-amino-acid repeats (FIG. 2a) that compete with the cell-adhesion molecule E-cadherin for β-catenin binding[115].

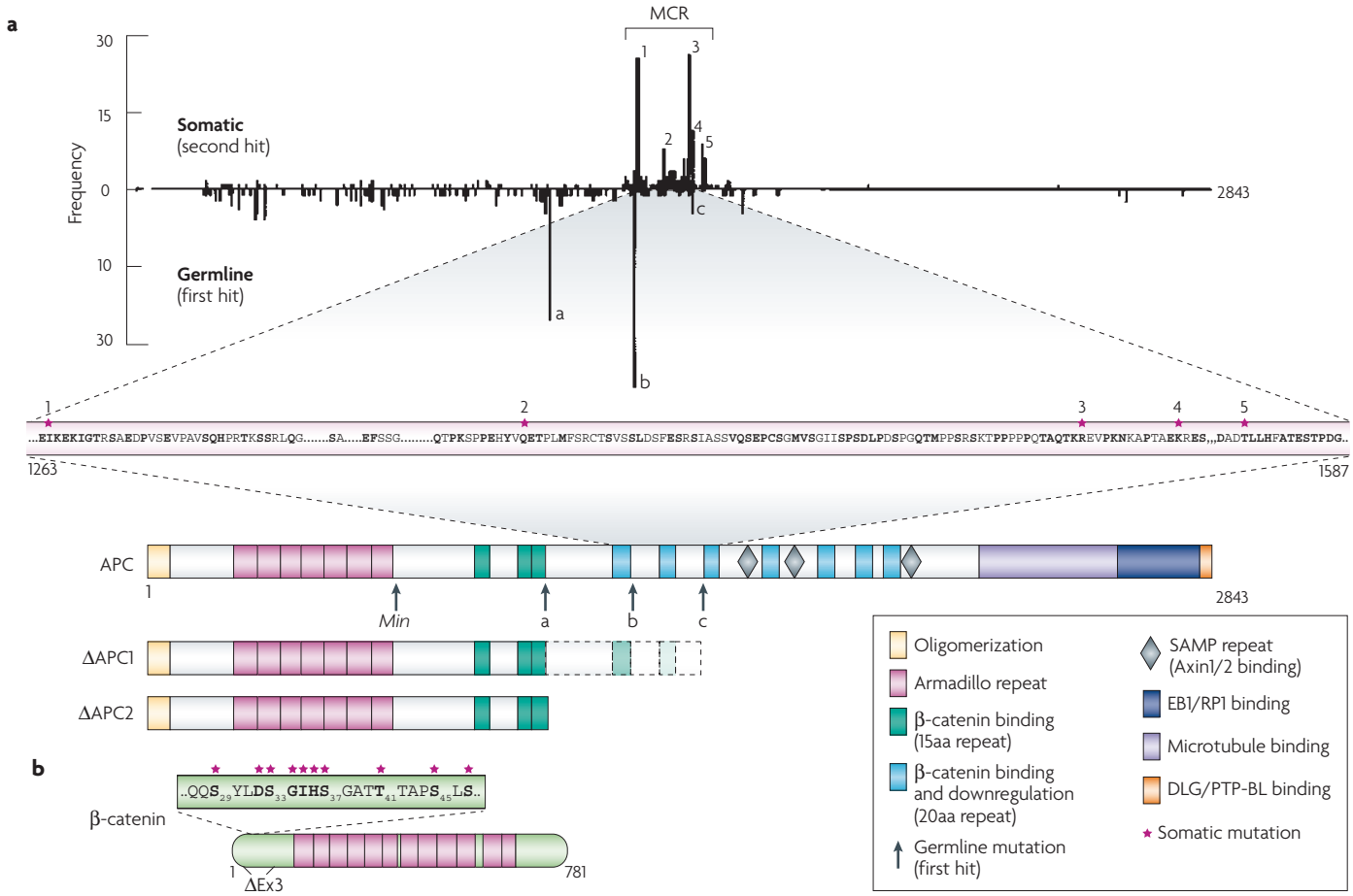


Figure 2 | **Human *APC* and *CTNNB1* mutations are associated with carcinogenesis. a** | Histogram indicating the frequency of somatic and germline mutations within human *APC* (adenomatous polyposis coli). Germline mutations are distributed all over the *APC* gene, with two preferential mutation sites at codon 1061 (a) and codon 1309 (b), which produce truncated APC proteins (ΔAPC1 and ΔAPC2) that have been associated with familial adenomatous polyposis (FAP). Somatic mutations in the mutation cluster region (MCR) produce truncated APC proteins that have been observed in sporadic colorectal cancers. People carrying one mutation in *APC* typically acquire a second mutation during adolescence and are predisposed to benign colorectal cancers. First-hit mutations at codon 1061 (a) accompanied by somatic mutations in the MCR (hot spots 1–5) produce truncated APC proteins (ΔAPC1) harbouring one or two 20-amino-acid repeats. Germline mutations at b or c that are followed by mutations in the MCR result in loss of the wild-type allele and produce a truncated APC protein (ΔAPC2).The *Min* mutation (nonsense mutation at codon 850) results in a stable truncated APC protein that predisposes to multiple intestinal neoplasia in mice. **b** | Somatic mutations and deletions (Δ) in the 5′ sequence (exon 3) of human *CTNNB1* (the gene that encodes β-catenin) that have been associated with human cancers. DLG, disc large; EB1/RP1, a family of microtubule-associated proteins; PTP-BL, protein tyrosine phosphatase BL. The data in the histogram in part **a** is adapted from REF. 121. The other data in part **a** is taken from REFS 155,219,220.

Adenomatous polyposis, a type of human colon cancer in which numerous polyps form in the epithelium of the large intestine, has been described since the mid eighteenth century, and its hereditary nature was recognized as early as 1900 (reviewed in REF. 116). The first clue to the molecular pathogenesis of colon cancer was the 1987 finding that the rare inherited disease, familial adenomatous polyposis (FAP), was associated with deletions of the specific chromosome region 5q21–22 (REFS 117,118) (TIMELINE). Patients with FAP develop hundreds to thousands of adenomatous polyps in the colon, and without surgical resection some of these polyps develop to malignant carcinomas. Only two years later, truncating mutations in *APC* were characterized in both patients with FAP and in frequent sporadic colorectal cancers; the latter represent approximately 85% of human colorectal cancers[119,120] (reviewed in REF. 116). A high frequency of *APC* mutations were frameshift, nonsense or splice-site mutations, which resulted in truncations of about 50% of the APC protein (reviewed in REF. 121) (FIG. 2a). However, single *APC* mutations are insufficient for the induction of adenomatous polyposis and a second mutation (or 'second hit') is always required: mutation of the second *APC* allele. Many *APC* mutations accumulate before the region that encodes the so-called SAMP repeats — regions that mediate the interaction of APC with scaffold proteins of the β-catenin destruction complex, Axin 1 and Axin 2 (REFS 37,122) (TIMELINE). In accordance with this, an *Apc* mouse mutant with a truncation mutation after the region that encodes the first SAMP repeat did not develop tumours[123]. *AXIN1* and *AXIN2* loss-of-function mutations have also been detected in rare cases of colorectal cancer[124,125]. A nonsense mutation of *Apc* was also produced in an ENU (the mutagen ethylnitrosourea)-treated mouse: the *Min* (Multiple intestinal neoplasia) mouse, which develops adenomatous polyposis[126,127] (TIMELINE). The *Min* mutant has since become an important animal model in cancer research.

Box 1 | **Wnt signalling in human disease**

Mutation and altered expression of components of the canonical Wnt pathway are linked to human diseases other than cancer (reviewed in REFS 141,218):

- Canonical Wnt signalling is involved in bone malformations. Osteoarthritis — a degenerative disorder of the joints — has been associated with augmented Wnt signalling: polymorphisms in *SFRP3* (secreted Frizzled-related protein 3), *LRP5* (LDL-receptor related protein 5) and *WISP1* (Wnt1-induced secreted protein 1), which is a direct Wnt target. Gain-of-function mutations in *LRP5* and loss of sclerostin, a secreted Wnt inhibitor, are known to affect the homeostatic balance of osteoblasts and osteoclasts after birth, which leads to high bone mass. Loss-of-function *LRP5* mutations lead to osteoporosis-pseudogliome syndrome (OPPG), which is associated with low bone mass.
- Loss-of-function mutations of *LRP5* are not only linked to low bone mass but also to eye defects, such as familial exudative vitreoretinopathy (FEVR). Many cases of FEVR are linked to loss-of-funtion mutations in the Wnt receptor FZ4 (Frizzled 4).
- Loss-of-function mutations in *WNT3* cause tetra-amelia, the loss of all four limbs.
- Acute renal failure and polycystic kidneys are associated with gain-of-function mutations in *WNT4* and aberrant expression of the target gene *PKD1* (polycystic kidney disease 1).
- Wnt–β-catenin signalling is involved in cardiogenesis and cardiovascular diseases such as cardiac hypertrophy (increased *FZ2*), as well as in neurodevelopment and neurodegenerative diseases such schizophrenia or Alzheimer disease (*WNT1* and *LRP6* are thought to be involved.

