

Wnt signalling in stem cells and cancer

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The canonical Wnt cascade has emerged as a critical regulator of stem cells. In many tissues, activation of Wnt signalling has also been associated with cancer. This has raised the possibility that the tightly regulated self-renewal mediated by Wnt signalling in stem and progenitor cells is subverted in cancer cells to allow malignant proliferation. Insights gained from understanding how the Wnt pathway is integrally involved in both stem cell and cancer cell maintenance and growth in the intestinal, epidermal and haematopoietic systems may serve as a paradigm for understanding the dual nature of self-renewal signals.

Stem cells are cells that have the unique ability to self-renew as well as to generate more differentiated progeny. The most primitive stem cell is the embryonic stem cell, which is derived from the inner cell mass of the blastocyst. This cell is pluripotent and can thus generate all the tissues of the body. Following the pioneering work on haematopoietic stem cells over the last five decades, a multitude of recent studies have indicated that most other adult tissues also harbour stem cells. These adult stem cells are normally involved in homeostatic self-renewal processes but can also be rapidly recruited to repair tissues upon injury. With the study of adult stem cell biology, recurring roles of a limited set of signalling cascades are rapidly being uncovered. One of these is the canonical Wnt cascade. Notably, in many of the same tissues where the Wnt cascade controls stem cells, cancer ensues upon dysregulated activation of this pathway. Conceptually, this indicates that an efficient road to cancer involves the hijacking of physiological regulators of stem cell function in these particular tissues. Below, we first outline the canonical Wnt cascade, and then describe its mirror image roles in the biology of stem cells and cancer.

The canonical Wnt signalling pathway in development

The discovery of the common origin of the *Drosophila* segment polarity gene *Wingless* and the murine proto-oncogene *Int-1* (ref. 1) laid the keystone of a signalling pathway now commonly referred to as the canonical Wnt cascade (Fig. 1). Wnt genes, of which the human genome harbours almost 20, occur throughout the animal kingdom. Signalling is initiated when Wnt ligands engage their cognate receptor complex, consisting of a serpentine receptor of the Frizzled family and a member of the LDL receptor family, Lrp5/6. The central player is a cytoplasmic protein termed β -catenin, the stability of which is regulated by the destruction complex. There are β -catenin-independent, non-canonical signalling pathways induced by Wnt, and their mechanism of action has been described elsewhere². When Wnt receptors are not engaged, two scaffolding proteins in the destruction complex—the tumour suppressors adenomatous polyposis coli (APC) and axin—bind newly synthesized β -catenin. CKI and GSK3, two kinases residing in the destruction complex, then sequentially phosphorylate a set of conserved Ser and Thr residues in the amino terminus of β -catenin. The resulting phosphorylated footprint recruits a β -TrCP-containing E3 ubiquitin ligase, which targets β -catenin for proteasomal degradation.

Receptor occupancy inhibits the kinase activity of the destruction complex by an incompletely understood mechanism involving the direct interaction of axin with Lrp5/6, and/or the actions of an axin-binding molecule, Dishevelled. As a consequence, β -catenin accumulates and travels into the nucleus where it engages the N terminus of DNA-binding proteins of the Tcf/Lef family³. The

vertebrate genome encodes four highly similar Tcf/Lef proteins. In the absence of a Wnt signal, Tcf/Lef proteins repress target genes through a direct association with co-repressors such as Groucho. The interaction with β -catenin transiently converts Tcf/Lef factors into transcriptional activators. *Drosophila* genetics has recently identified two additional nuclear components, Pygopus and Bcl9 (also known as legless), conserved in vertebrates. Pygopus is essential for transcriptional activation of Tcf/Lef target genes, whereas Bcl9 seems to bridge Pygopus to Tcf-bound β -catenin. In sum, the canonical pathway translates a Wnt signal into the transient transcription of a Tcf/Lef target gene programme⁴.