In a fraction of sporadic colorectal human cancers, gain-of-function mutations of *CTNNB1* (the β-catenin gene) have been discovered; their effects are to prevent phosphorylation, subsequent ubiquitylation, and proteasomal degradation of β-catenin. Initially, it was reported in a rather limited sampling that about 10% of sporadic colorectal cancers contain activating mutations in *CTNNB1* (REFS 128–130). More recent and far more extensive mutational surveys indicate that the frequency in sporadic colorectal cancers is actually closer to 1% (reviewed in REF. 131). Over 3,500 different human cancers were examined for the occurrence of *CTNNB1* mutations (colon cancer, melanoma, pilomatrixoma (hair tumours), hepatocellular carcinoma, medulloblastoma, hepatoblastoma, gastrointestinal tumours, Wilms tumours[132] and others), and in over 700 cases mutations were found that were predominantly centered in the N terminus of *CTNNB1* (REFS 121,133). The mutations in the N terminus either caused the deletion of an N-terminal fragment (encoded by exon 3), or altered the N-terminal phosphorylation sites Ser45, Thr41, Ser37, Ser33 or neighbouring residues[121] (FIG. 2b). If exon 3 of *Ctnnb1* is deleted by a conditional mutation in mice, adenomatous polyposis or other cancers develop[134]. Therefore, the tumour-causing mutations in *APC*, *AXIN1*, *AXIN2* and *CTNNB1* generally lead to inappropriate stabilization of β-catenin. Surplus β-catenin then translocates to the nucleus, interacts with the LEF and TCF transcription factors, and persistently transactivates genes associated with the regulation of cell proliferation, such as *MYC* and cyclin D1 (REFS 30,32,106,135–137). Loss-of-function mutations also occur in *WTX* (X-chromosome-linked Wilms tumour; also known as *FAM123B*), which encodes a recently discovered component of the β-catenin destruction complex[138,139]. Moreover, uncontrolled Wnt–β-catenin signalling that is associated with elevated β-catenin levels is also linked to aggressive fibromatosis and pulmonary fibrosis[140,141]. In recent years, other mechanisms of activation of the canonical Wnt pathway in tumours have been discovered; for example, silencing of the genes that encode the inhibitory Wnt ligands SFRPs and DKKs by hypermethylation, or by overexpression of Wnt proteins (WNT2B for example), Frizzleds (FZD10 for example) or Dishevelled[142–149] (TIMELINE). Recently, activating *LRP5* mutations were discovered in thyroid tumours[150] (TIMELINE). Inappropriate mutation or deregulated expression of various genes of the Wnt pathway are also the cause of many other diseases that affect the cardiovascular, nervous, bone, kidney and other systems (BOX 1).

It is now widely accepted that multiple mutations are necessary for the development of human malignancy. In colon cancer, *APC* mutations represent early events in tumour progression (that is, they represent the 'gatekeeper'), but other mutations that affect *KRAS*, *SMAD2*, *SMAD4* and *TP53* follow (see the Kinzler–Vogelstein model of the adenoma–carcinoma sequence in colorectal cancer, reviewed in REFS 116,151–155). KRAS functions as a component of receptor tyrosine kinases, and SMAD2 and SMAD4 are components of the TGFβ signalling pathway. In a recent mouse tumour model, the double mutation of *Apc* and *Smad4* leads to activation of the expression of the chemokine CCL9, which recruits immature myeloid cells from the tumour stroma and promotes tumour progression[156]. Mutations of components of the Hedgehog signalling pathway, such as loss of Indian hedgehog, also contribute to the progression of colorectal cancer[157]. It therefore seems that it is the inappropriate activation of developmental signalling pathways that are responsible for and promote tumour progression.

Wnt therapeutics

Basic researchers and pharmaceutical and biotechnology companies have been interested in developing Wnt pathway inhibitors since inappropriate activation of the Wnt pathway was first linked to human cancer in the late 1990s (FIG. 3). It was also realized that a number of existing drugs and recently developed derivatives of non-steroidal anti-inflammatory drugs (NSAIDs; for example, aspirin, indomethacin, sulindac, celecoxib, rofecoxib and others), or vitamins (such as vitamin A and D derivatives) seem to target the Wnt pathway, directly or indirectly, for example by inhibiting the Wnt target enzyme cyclooxygenase 2 (COX2) or activating E-cadherin[158–160] (reviewed in REFS 161,162). Some of the NSAIDs also seem to affect the level of β-catenin or its cellular distribution. These drugs were originally developed for the treatment of other diseases and are approved by the US Food and Drug Administration (FDA) and European Medicines Agency, as pain killers for example. NSAIDs can inhibit colorectal tumours in rats and have cancer-preventive properities in epidemiological studies. Celecoxib has been approved for the treatment of patients with FAP since 1999 (REF. 163). In 2004, a high-throughput screening method to search for low-molecular-weight antagonists that target the interaction between β-catenin and TCF4 was reported, and fungal derivatives were found that suppress the transcriptional activity of β-catenin[164] (TIMELINE). To our knowledge, these compounds have not yet entered clinical trials, and it is not clear whether their development has been carried further. The recent efforts of biotech companies and the pharmaceutical industry to develop effective inhibitors of the Wnt pathway for the treatment of patients with cancer and other diseases can be seen on their webpages, such as The Genetics Company, Nuvelo, Avalon and Curis.

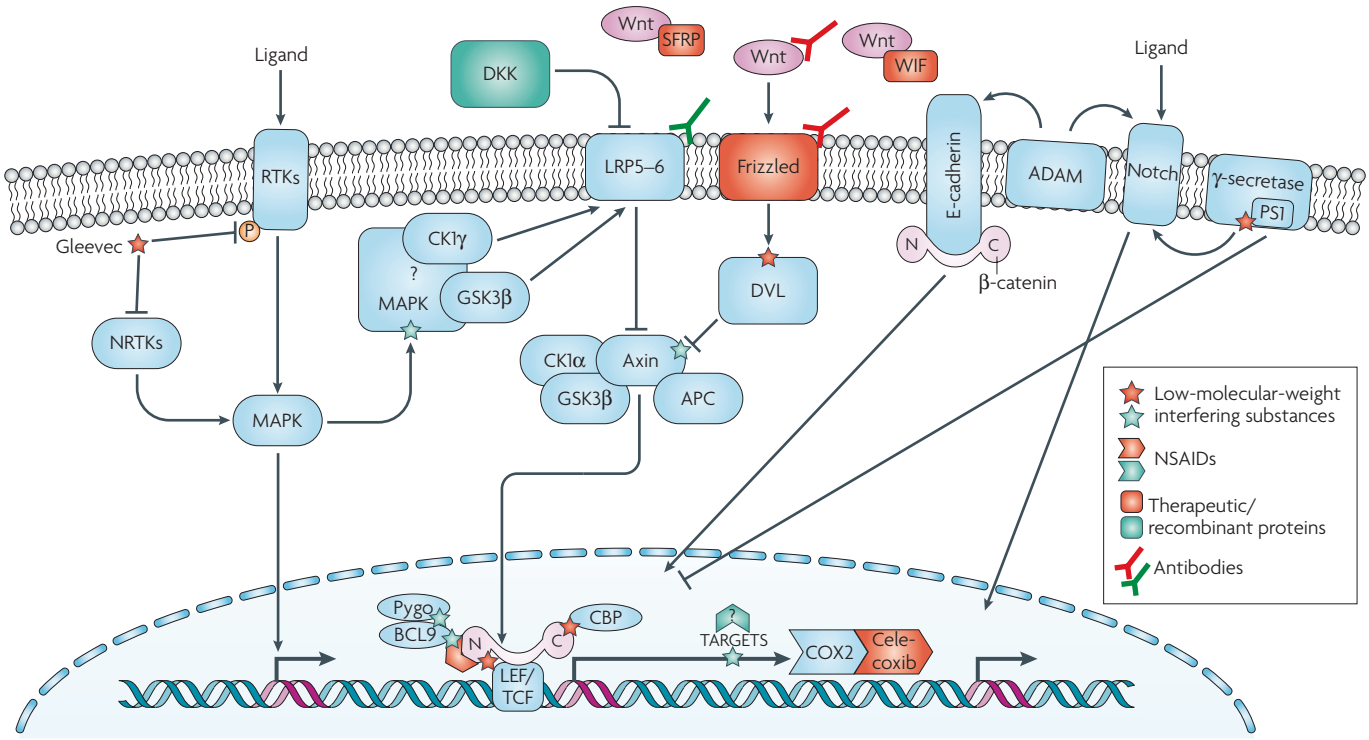


Figure 3 | **Cancer therapeutics that target components of the canonical Wnt pathway.** Schematic representation of canonical Wnt pathway components that are already used as targets (red) or are future targets (green) for cancer therapeutics in animals and humans. The non-steroidal anti-inflammatory drug (NSAID) celecoxib, which targets the Wnt target gene *COX2* (cyclooxygenase 2), is already approved for the treatment of patients with familial adenomatous polyposis (FAP)[163]. New NSAIDs, therapeutic proteins, antibodies and low-molecular-weight products that interfere with aberrant activity of the canonical Wnt pathway are currently being developed or might be future candidates. Transcriptional co-activators of the Wnt pathway might also be future candidates for Wnt therapeutics. Tissue-specific Wnt target genes might allow the development of drugs that target specific types of Wnt-induced tumour. Imatinib mesylate (Gleevec), a tyrosine kinase inhibitor and an approved therapeutic for chronic myeloid leukaemia (CML), shows promise for the treatment of Wnt-induced gastrointestinal tumours[216,221]. Interference with members of other signalling pathways, which directly or indirectly control β-catenin stability (such as γ-secretase or ADAM, a disintegrin and metalloproteinase[177,222]) might also provide therapeutics against disease. APC, adenomatous polyposis coli; BCL9, B-cell lymphoma 9; CBP, CREB-binding protein; CK1α, casein kinase 1α; DKK, Dickkopf; DVL, Dishevelled; GSK3β, glycogen synthase kinase 3β; LEF, lymphoid enhancer factor; LRP5, LDL-receptor related protein 5; MAPK, mitogen-activated protein kinase; NRTKs, non-receptor tyrosine kinases; SFRP, secreted Frizzled-related protein; TCF, T-cell factor; P, phosphorylation; PS1, presenilin 1; Pygo, Pygopus; RTKs, receptor tyrosine kinases; WIF, Wnt inhibitory factor 1.

Recently, antibody-based therapies have also been developed that target molecules of the Wnt pathway (such as Wnts or Frizzleds) that are overexpressed in disease[165,166]. Moreover, therapeutic proteins such as SFRPs, which function as inhibitors of the Wnt pathway, are presently being developed and tested in preclinical tumour models[167] (FIG. 3). TCF–β-catenin-restrictive oncolytic viruses are also under development[168,169] (reviewed in REF. 161).

X-ray structure analysis of many components of the Wnt pathway, such as β-catenin, axins, APC, TCFs, Dishevelled, BCL9 and their complexes should allow the future design and testing of low-molecular-weight substances that interfere with their activity, and many high-throughput screening programmes to discover such compounds are running or planned[164,170–175] (FIG. 3; Supplementary information S1 (table)). Low-molecular-weight compounds that target transcriptional co-activators of the Wnt pathway such as BCL9, CBP, CREB, BRG1, Pygopus, Hyrax and components of the Mediator complex are also under scrutiny for potential therapeutic applications[176,177] (reviewed in REFS 161,162). However, it could take a while until such novel low-molecular-weight inhibitors and therapeutic proteins are in the clinic.

Wnt signalling and stem cells

Recent findings have demonstrated that the Wnt signalling pathway has an important role in the specification and maintenance of precursor cell and stem cell lineages in various tissues and organs. In 1998, the Clevers laboratory reported the amazing finding that the mutation of the β-catenin interaction partner *Tcf4* in mice resulted in the complete absence of the stem cell compartment in the small intestine[178] (TIMELINE). Further work has shown that canonical Wnt signalling cooperates with BMP and Notch signalling in the intestinal stem cell niche to control stem cell self-renewal[179–181]. Since then, Wnt signalling has been shown to have an important role in the stem cell compartments of various other tissues. We have shown that in the skin, loss-of-function mutation of *Ctnnb1* prevents the generation of hair cell progenitors, but not epidermal progenitors in the stem cell niche of the follicular bulge[182]. This is in accordance with the finding that activating mutations of *Ctnnb1* in the skin lead to an expansion of hair precursor cells and are associated with the formation of 'hair tumours': pilomatrixomas and trichofolliculomas[183,184]. In addition, Wnt–β-catenin signalling has been proposed to increase haematopoietic stem cell renewal[58,185–187]. Here, Wnt signals also join forces with Notch signalling[188].

Wnt–β-catenin signalling also regulates precursor cell maintenance in the central and peripheral nervous system[189,190]. For

example, the balance of neuronal progenitor cell proliferation and patterning is disturbed in the dorsal spinal cord of loss- and gain-of-function *Ctnnb1* mutant mice. We have shown that Wnt–β-catenin signalling in the dorsal spinal cord is dependent on BMP and controls the action of the essential basic helix-loop-helix transcription factor OLIG3 in the domain 2 and 3 postmitotic neurons[190]. Recently, Wnt–β-catenin signalling has also been shown to control self-renewal and differentiation of *Islet1*-expressing precursor cells in neo- and postnatal hearts, which might be useful in the development of cell-based therapies for regenerative medicine[191,192] (reviewed in REF. 193). Taken together, these data demonstrate that the Wnt pathway controls specification and maintenance of particular progenitor and stem cell lineages in various tissues and organs during development and in the adult. Recent results show that the Wnt pathway is involved not only in development and disease but also in regeneration and ageing processes[194–199].

The recently identified cancer stem cells share many characteristics with normal stem cells. These are the capacity for self-renewal and differentiation into specific cell types, as well as their dependence on a particular environment, the (cancer) stem cell niche (reviewed in REF. 200). A crucial role of Wnt–β-catenin signalling in cancer stem cells of the mammary gland and epidermis has been identified. It was shown that the frequency of $CD29^+CD24^+$ stem cells in the mammary gland increases by a factor of 6.4 following activation of WNT1 (REF. 201). Malanchi *et al.* have identified a population of stem cells in early mouse epidermal tumours that are characterized by phenotypic and functional similarities to normal follicular bulge epidermal stem cells[202]. In normal mouse skin, $CD34^+$ follicular bulge stem cells account for approximately 1.8% of the keratinocytes. However, in cutaneous tumours derived by chemical (dimethylbenzanthracene (DMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)) carcinogenesis or by overexpression of mutant Ras (HRAS-R12T59), a 9-fold increase of this $CD34^+$ cell population was seen. The tumorogenic capacity of the $CD34^+$ cells was over 100-fold greater than that of the unsorted cells, and the tumours resembled the architecture of the parental tumours, including the maintenance of a small population of the $CD34^+$ cells. Remarkably, *Ctnnb1* deletion in DMBA–TPA or Ras-induced tumours by conditional mutagenesis resulted in a complete regression of the tumours[202]. These data demonstrate that Wnt–β-catenin signalling has an essential function in the maintenance of the mammary gland and skin cancer stem cells, and that the differentiation potential of β-catenin in cancer versus normal stem cells could be exploited for therapy.

It has also been shown that cytotoxic drugs or irradiation often kill tumour cells, whereas putative cancer stem cells are resistant (reviewed in REFS 203,204). It therefore needs to be determined whether the Wnt-inhibiting drugs that are presently being developed (see above) have a preference for targeting cancer stem cells in the different tumour types. It has also been shown that the Wnt–β-catenin pathway is activated further at late stages of tumour progression, for example in colon carcinoma formation and metastasis[155,205]. Therefore, Wnt-inhibitory drugs might also interfere with metastasis formation and maintenance.

The next 25 years

In the past 25 years, many components and target genes of the Wnt signalling pathway have been identified. Clearly the future will lead to the identification of new partners — we will gain a better understanding of how components of the Wnt pathway function, and how they cooperate with the many other proteins in cells[206–209]. We will also gain a better understanding of how the major signalling pathways interact during development and why mutations in their components lead to tumour progression and other diseases. A few specific unknown details that are likely to be resolved in the near future are, for example, the mechanism of action of *BCL9* and *PYGO* in vertebrate development and disease, the role of tyrosine phosphorylation of different Wnt components in these processes, the roles of activating serine/threonine kinases in the Wnt pathway and the actual mechanisms by which the Wnt pathway affects gene regulation and chromatin remodelling (reviewed in REF. 210).

Over 90% of colon cancers and a high percentage of other cancers (reviewed in REF. 211) originate from activating mutations in the Wnt pathway. Cell culture studies reveal that inhibition of the Wnt pathway (by WIF1 or β-catenin silencing, RNA interference targeting GSK3β, Pygopus 2 antisense oligonucleotides or Vitamin D3, for example) can normalize cancer cells — that is, inhibit proliferation and induce differentiation[159,212–214]. Therefore, it is likely that inhibiting the Wnt pathway could make a great contribution to cures for cancer or other diseases. There will soon be a broad spectrum of tools to do this: therapeutic proteins (such as SFRPs and DKKs), nucleic-acid-based substances (vaccines, interfering viruses and small interfering RNAs for example) and many low-molecular-weight interfering substances of Wnt pathway components. Effective therapies might necessitate interfering with protein–protein interactions at crucial steps of the pathway, which is still a great challenge for present-day drug development[215]. New protein kinases that activate the Wnt pathway have recently been discovered[96], and these hold great promise for the development of specific inhibitors of these enzymes. Last but not least, the combined use of inhibitors of different pathways will be examined, such as inhibitors of receptor tyrosine kinases and Notch, Hedgehog, or TGF-β inhibitors in combination with Wnt inhibitors. For example, tyrosine kinase inhibitors such as imatinib mesylate (Gleevec) and γ-secretase inhibitors perturb the Wnt signalling pathway (the Ras and the Notch pathways cooperate with the Wnt pathway in tumour formation)[216,217] (FIG. 3), and these will be developed further. It might also be possible to interfere at the target gene level.

Recent studies suggest that so-called cancer stem cells, which represent only a minor fraction of tumour cells, might be solely responsible for the generation and maintenance of tumours. These cancer stem cells seem to share many characteristics with normal stem cells, including the capacity for self-renewal and differentiation (see above). Perhaps these have a similar role in tumours. This emerging concept provides exciting possibilities for both understanding tumour progression and possible therapeutic interference. Future research will therefore demonstrate how important the Wnt–β-catenin signalling pathway is for the self-renewal of cancer stem cells.

The past two decades have seen a number of cases in which processes that regulate embryonic development have been implicated in disease. The ancient Wnt signalling pathway controls many of the processes crucial for the growth, differentiation, and regulation of animal cells, so it is little wonder that mutations in its components have been linked to the deregulation of those processes in disease. Learning to manipulate this pathway through new low-molecular-weight substances or recombinant or therapeutic molecules is of great promise for therapy in the future.