From crypt physiology to colon cancer

The intestinal tract consists of the small intestine (duodenum,

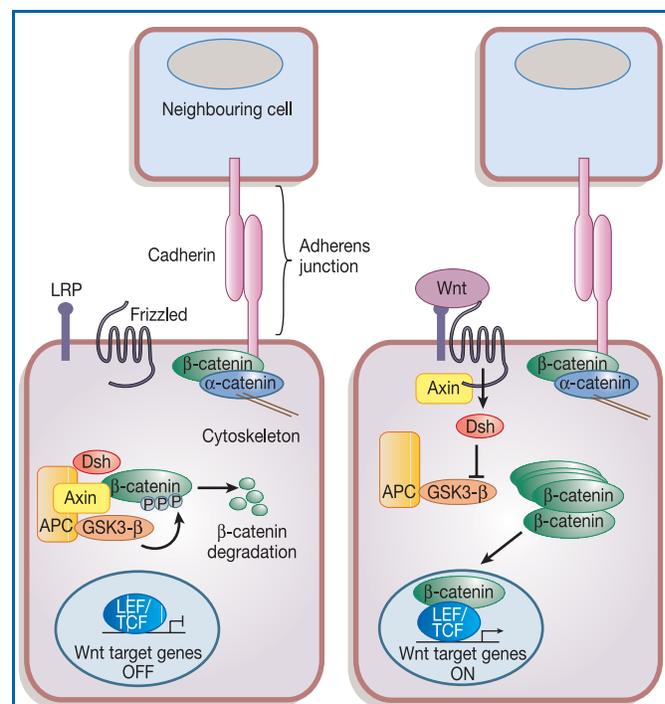


Figure 1 The canonical Wnt signalling pathway. In the absence of Wnt signalling (left panel), β -catenin is in a complex with axin, APC and GSK3- β , and gets phosphorylated and targeted for degradation. β -Catenin also exists in a cadherin-bound form and regulates cell–cell adhesion. In the presence of Wnt signalling (right panel), β -catenin is uncoupled from the degradation complex and translocates to the nucleus, where it binds Lef/Tcf transcription factors, thus activating target genes. (Adapted from ref. 44.)

jejunum and ileum) and the large intestine or colon. The absorptive epithelium of the small intestine is ordered into villi and crypts of Lieberkühn. Differentiated cells (enterocytes, enteroendocrine cells and goblet cells) occupy the villi (Fig. 2). A fourth differentiated type, the Paneth cell, resides at the bottom of crypts and secretes antimicrobial agents⁵. The remainder of the crypts constitutes the stem/progenitor compartment. The mucosa of the colon has a flat surface epithelium instead of villi (Fig. 3). Proliferative stem and precursor cells occupy the bottom two-thirds of crypts, whereas differentiated cells constitute the surface epithelium and top third of the crypts.

The life cycle of an individual epithelial cell spans less than a week. In the mouse, some 200 cells are generated per crypt every day. This is compensated by cell loss at the villus tip. The epithelium forms a contiguous two-dimensional sheet that is in a perpetual upward movement⁶. Stem cells and Paneth cells at the crypt bottom escape this flow⁷. Although no unique markers have been identified for intestinal stem cells, they can be efficiently labelled *in vivo* with 3H-thymidine or 5-bromodeoxyuridine (BrdU) during early postnatal life or after irradiation⁸. Long-term label retention,