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1. Nusse, R. & Varmus, H. E. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. *Cell* **31**, 99–109 (1982).
2. Lane, P. W. Swaying. *Mouse News Lett.* **36**, 40 (1967).
3. Thomas, K. R. & Capecchi, M. R. Targeted disruption of the murine *int-1* proto-oncogene resulting in severe abnormalities in midbrain and cerebellar development. *Nature* **346**, 847–850 (1990).
4. Thomas, K. R., Musci, T. S., Neumann, P. E. & Capecchi, M. R. Swaying is a mutant allele of the proto-oncogene *Wnt-1*. *Cell* **67**, 969–976 (1991).
5. Sharma, R. P. Wingless, a new mutant in *D. melanogaster*. *Drosoph. Inf. Serv.* **50**, 134 (1973).
6. Rijsewijk, F. *et al.* The *Drosophila* homolog of the mouse mammary oncogene *int-1* is identical to the segment polarity gene wingless. *Cell* **50**, 649–657 (1987).
7. Cabrera, C. V., Alonso, M. C., Johnston, P., Phillips, R. G. & Lawrence, P. A. Phenocopies induced with antisense RNA identify the wingless gene. *Cell* **50**, 659–663 (1987).
8. Nüsslein-Volhard, C. & Wieschaus, E. Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795–801 (1980).
9. Nüsslein-Volhard, C., Wieschaus, E. & Kluding, H. Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. I. Zygotic loci on the second chromosome. *Rouxs Arch. Dev. Biol.* **193**, 267–282 (1984).
10. Nusse, R. *et al.* A new nomenclature for *int-1* and related genes: the *Wnt* gene family. *Cell* **64**, 231 (1991).
11. Bittner, J. J. Some possible effects of nursing on the mammary gland tumor incidence in mice. *Science* **84**, 162 (1936).
12. Spemann, H. & Mangold, H. Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren. *Wilhelm Roux Arch. Entw. Mech. Org.* **100**, 599–638 (1924) (in German).
13. Glinka, A., Delius, H., Blumenstock, C. & Niehrs, C. Combinatorial signalling by Xwnt-11 and Xnr3 in the organizer epithelium. *Mech. Dev.* **60**, 221–231 (1996).
14. Morgan, T. H. The relation between normal and abnormal development of the embryo of the frog, as determined by the effect of lithium chloride in solution. *Archiv fur Entwicklungsmechanik* **16**, 691–716 (1902).
15. Klein, P. S. & Melton, D. A. A molecular mechanism for the effect of lithium on development. *Proc. Natl Acad. Sci. USA* **93**, 8455–8459 (1996).
16. Perrimon, N. & Mahowald, A. P. Multiple functions of segment polarity genes in *Drosophila*. *Dev. Biol.* **119**, 587–600 (1987).
17. Perrimon, N., Engstrom, L. & Mahowald, A. P. Zygotic lethals with specific maternal effect phenotypes in *Drosophila melanogaster*. I. Loci on the X chromosome. *Genetics* **121**, 333–352 (1989).
18. Wieschaus, E. & Riggleman, R. Autonomous requirements for the segment polarity gene armadillo during *Drosophila* embryogenesis. *Cell* **49**, 177–184 (1987).
19. Riggleman, B., Schedl, P. & Wieschaus, E. Spatial expression of the *Drosophila* segment polarity gene armadillo is posttranscriptionally regulated by wingless. *Cell* **63**, 549–560 (1990).
20. Klingensmith, J., Nusse, R. & Perrimon, N. The *Drosophila* segment polarity gene dishevelled encodes a novel protein required for response to the wingless signal. *Genes Dev.* **8**, 118–130 (1994).
21. Noordermeer, J., Klingensmith, J., Perrimon, N. & Nusse, R. Dishevelled and armadillo act in the wingless signalling pathway in *Drosophila*. *Nature* **367**, 80–83 (1994).
22. Siegfried, E., Chou, T. B. & Perrimon, N. Wingless signaling acts through zeste-white 3, the *Drosophila* homolog of glycogen synthase kinase-3, to regulate engrailed and establish cell fate. *Cell* **71**, 1167–1179 (1992).
23. Peifer, M., Sweeton, D., Casey, M. & Wieschaus, E. Wingless signal and Zeste-white 3 kinase trigger opposing changes in the intracellular distribution of Armadillo. *Development* **120**, 369–380 (1994).
24. Siegfried, E., Wilder, E. L. & Perrimon, N. Components of wingless signalling in *Drosophila*. *Nature* **367**, 76–80 (1994).
25. Peifer, M., Pai, L. M. & Casey, M. Phosphorylation of the *Drosophila* adherens junction protein Armadillo: roles for wingless signal and zeste-white 3 kinase. *Dev. Biol.* **166**, 543–556 (1994).
26. McMahon, A. P. & Moon, R. T. Ectopic expression of the proto-oncogene *int-1* in *Xenopus* embryos leads to duplication of the embryonic axis. *Cell* **58**, 1075–1084 (1989).
27. Dominguez, I., Itoh, K. & Sokol, S. Y. Role of glycogen synthase kinase 3 β as a negative regulator of dorsoventral axis formation in *Xenopus* embryos. *Proc. Natl Acad. Sci. USA* **92**, 8498–8502 (1995).
28. Guger, K. A. & Gumbiner, B. M. β-Catenin has Wnt-like activity and mimics the Nieuwkoop signaling center in *Xenopus* dorsal–ventral patterning. *Dev. Biol.* **172**, 115–125 (1995).
29. He, X., Saint-Jeannet, J. P., Woodgett, J. R., Varmus, H. E. & Dawid, I. B. Glycogen synthase kinase-3 and dorsoventral patterning in *Xenopus* embryos. *Nature* **374**, 617–622 (1995).
30. Behrens, J. *et al.* Functional interaction of β-catenin with the transcription factor LEF-1. *Nature* **382**, 638–642 (1996).
31. Huber, O. *et al.* Nuclear localization of β-catenin by interaction with transcription factor LEF-1. *Mech. Dev.* **59**, 3–10 (1996).
32. Molenaar, M. *et al.* XTcf-3 transcription factor mediates β-catenin-induced axis formation in *Xenopus* embryos. *Cell* **86**, 391–399 (1996).
33. Travis, A., Amsterdam, A., Belanger, C. & Grosschedl, R. *LEF-1*, a gene encoding a lymphoid-specific protein with an HMG domain, regulates T-cell receptor α enhancer function [corrected]. *Genes Dev.* **5**, 880–894 (1991).
34. van de, W. M., Oosterwegel, M., Dooijes, D. & Clevers, H. Identification and cloning of TCF-1, a T lymphocyte-specific transcription factor containing a sequence-specific HMG box. *EMBO J.* **10**, 123–132 (1991).
35. Waterman, M. L., Fischer, W. H. & Jones, K. A. A thymus-specific member of the HMG protein family regulates the human T cell receptor C α enhancer. *Genes Dev.* **5**, 656–669 (1991).
36. Zeng, L. *et al.* The mouse Fused locus encodes Axin, an inhibitor of the Wnt signaling pathway that regulates embryonic axis formation. *Cell* **90**, 181–192 (1997).
37. Behrens, J. *et al.* Functional interaction of an axin homolog, conductin, with β-catenin, APC, and GSK3β. *Science* **280**, 596–599 (1998).
38. Itoh, K., Krupnik, V. E. & Sokol, S. Y. Axis determination in *Xenopus* involves biochemical interactions of axin, glycogen synthase kinase 3 and β-catenin. *Curr. Biol.* **8**, 591–594 (1998).
39. Huelsken, J. *et al.* Requirement for β-catenin in anterior-posterior axis formation in mice. *J. Cell Biol.* **148**, 567–578 (2000).
40. Gluecksohn-Schoenheimer, S. The effects of a lethal mutation responsible for duplications and twinning in mouse embryos. *J. Exp. Zool.* **110**, 47–76 (1949).
41. Bhanot, P. *et al.* A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. *Nature* **382**, 225–230 (1996).
42. Wang, Y. *et al.* A large family of putative transmembrane receptors homologous to the product of the *Drosophila* tissue polarity gene frizzled. *J. Biol. Chem.* **271**, 4468–4476 (1996).
43. He, X. *et al.* A member of the Frizzled protein family mediating axis induction by Wnt-5A. *Science* **275**, 1652–1654 (1997).
44. Pinson, K. I., Brennan, J., Monkley, S., Avery, B. J. & Skarnes, W. C. An LDL-receptor-related protein mediates Wnt signalling in mice. *Nature* **407**, 535–538 (2000).
45. Tamai, K. *et al.* LDL-receptor-related proteins in Wnt signal transduction. *Nature* **407**, 530–535 (2000).
46. Wehrli, M. *et al.* arrow encodes an LDL-receptor-related protein essential for Wingless signalling. *Nature* **407**, 527–530 (2000).
47. Belenkaya, T. Y. *et al.* pygopus Encodes a nuclear protein essential for wingless/Wnt signaling. *Development* **129**, 4089–4101 (2002).
48. Kramps, T. *et al.* Wnt/wingless signaling requires BCL9/legless-mediated recruitment of pygopus to the nuclear β-catenin-TCF complex. *Cell* **109**, 47–60 (2002).
49. Parker, D. S., Jemison, J. & Cadigan, K. M. Pygopus, a nuclear PHD-finger protein required for Wingless signaling in *Drosophila*. *Development* **129**, 2565–2576 (2002).
50. Thompson, B., Townsley, F., Rosin-Arbesfeld, R., Musisi, H. & Bienz, M. A new nuclear component of the Wnt signalling pathway. *Nature Cell Biol.* **4**, 367–373 (2002).
51. Brembeck, F. H. *et al.* Essential role of BCL9–2 in the switch between β-catenin's adhesive and transcriptional functions. *Genes Dev.* **18**, 2225–2230 (2004).
52. Coudreuse, D. Y., Roel, G., Betist, M. C., Destree, O. & Korswagen, H. C. Wnt gradient formation requires retromer function in Wnt-producing cells. *Science* **312**, 921–924 (2006).
53. Prasad, B. C. & Clark, S. G. Wnt signaling establishes anteroposterior neuronal polarity and requires retromer in *C. elegans*. *Development* **133**, 1757–1766 (2006).
54. Kadowaki, T., Wilder, E., Klingensmith, J., Zachary, K. & Perrimon, N. The segment polarity gene porcupine encodes a putative multitransmembrane protein involved in Wingless processing. *Genes Dev.* **10**, 3116–3128 (1996).
55. Haerry, T. E., Heslip, T. R., Marsh, J. L. & O'Connor, M. B. Defects in glucuronate biosynthesis disrupt Wingless signaling in *Drosophila*. *Development* **124**, 3055–3064 (1997).
56. Tanaka, K., Kitagawa, Y. & Kadowaki, T. *Drosophila* segment polarity gene product porcupine stimulates the posttranslational *N*-glycosylation of wingless in the endoplasmic reticulum. *J. Biol. Chem.* **277**, 12816–12823 (2002).
57. Reichsman, F., Smith, L. & Cumberledge, S. Glycosaminoglycans can modulate extracellular localization of the wingless protein and promote signal transduction. *J. Cell Biol.* **135**, 819–827 (1996).
58. Willert, K. *et al.* Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* **423**, 448–452 (2003).
59. Kitajewski, J., Mason, J. O. & Varmus, H. E. Interaction of Wnt-1 proteins with the binding protein BiP. *Mol. Cell. Biol.* **12**, 784–790 (1992).
60. Culi, J. & Mann, R. S. Boca, an endoplasmic reticulum protein required for wingless signaling and trafficking of LDL receptor family members in *Drosophila*. *Cell* **112**, 343–354 (2003).
61. Hsieh, J. C. *et al.* Mesd encodes an LRP5/6 chaperone essential for specification of mouse embryonic polarity. *Cell* **112**, 355–367 (2003).
62. Banziger, C. *et al.* Wntless, a conserved membrane protein dedicated to the secretion of Wnt proteins from signaling cells. *Cell* **125**, 509–522 (2006).
63. Bartscherer, K., Pelte, N., Ingelfinger, D. & Boutros, M. Secretion of Wnt ligands requires Evi, a conserved transmembrane protein. *Cell* **125**, 523–533 (2006).
64. Goodman, R. M. *et al.* Sprinter: a novel transmembrane protein required for Wg secretion and signaling. *Development* **133**, 4901–4911 (2006).
65. Binari, R. C. *et al.* Genetic evidence that heparin-like glycosaminoglycans are involved in wingless signaling. *Development* **124**, 2623–2632 (1997).
66. Hacker, U., Lin, X. & Perrimon, N. The *Drosophila* sugarless gene modulates Wingless signaling and encodes an enzyme involved in polysaccharide biosynthesis. *Development* **124**, 3565–3573 (1997).
67. Mikels, A. J. & Nusse, R. Wnts as ligands: processing, secretion and reception. *Oncogene* **25**, 7461–7468 (2006).
68. Hausmann, G., Banziger, C. & Basler, K. Helping Wingless take flight: how WNT proteins are secreted. *Nature Rev. Mol. Cell Biol.* **8**, 331–336 (2007).
69. Eaton, S. Retromer retrieves wntless. *Dev. Cell* **14**, 4–6 (2008).
70. Takada, R. *et al.* Monounsaturated fatty acid modification of Wnt protein: its role in Wnt secretion. *Dev. Cell* **11**, 791–801 (2006).
71. Finch, P. W. *et al.* Purification and molecular cloning of a secreted, Frizzled-related antagonist of Wnt action. *Proc. Natl Acad. Sci. USA* **94**, 6770–6775 (1997).
72. Leyns, L., Bouwmeester, T., Kim, S. H., Piccolo, S. & De Robertis, E. M. Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. *Cell* **88**, 747–756 (1997).
73. Rattner, A. *et al.* A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. *Proc. Natl Acad. Sci. USA* **94**, 2859–2863 (1997).
74. Wang, S., Krinks, M., Lin, K., Luyten, F. P. & Moos, M., Jr. Frzb, a secreted protein expressed in the Spemann

organizer, binds and inhibits Wnt-8. *Cell* **88**, 757–766 (1997).
75. Glinka, A. *et al.* Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* **391**, 357–362 (1998).
76. Hsieh, J. C. *et al.* A new secreted protein that binds to Wnt proteins and inhibits their activities. *Nature* **398**, 431–436 (1999).
77. Mukhopadhyay, M. *et al.* Dickkopf1 is required for embryonic head induction and limb morphogenesis in the mouse. *Dev. Cell* **1**, 423–434 (2001).
78. Mao, B. *et al.* Kremen proteins are Dickkopf receptors that regulate Wnt/β-catenin signalling. *Nature* **417**, 664–667 (2002).
79. Kazanskaya, O. *et al.* R-Spondin2 is a secreted activator of Wnt/β-catenin signaling and is required for *Xenopus* myogenesis. *Dev. Cell* **7**, 525–534 (2004).
80. Xu, Q. *et al.* Vascular development in the retina and inner ear: control by Norrin and Frizzled-4, a high-affinity ligand-receptor pair. *Cell* **116**, 883–895 (2004).
81. Munemitsu, S., Albert, I., Souza, B., Rubinfeld, B. & Polakis, P. Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. *Proc. Natl Acad. Sci. USA* **92**, 3046–3050 (1995).
82. Peifer, M., Sweeton, D., Casey, M. & Wieschaus, E. Wingless signal and Zeste-white 3 kinase trigger opposing changes in the intracellular distribution of Armadillo. *Development* **120**, 369–380 (1994).
83. van Leeuwen, F., Samos, C. H. & Nusse, R. Biological activity of soluble wingless protein in cultured *Drosophila* imaginal disc cells. *Nature* **368**, 342–344 (1994).
84. Peters, J. M., McKay, R. M., McKay, J. P. & Graff, J. M. Casein kinase I transduces Wnt signals. *Nature* **401**, 345–350 (1999).
85. Sakanaka, C., Leong, P., Xu, L., Harrison, S. D. & Williams, L. T. Casein kinase iε in the wnt pathway: regulation of β-catenin function. *Proc. Natl Acad. Sci. USA* **96**, 12548–12552 (1999).
86. Amit, S. *et al.* Axin-mediated CKI phosphorylation of β-catenin at Ser 45: a molecular switch for the Wnt pathway. *Genes Dev.* **16**, 1066–1076 (2002).
87. Liu, C. *et al.* Control of β-catenin phosphorylation/degradation by a dual-kinase mechanism. *Cell* **108**, 837–847 (2002).
88. Yanagawa, S. *et al.* Casein kinase I phosphorylates the Armadillo protein and induces its degradation in *Drosophila*. *EMBO J.* **21**, 1733–1742 (2002).
89. Aberle, H., Bauer, A., Stappert, J., Kispert, A. & Kemler, R. β-catenin is a target for the ubiquitin-proteasome pathway. *EMBO J.* **16**, 3797–3804 (1997).
90. Salomon, D. *et al.* Regulation of β-catenin levels and localization by overexpression of plakoglobin and inhibition of the ubiquitin-proteasome system. *J. Cell Biol.* **139**, 1325–1335 (1997).
91. Jiang, J. & Struhl, G. Regulation of the Hedgehog and Wingless signalling pathways by the F-box/WD40-repeat protein Slimb. *Nature* **391**, 493–496 (1998).
92. Marikawa, Y. & Elinson, R. P. β-TrCP is a negative regulator of Wnt/β-catenin signaling pathway and dorsal axis formation in *Xenopus* embryos. *Mech. Dev.* **77**, 75–80 (1998).
93. Cavallo, R. A. *et al. Drosophila* Tcf and Groucho interact to repress Wingless signalling activity. *Nature* **395**, 604–608 (1998).
94. Levanon, D. *et al.* Transcriptional repression by AML1 and LEF-1 is mediated by the TLE/Groucho corepressors. *Proc. Natl Acad. Sci. USA* **95**, 11590–11595 (1998).
95. Roose, J. *et al.* The *Xenopus* Wnt effector XTcf-3 interacts with Groucho-related transcriptional repressors. *Nature* **395**, 608–612 (1998).
96. Davidson, G. *et al.* Casein kinase 1 γ couples Wnt receptor activation to cytoplasmic signal transduction. *Nature* **438**, 867–872 (2005).
97. Zeng, X. *et al.* A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. *Nature* **438**, 873–877 (2005).
98. Bilic, J. *et al.* Wnt induces LRP6 signalosomes and promotes dishevelled-dependent LRP6 phosphorylation. *Science* **316**, 1619–1622 (2007).
99. Schwarz-Romond, T. *et al.* The DIX domain of Dishevelled confers Wnt signaling by dynamic polymerization. *Nature Struct. Mol. Biol.* **14**, 484–492 (2007).
100. Yamamoto, H., Komekado, H. & Kikuchi, A. Caveolin is necessary for Wnt-3a-dependent internalization of LRP6 and accumulation of β-catenin. *Dev. Cell* **11**, 213–223 (2006).
101. Hecht, A., Vleminckx, K., Stemmler, M. P., van Roy, F. & Kemler, R. The p300/CBP acetyltransferases function as transcriptional coactivators of β-catenin in vertebrates. *EMBO J.* **19**, 1839–1850 (2000).
102. Mosimann, C., Hausmann, G. & Basler, K. Parafibromin/Hyrax activates Wnt/Wg target gene transcription by direct association with β-catenin/Armadillo. *Cell* **125**, 327–341 (2006).
103. Wolf, D., Rodova, M., Miska, E. A., Calvet, J. P. & Kouzarides, T. Acetylation of β-catenin by CREB-binding protein (CBP). *J. Biol. Chem.* **277**, 25562–25567 (2002).
104. de la Roche, M. & Bienz, M. Wingless-independent association of Pygopus with dTCF target genes. *Curr. Biol.* **17**, 556–561 (2007).
105. Daniels, D. L. & Weis, W. I. β-catenin directly displaces Groucho/TLE repressors from Tcf/Lef in Wnt-mediated transcription activation. *Nature Struct. Mol. Biol.* **12**, 364–371 (2005).
106. He, T. C. *et al.* Identification of c-MYC as a target of the APC pathway. *Science* **281**, 1509–1512 (1998).
107. Riese, J. *et al.* LEF-1, a nuclear factor coordinating signaling inputs from wingless and decapentaplegic. *Cell* **88**, 777–787 (1997).
108. Krasnow, R. E., Wong, L. L. & Adler, P. N. Dishevelled is a component of the frizzled signaling pathway in *Drosophila*. *Development* **121**, 4095–4102 (1995).
109. Seifert, J. R. & Mlodzik, M. Frizzled/PCP signalling: a conserved mechanism regulating cell polarity and directed motility. *Nature Rev. Genet.* **8**, 126–138 (2007).
110. Veeman, M. T., Axelrod, J. D. & Moon, R. T. A second canon. Functions and mechanisms of β-catenin-independent Wnt signaling. *Dev. Cell* **5**, 367–377 (2003).
111. Weeraratna, A. T. *et al.* Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell* **1**, 279–288 (2002).
112. Ouko, L., Ziegler, T. R., Gu, L. H., Eisenberg, L. M. & Yang, V. W. Wnt11 signaling promotes proliferation, transformation, and migration of IEC6 intestinal epithelial cells. *J. Biol. Chem.* **279**, 26707–26715 (2004).
113. Rubinfeld, B. *et al.* Association of the APC gene product with β-catenin. *Science* **262**, 1731–1734 (1993).
114. Su, L. K., Vogelstein, B. & Kinzler, K. W. Association of the APC tumor suppressor protein with catenins. *Science* **262**, 1734–1737 (1993).
115. Hulsken, J., Birchmeier, W. & Behrens, J. E-cadherin and APC compete for the interaction with β-catenin and the cytoskeleton. *J. Cell Biol.* **127**, 2061–2069 (1994).
116. Kinzler, K. W. & Vogelstein, B. Lessons from hereditary colorectal cancer. *Cell* **87**, 159–170 (1996).
117. Bodmer, W. F. *et al.* Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* **328**, 614–616 (1987).
118. Leppert, M. *et al.* The gene for familial polyposis coli maps to the long arm of chromosome 5. *Science* **238**, 1411–1413 (1987).
119. Ashton-Rickardt, P. G. *et al.* High frequency of *APC* loss in sporadic colorectal carcinoma due to breaks clustered in 5q21–22. *Oncogene* **4**, 1169–1174 (1989).
120. Groden, J. *et al.* Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* **66**, 589–600 (1991).
121. Polakis, P. Wnt signaling and cancer. *Genes Dev.* **14**, 1837–1851 (2000).
122. Hart, M. J., de los, S. R., Albert, I. N., Rubinfeld, B. & Polakis, P. Downregulation of β-catenin by human Axin and its association with the APC tumor suppressor, β-catenin and GSK3 β. *Curr. Biol.* **8**, 573–581 (1998).
123. Smits, R. *et al.* Apc1638T: a mouse model delineating critical domains of the adenomatous polyposis coli protein involved in tumorigenesis and development. *Genes Dev.* **13**, 1309–1321 (1999).
124. Liu, W. *et al.* Mutations in AXIN2 cause colorectal cancer with defective mismatch repair by activating β-catenin/TCF signalling. *Nature Genet.* **26**, 146–147 (2000).
125. Satoh, S. *et al.* AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. *Nature Genet.* **24**, 245–250 (2000).
126. Moser, A. R., Pitot, H. C. & Dove, W. F. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science* **247**, 322–324 (1990).
127. Su, L. K. *et al.* Multiple intestinal neoplasia caused by a mutation in the murine homolog of the *APC* gene. *Science* **256**, 668–670 (1992).
128. Korinek, V. *et al.* Constitutive transcriptional activation by a β-catenin-Tcf complex in *APC*$^{-/-}$ colon carcinoma. *Science* **275**, 1784–1787 (1997).
129. Morin, P. J. *et al.* Activation of β-catenin–Tcf signaling in colon cancer by mutations in β-catenin or APC. *Science* **275**, 1787–1790 (1997).
130. Rubinfeld, B. *et al.* Stabilization of β-catenin by genetic defects in melanoma cell lines. *Science* **275**, 1790–1792 (1997).
131. Polakis, P. The many ways of Wnt in cancer. *Curr. Opin. Genet. Dev.* **17**, 45–51 (2007).
132. Koesters, R. *et al.* Mutational activation of the β-catenin proto-oncogene is a common event in the development of Wilms' tumors. *Cancer Res.* **59**, 3880–3882 (1999).
133. Schwarz-Romond, T. *Diversin, eine neue Komponente des Wnt-Signalweges*. Thesis, Free Univ. Berlin 1–89 (2002) (in German).
134. Harada, N. *et al.* Intestinal polyposis in mice with a dominant stable mutation of the β-catenin gene. *EMBO J.* **18**, 5931–5942 (1999).
135. Inomata, M., Ochiai, A., Akimoto, S., Kitano, S. & Hirohashi, S. Alteration of β-catenin expression in colonic epithelial cells of familial adenomatous polyposis patients. *Cancer Res.* **56**, 2213–2217 (1996).
136. Shtutman, M. *et al.* The cyclin D1 gene is a target of the β-catenin/LEF-1 pathway. *Proc. Natl Acad. Sci. USA* **96**, 5522–5527 (1999).
137. Tetsu, O. & McCormick, F. β-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* **398**, 422–426 (1999).
138. Major, M. B. *et al.* Wilms tumor suppressor WTX negatively regulates WNT/β-catenin signaling. *Science* **316**, 1043–1046 (2007).
139. Rivera, M. N. *et al.* An X chromosome gene, *WTX*, is commonly inactivated in Wilms tumor. *Science* **315**, 642–645 (2007).
140. Cheon, S. S. *et al.* β-Catenin stabilization dysregulates mesenchymal cell proliferation, motility, and invasiveness and causes aggressive fibromatosis and hyperplastic cutaneous wounds. *Proc. Natl Acad. Sci. USA* **99**, 6973–6978 (2002).
141. Moon, R. T., Kohn, A. D., De Ferrari, G. V. & Kaykas, A. WNT and β-catenin signalling: diseases and therapies. *Nature Rev. Genet.* **5**, 691–701 (2004).
142. Vider, B. Z. *et al.* Evidence for the involvement of the *Wnt 2* gene in human colorectal cancer. *Oncogene* **12**, 153–158 (1996).
143. Katoh, M., Kirikoshi, H., Terasaki, H. & Shiokawa, K. *WNT2B2* mRNA, up-regulated in primary gastric cancer, is a positive regulator of the WNT–β-catenin–TCF signaling pathway. *Biochem. Biophys. Res. Commun.* **289**, 1093–1098 (2001).
144. Kirikoshi, H., Sekihara, H. & Katoh, M. Expression of *WNT14* and *WNT14B* mRNAs in human cancer, up-regulation of WNT14 by IFNγ and up-regulation of WNT14B by β-estradiol. *Int. J. Oncol.* **19**, 1221–1225 (2001).
145. Terasaki, H., Saitoh, T., Shiokawa, K. & Katoh, M. Frizzled-10, up-regulated in primary colorectal cancer, is a positive regulator of the. *Int. J. Mol. Med.* **9**, 107–112 (2002).
146. 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.* **10**, 1219–1223 (2003).
147. Caldwell, G. M. *et al.* The Wnt antagonist sFRP1 in colorectal tumorigenesis. *Cancer Res.* **64**, 883–888 (2004).
148. Suzuki, H. *et al.* Epigenetic inactivation of SFRP genes allows constitutive WNT signaling in colorectal cancer. *Nature Genet.* **36**, 417–422 (2004).
149. Sato, H. *et al.* Frequent epigenetic inactivation of DICKKOPF family genes in human gastrointestinal tumors. *Carcinogenesis* **28**, 2459–2466 (2007).
150. Bjorklund, P., Akerstrom, G. & Westin, G. An LRP5 receptor with internal deletion in hyperparathyroid tumors with implications for deregulated WNT/β-catenin signaling. *PLoS. Med.* **4**, e328 (2007).
151. Bos, J. L. *et al.* Prevalence of ras gene mutations in human colorectal cancers. *Nature* **327**, 293–297 (1987).
152. Forrester, K., Almoguera, C., Han, K., Grizzle, W. E. & Perucho, M. Detection of high incidence of *K-ras* oncogenes during human colon tumorigenesis. *Nature* **327**, 298–303 (1987).