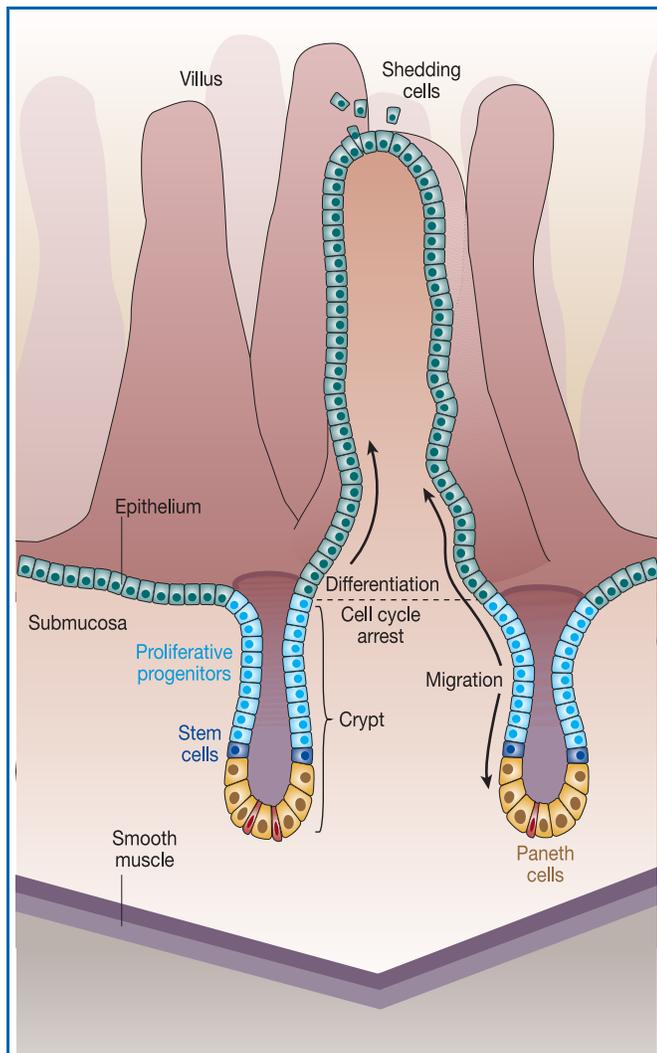


Figure 2 Tissue anatomy of the adult small intestine. Putative stem cells (dark blue) reside immediately above the Paneth cells (yellow) near the crypt bottom. Proliferating progenitor cells occupy the remainder of the crypt. Differentiated cells (green) populate the villus, and include goblet cells, enterocytes and enteroendocrine cells. (Adapted from ref. 89.)

mouse chimaeras^{9,10} and the study of regeneration upon injury have allowed an operational definition of numbers and localization. The estimated number of stem cells is between one and six per crypt⁷. Colon stem cells reside at the crypt bottom, whereas small intestinal stem cells are at 'position +4' above the Paneth cells (reviewed in ref. 11). These stem cells cycle slowly, continuously producing rapidly proliferating 'transit amplifying' cells capable of differentiating towards all lineages. They not only self-renew throughout life but also regenerate the epithelium after injury. Committed progenitors undergo cell cycle arrest and start expressing differentiation markers when they reach the top one-third of colorectal crypts or the crypt–villus junction in the small intestine. The differentiated cells continue their migration on the villus in coherent bands stretching along the crypt–villus axis. Although crypts are monoclonal, each villus receives cells from multiple crypts and is therefore polyclonal^{10,12}.

Current evidence indicates that the Wnt cascade is the single most dominant force in controlling cell fate along the crypt–villus axis. Nuclear β -catenin, the hallmark of Wnt signalling, is observed throughout the crypts of the intestine¹³. In *Tcf4*^{-/-} neonatal mice, the villus epithelial compartment appears unaffected but the crypt progenitor compartment is entirely absent, implying that physiological Wnt signalling is required for maintenance of the crypt progenitor phenotype¹⁴ (Table 1). In concordance, inhibition of the Wnt receptor complex by transgenic Dickkopf-1 expression induces the complete loss of crypts in adult mice^{15,16}. As mentioned below, a constitutive β -catenin–Tcf4 complex drives a genetic programme in colorectal cancer cells that is physiologically expressed in crypt stem/progenitor cells¹⁷. The Wnt signalling gradient also controls expression of the EphB/ephrinB sorting receptors and ligands¹³. The resulting EphB/ephrinB counter-gradients allow the establishment of crypt–villus boundaries as well as the positioning of Paneth cells at the crypt bottom.