153. Baker, S. J. *et al.* Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science* **244**, 217–221 (1989).
154. Fearon, E. R. & Vogelstein, B. A genetic model for colorectal tumorigenesis. *Cell* **61**, 759–767 (1990).
155. Fodde, R., Smits, R. & Clevers, H. APC, signal transduction and genetic instability in colorectal cancer. *Nature Rev. Cancer* **1**, 55–67 (2001).
156. Kitamura, T. *et al.* SMAD4-deficient intestinal tumors recruit CCR1 + myeloid cells that promote invasion. *Nature Genet.* **39**, 467–475 (2007).
157. van den Brink, G. R. *et al.* Indian Hedgehog is an antagonist of Wnt signaling in colonic epithelial cell differentiation. *Nature Genet.* **36**, 277–282 (2004).
158. Reddy, B. S. *et al.* Chemoprevention of colon cancer by specific cyclooxygenase-2 inhibitor, celecoxib, administered during different stages of carcinogenesis. *Cancer Res.* **60**, 293–297 (2000).
159. Palmer, H. G. *et al.* Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of β-catenin signaling. *J. Cell Biol.* **154**, 369–387 (2001).
160. Boon, E. M. *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* **90**, 224–229 (2004).
161. Dihlmann, S. & von Knebel, D. M. Wnt/β-catenin-pathway as a molecular target for future anti-cancer therapeutics. *Int. J. Cancer* **113**, 515–524 (2005).
162. Barker, N. & Clevers, H. Mining the Wnt pathway for cancer therapeutics. *Nature Rev. Drug Discov.* **5**, 997–1014 (2006).
163. Smigel, K. Arthritis drug approved for polyp prevention blazes trail for other prevention trials. *J. Natl Cancer Inst.* **92**, 297–299 (2000).
164. Lepourcelet, M. *et al.* Small-molecule antagonists of the oncogenic Tcf/β-catenin protein complex. *Cancer Cell* **5**, 91–102 (2004).
165. You, L. *et al.* An anti-Wnt-2 monoclonal antibody induces apoptosis in malignant melanoma cells and inhibits tumor growth. *Cancer Res.* **64**, 5385–5389 (2004).
166. Nagayama, S. *et al.* Therapeutic potential of antibodies against FZD 10, a cell-surface protein, for synovial sarcomas. *Oncogene* **24**, 6201–6212 (2005).
167. DeAlmeida, V. I. *et al.* The soluble wnt receptor Frizzled8CRD-hFc inhibits the growth of teratocarcinomas *in vivo*. *Cancer Res.* **67**, 5371–5379 (2007).
168. Chen, R. H. & McCormick, F. Selective targeting to the hyperactive β-catenin/T-cell factor pathway in colon cancer cells. *Cancer Res.* **61**, 4445–4449 (2001).
169. Malerba, M., Daeffler, L., Rommelaere, J. & Iggo, R. D. Replicating parvoviruses that target colon cancer cells. *J. Virol.* **77**, 6683–6691 (2003).
170. Graham, T. A., Weaver, C., Mao, F., Kimelman, D. & Xu, W. Crystal structure of a β-catenin/Tcf complex. *Cell* **103**, 885–896 (2000).
171. Huber, A. H. & Weis, W. I. The structure of the β-catenin/E-cadherin complex and the molecular basis of diverse ligand recognition by β-catenin. *Cell* **105**, 391–402 (2001).
172. Xing, Y., Clements, W. K., Kimelman, D. & Xu, W. Crystal structure of a β-catenin/axin complex suggests a mechanism for the β-catenin destruction complex. *Genes Dev.* **17**, 2753–2764 (2003).
173. Liu, J. *et al.* A small-molecule agonist of the Wnt signaling pathway. *Angew. Chem. Int. Ed. Engl.* **44**, 1987–1990 (2005).
174. Shan, J., Shi, D. L., Wang, J. & Zheng, J. Identification of a specific inhibitor of the dishevelled PDZ domain. *Biochemistry* **44**, 15495–15503 (2005).
175. Sampietro, J. *et al.* Crystal structure of a β-catenin/BCL9/Tcf4 complex. *Mol. Cell* **24**, 293–300 (2006).
176. Stadeli, R. & Basler, K. Dissecting nuclear Wingless signalling: recruitment of the transcriptional co-activator Pygopus by a chain of adaptor proteins. *Mech. Dev.* **122**, 1171–1182 (2005).
177. Teo, J. L., 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* **102**, 12171–12176 (2005).
178. Korinek, V. *et al.* Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nature Genet.* **19**, 379–383 (1998).
179. Haramis, A. P. *et al. De novo* crypt formation and juvenile polyposis on BMP inhibition in mouse intestine. *Science* **303**, 1684–1686 (2004).
180. He, X. C. *et al.* BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt–β-catenin signaling. *Nature Genet.* **36**, 1117–1121 (2004).
181. van Es, J. H. *et al.* Notch/γ-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature* **435**, 959–963 (2005).
182. Huelsken, J., Vogel, R., Erdmann, B., Cotsarelis, G. & Birchmeier, W. β-Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. *Cell* **105**, 533–545 (2001).
183. Gat, U., DasGupta, R., Degenstein, L. & Fuchs, E. *De novo* hair follicle morphogenesis and hair tumors in mice expressing a truncated β-catenin in skin. *Cell* **95**, 605–614 (1998).
184. Chan, E. F., Gat, U., McNiff, J. M. & Fuchs, E. A common human skin tumour is caused by activating mutations in β-catenin. *Nature Genet.* **21**, 410–413 (1999).
185. Reya, T. *et al.* A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* **423**, 409–414 (2003).
186. Kirstetter, P., Anderson, K., Porse, B. T., Jacobsen, S. E. & Nerlov, C. Activation of the canonical Wnt pathway leads to loss of hematopoietic stem cell repopulation and multilineage differentiation block. *Nature Immunol.* **7**, 1048–1056 (2006).
187. Scheller, M. *et al.* Hematopoietic stem cell and multilineage defects generated by constitutive β-catenin activation. *Nature Immunol.* **7**, 1037–1047 (2006).
188. Duncan, A. W. *et al.* Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. *Nature Immunol.* **6**, 314–322 (2005).
189. Lee, H. Y. *et al.* Instructive role of Wnt/β-catenin in sensory fate specification in neural crest stem cells. *Science* **303**, 1020–1023 (2004).
190. Zechner, D. *et al.* Bmp and Wnt/β-catenin signals control expression of the transcription factor Olig3 and the specification of spinal cord neurons. *Dev. Biol.* **303**, 181–190 (2007).
191. Klaus, A., Saga, Y., Taketo, M. M., Tzahor, E. & Birchmeier, W. Distinct roles of Wnt/β-catenin and Bmp signaling during early cardiogenesis. *Proc. Natl Acad. Sci. USA* **104**, 18531–18536 (2007).
192. Qyang, Y. *et al.* The Renewal and differentiation of Isl1 + cardiovascular progenitors are controlled by a Wnt/β-catenin pathway. *Cell Stem Cell* **1**, 1–15 (2007).
193. Tzahor, E. Wnt/β-catenin signaling and cardiogenesis: timing does matter. *Dev. Cell* **13**, 10–13 (2007).
194. Kawakami, Y. *et al.* Wnt/β-catenin signaling regulates vertebrate limb regeneration. *Genes Dev.* **20**, 3232–3237 (2006).
195. Stoick-Cooper, C. L. *et al.* Distinct Wnt signaling pathways have opposing roles in appendage regeneration. *Development* **134**, 479–489 (2007).
196. Brack, A. S. *et al.* Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science* **317**, 807–810 (2007).
197. Liu, H. *et al.* Augmented Wnt signaling in a mammalian model of accelerated aging. *Science* **317**, 803–806 (2007).
198. Gurley, K. A., Rink, J. C. & Sanchez, A. A. β-catenin defines head versus tail identity during planarian regeneration and homeostasis. *Science* **319**, 323–327 (2008).
199. Petersen, C. P. & Reddien, P. W. Smed-βcatenin-1 is required for anteroposterior blastema polarity in planarian regeneration. *Science* **319**, 327–330 (2008).
200. Sneddon, J. B. & Werb, Z. Location, location, location: the cancer stem cell niche. *Cell Stem Cell* **1**, 607–611 (2007).
201. Shackleton, M. *et al.* Generation of a functional mammary gland from a single stem cell. *Nature* **439**, 84–88 (2006).
202. Malanchi, I. *et al.* Cutaneous cancer stem cell maintenance is dependent on β-catenin signaling. *Nature* **452**, 650–653 (2008).
203. Lobo, N. A., Shimono, Y., Qian, D. & Clarke, M. F. The biology of cancer stem cells. *Annu. Rev. Cell Dev. Biol.* **23**, 675–699 (2007).
204. Wang, J. C. & Dick, J. E. Cancer stem cells: lessons from leukemia. *Trends Cell Biol.* **15**, 494–501 (2005).
205. Stein, U. *et al.* The metastasis-associated gene *S100A4* is a novel target of β-catenin/T-cell factor signaling in colon cancer. *Gastroenterology* **131**, 1486–1500 (2006).
206. Biemar, F. *et al.* Comprehensive identification of *Drosophila* dorsal–ventral patterning genes using a whole-genome tiling array. *Proc. Natl Acad. Sci. USA* **103**, 12763–12768 (2006).
207. DasGupta, R., Kaykas, A., Moon, R. T. & Perrimon, N. Functional genomic analysis of the Wnt–wingless signaling pathway. *Science* **308**, 826–833 (2005).
208. Srahna, M. *et al.* A signaling network for patterning of neuronal connectivity in the *Drosophila* brain. *PLoS Biol.* **4**, e348 (2006).
209. Stelzl, U. *et al.* A human protein–protein interaction network: a resource for annotating the proteome. *Cell* **122**, 957–968 (2005).
210. Willert, K. & Jones, K. A. Wnt signaling: is the party in the nucleus? *Genes Dev.* **20**, 1394–1404 (2006).
211. Giles, R. H., van Es, J. H. & Clevers, H. Caught up in a Wnt storm: Wnt signaling in cancer. *Biochim. Biophys. Acta* **1653**, 1–24 (2003).
212. Shakoori, A. *et al.* Deregulated GSK3β activity in colorectal cancer: its association with tumor cell survival and proliferation. *Biochem. Biophys. Res. Commun.* **334**, 1365–1373 (2005).
213. Popadiuk, C. M. *et al.* Antisense suppression of pygopus2 results in growth arrest of epithelial ovarian cancer. *Clin. Cancer Res.* **12**, 2216–2223 (2006).
214. Lin, Y. C. *et al.* Wnt inhibitory factor-1 gene transfer inhibits melanoma cell growth. *Hum. Gene Ther.* **18**, 379–386 (2007).
215. Trosset, J. Y. *et al.* Inhibition of protein–protein interactions: the discovery of druglike β-catenin inhibitors by combining virtual and biophysical screening. *Proteins* **64**, 60–67 (2006).
216. Zhou, L. *et al.* Tyrosine kinase inhibitor STI-571/Gleevec down-regulates the β-catenin signaling activity. *Cancer Lett.* **193**, 161–170 (2003).
217. Takahashi-Yanaga, F. & Sasaguri, T. The Wnt/β-catenin signaling pathway as a target in drug discovery. *J. Pharmacol. Sci.* **104**, 293–302 (2007).
218. Schedl, A. Renal abnormalities and their developmental origin. *Nature Rev. Genet.* **8**, 791–802 (2007).
219. Gaspar, C. & Fodde, R. APC dosage effects in tumorigenesis and stem cell differentiation. *Int. J. Dev. Biol.* **48**, 377–386 (2004).
220. Bienz, M. The subcellular destinations of APC proteins. *Nature Rev. Mol. Cell Biol.* **3**, 328–338 (2002).
221. Gleevec approved for first-line treatment of CML. *FDA Consum.* **37**, 5 (2003).
222. Maretzky, T. *et al.* ADAM10 mediates E-cadherin shedding and regulates epithelial cell–cell adhesion, migration, and β-catenin translocation. *Proc. Natl Acad. Sci. USA* **102**, 9182–9187 (2005).
223. Nishisho, I. *et al.* Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* **253**, 665–669 (1991).
224. McCrea, P. D., Turck, C. W. & Gumbiner, B. A homolog of the armadillo protein in *Drosophila* (plakoglobin) associated with E-cadherin. *Science* **254**, 1359–1361 (1991).
225. Schneider, S., Steinbeisser, H., Warga, R. M. & Hausen, P. β-catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. *Mech. Dev.* **57**, 191–198 (1996).
226. Yost, C. *et al.* The axis-inducing activity, stability, and subcellular distribution of β-catenin is regulated in *Xenopus* embryos by glycogen synthase kinase 3. *Genes Dev.* **10**, 1443–1454 (1996).
227. Huber, A. H., Nelson, W. J. & Weis, W. I. Three-dimensional structure of the armadillo repeat region of β-catenin. *Cell* **90**, 871–882 (1997).
228. Sakanaka, C., Leong, P., Xu, L., Harrison, S. D. & Williams, L. T. Casein kinase iepsilon in the wnt pathway: regulation of β-catenin function. *Proc. Natl Acad. Sci. USA* **96**, 12548–12552 (1999).
229. Hsu, W., Zeng, L. & Costantini, F. Identification of a domain of Axin that binds to the serine/threonine protein phosphatase 2A and a self-binding domain. *J. Biol. Chem.* **274**, 3439–3445 (1999).
230. Seeling, J. M. *et al.* Regulation of β-catenin signaling by the B56 subunit of protein phosphatase 2A. *Science* **283**, 2089–2091 (1999).
231. Lin, X. & Perrimon, N. Dally cooperates with *Drosophila* Frizzled 2 to transduce Wingless signalling. *Nature* **400**, 281–284 (1999).
232. Tsuda, M. *et al.* The cell-surface proteoglycan Dally regulates Wingless signalling in *Drosophila*. *Nature* **400**, 276–280 (1999).
233. Mao, J. *et al.* Low-density lipoprotein receptor-related protein-5 binds to Axin and regulates the canonical Wnt signaling pathway. *Mol. Cell* **7**, 801–809 (2001).
234. To, K. F. *et al.* Alterations of frizzled (FzE3) and secreted frizzled related protein (hsFRP) expression in gastric cancer. *Life Sci.* **70**, 483–489 (2001).

235. Ugolini, F. *et al.* WNT pathway and mammary carcinogenesis: loss of expression of candidate tumor suppressor gene *SFRP1* in most invasive carcinomas except of the medullary type. *Oncogene* **20**, 5810–5817 (2001).
236. Uematsu, K. *et al.* Activation of the Wnt pathway in non small cell lung cancer: evidence of dishevelled overexpression. *Oncogene* **22**, 7218–7221 (2003).
237. Uematsu, K. *et al.* Wnt pathway activation in mesothelioma: evidence of Dishevelled overexpression and transcriptional activity of β-catenin. *Cancer Res.* **63**, 4547–4551 (2003).
238. Wissmann, C. *et al.* WIF1, a component of the Wnt pathway, is down-regulated in prostate, breast, lung, and bladder cancer. *J. Pathol.* **201**, 204–212 (2003).
239. Yoshikawa, S., McKinnon, R. D., Kokel, M. & Thomas, J. B. Wnt-mediated axon guidance via the *Drosophila* Derailed receptor. *Nature* **422**, 583–588 (2003).
240. Tao, Q. *et al.* Maternal wnt11 activates the canonical wnt signaling pathway required for axis formation in *Xenopus* embryos. *Cell* **120**, 857–871 (2005).
241. Li, X. *et al.* Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. *J. Biol. Chem.* **280**, 19883–19887 (2005).
242. Semenov, M., Tamai, K. & He, X. SOST is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor. *J. Biol. Chem.* **280**, 26770–26775 (2005).
243. Takeda, H. *et al.* Human sebaceous tumors harbor inactivating mutations in LEF1. *Nature Med.* **12**, 395–397 (2006).
244. Martello, G. *et al.* MicroRNA control of Nodal signalling. *Nature* **449**, 183–188 (2007).
245. Barker, N. *et al.* Identification of stem cells in small intestine and colon by marker gene *Lgr5*. *Nature* **449**, 1003–1007 (2007).

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DATABASES
Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Apc | *armadillo* | β-TrCP | caveolin | CBP | CCL9 | CK1α | *CTNNB1* | *dishevelled* | DKK1 | *FAM123B* | *FGF4* | *FGF18* | Frizzled | FZD10 | Hyrax | Indian hedgehog | *Int1* | *Islet1* | *KRAS* | Kremen | *lef1* | Legless | LRP5 | LRP6 | *MYC* | Norrin | *NRCAM* | OLIG3 | *porcupine* | Pygopus | *SMAD2* | *SMAD4* | *TP53* | *Ultrabithorax* | *VEGF* | WIF1 | WNT1 | WNT2B | *Wnt5a* | Wntless | *zeste white 3*
National Cancer Institute: http://www.cancer.gov/
colon cancer | hepatocellular carcinoma | medulloblastoma | melanoma | thyroid tumours | Wilms tumours

FURTHER INFORMATION
W. Birchmeier's homepage: http://www.mdc-berlin.de/en/research/research_teams/signal_transduction_invasion_and_metastasis_of_epithelial_cells/index.html
Avalon: http://www.avalonrx.com/content.aspx?id=36
Curis: http://www.curis.com/pipeline.php
Nuvelo: http://www.nuvelo.com
Roel Nusse's webpage:
http://www.stanford.edu/~rnusse/wntwindow.html
The Genetics Company: http://www.the-genetics.com/?menu=therapeutics&sub=wntinhibitors&doc=main

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SCIENCE AND SOCIETY

The challenge of cancer control in Africa

Rebecca J. Lingwood, Peter Boyle, Alan Milburn, Twalib Ngoma, John Arbuthnott, Ruth McCaffrey, Stewart H. Kerr and David J. Kerr

Abstract | While the world is focused on controlling the spread of diseases such as HIV and malaria in the developing world, another approaching epidemic has been largely overlooked. The World Heath Organization predicts that there will be 16 million new cancer cases per year in 2020 and 70% of these will be in the developing world. Many of these cancers are preventable, or treatable when detected early enough. Establishing effective, affordable and workable cancer control plans in African countries is one step in the right direction toward limiting this epidemic.

In the developing world, one-third of cancers are potentially preventable and another third are treatable if detected early[1]. However, in many developing countries, governments and institutions face a wide range of serious health problems and cancer is often not a priority in limited-resource settings. Currently, a cancer diagnosis in the developing world means a painful and distressing death in most cases. Although there is increasing awareness of the magnitude of the growing cancer problem in the developing world, the challenges posed are substantial (BOX 1).

The world is focused on controlling the spread of HIV, tuberculosis (TB) and malaria, which are all acknowledged to be major killers in the developing world, and huge sums of money are currently available to help combat these diseases[2]. Cancer is set to become the newest epidemic in the developing world, with the potential to claim a vast number of lives, but currently there is limited funding available to tackle this disease. Raising awareness of this looming epidemic in Africa is the first step. If the international cancer community takes concerted action now, working in partnership with the African Health Ministries, another tragedy can be prevented. To establish cancer care programmes in African countries requires the integration of clinical and public-health systems so as to be truly comprehensive, and must bring together prevention, early detection and diagnosis, treatment, palliative care and the investment needed to deliver these services. This will require trained staff, equipment, relevant drugs and information systems, supported by broad and effective partnerships between local health-care delivery systems, research institutions, international organizations, non-governmental organizations (NGOs), national governments in developed and developing countries, and the pharmaceutical industry. The relevant organizations and individuals must be brought together to develop achievable and sustainable national cancer plans that are evidence-based, priority-driven and resource-appropriate for African countries.

Cancer burden

In 2002, 7.6 million people worldwide died of cancer. This was 13% of the global mortality burden and, perhaps surprisingly, more than the number of deaths from HIV/AIDS, TB and malaria combined (~5.6 million)[3] (FIG. 1a).

The World Health Organization (WHO) has estimated that the global cancer burden will increase, according to current trends, from 10 million new cases per year in 2000 to 16 million in 2020. Remarkably, 70% of these cases will be in the developing world, rising from 5.2 million annually to 8.8 million by 2020, an increase of ~60%. Sub-Saharan Africa will account for >1 million of these cases by 2020 (REF. 4).

Although the AIDS epidemic has seen the relatively indolent tumour Kaposi sarcoma leap to the top of the cancer league tables for Uganda, Swaziland, Malawi and Zimbabwe, FIG. 2 shows the other prevalent tumour types, with cervical and breast carcinoma predominating in women and prostate and liver cancer in men[5].

Cancer infrastructure in Africa

One of the levers used to promote investment in cancer control in developed countries was the international comparison of relative spend and infrastructure in neighbouring nations, the lobbyists using these