The Wnt pathway in colon cancer

The *APC* gene was originally discovered to be the culprit in a hereditary cancer syndrome termed familial adenomatous polyposis (FAP). FAP patients, inheriting one defective *APC* allele, develop large numbers of colon polyps, or adenomas, early in life. Individual polyps are clonal outgrowths of epithelial cells in which the second *APC* allele is inactivated. The large burden of FAP adenomas inevitably results in the appearance of adenocarcinomas through clonal evolution, evident as a more or less ordered accumulation of mutations in additional oncogenes or tumour suppressor genes, such as *K-Ras*, *p53* and *Smad4*. Loss of APC also occurs in most sporadic colorectal cancers¹⁸. Mutational inactivation of APC leads to the inappropriate stabilization of β -catenin¹⁹, implying that the absence of functional APC transforms epithelial cells through activation of the Wnt cascade. Indeed, reporter plasmids containing concatemeric Tcf binding sites such as pTOPFLASH, normally transcribed only upon Wnt signalling, are inappropriately transcribed in APC mutant cancer cells through the action of constitutive β -catenin–Tcf4 complexes²⁰. In some cases of colorectal cancer in which APC is not mutated, the scaffolding protein axin 2 is mutant²¹, or activating (oncogenic) point mutations in β -catenin remove its N-terminal Ser/Thr destruction motif²². As exceptions to the rule that APC inactivation initiates colon cancer, these rare mutations underscore the central role of the inappropriate persistence of β -catenin–Tcf4 complexes in the transformation of epithelial cells.

Multiple studies have used a candidate gene approach to address the nature of the Tcf4 target gene programme in colorectal cancer. Among others, *c-Myc*²³ and cyclin D1 (ref. 24) reportedly are essential components of this programme in transformed intestinal epithelial cells. We recently induced expression of dominant-negative Tcf4 in colorectal cell lines and carried out a global assessment of the target gene programme using DNA microarrays¹⁷.

This study revealed that Tcf4 drives the same genetic programme in colorectal cancer cells as in crypt stem and progenitor cells. *APC*^{-/-} adenoma cells thus represent the transformed counterparts of the proliferative crypt progenitor. Once the Wnt cascade is mutationally activated, the adenoma cells maintain their progenitor status indefinitely. This allows the adenomas to persist for many years, providing ample opportunity for the acquisition of further mutations. In an elegant *in vivo* study, the Wnt cascade was mutationally activated by inducible deletion of an *APC* allele. Within 1–2 days, villi were entirely populated by crypt-like cells. DNA array analysis revealed the induction of many of the crypt markers previously identified as Tcf4 targets in human colorectal cancer cell lines²⁵.

Colorectal cancer almost invariably initiates with an activating mutation in the Wnt cascade. Current evidence indicates that the transformation process exploits the unique physiological dependence of the intestinal progenitor/stem cell on the Wnt cascade. A surprising symmetry ensues between the crypt progenitor and adenoma cells.

Wnt signalling in epidermal development and cancer

The hair follicle and the associated sebaceous gland are appendices of the squamous interfollicular epidermis of the skin²⁶. Hair follicles

are far more complex structures than the crypt–villus unit described above. Multipotent epidermal stem cells reside in the bulge region, a structure situated below the sebaceous gland at the site of attachment of the arrector pili muscle (Fig. 4). Bulge stem cells can generate the hair lineages, sebocytes, as well as the stem cells of the interfollicular epidermis. Distinct stem cell populations may exist also in the interfollicular epidermis and sebaceous gland (see ref. 26). To form a hair, cells flow downward from the bulge through the outer root sheath. At the base of the follicle, the cells enter a transit amplifying compartment termed the germinative matrix. After exiting the matrix, the cells undergo terminal differentiation in the precortex compartment. Bulge-derived cells can also flow upward through the outer root sheath to yield sebocytes, or to seed the basal layer of the interfollicular epidermis with slow-cycling keratinocyte stem cells. These latter cells maintain a transit amplifying compartment, in which cells divide several times before the onset of terminal keratinocyte differentiation.

Mice with a mutation in one of the four *Tcf/Lef* genes, *Lef1*, display a paucity of hair follicles²⁷ (Table 1). Conversely, overexpression of transgenic *Lef1* in bulge and interfollicular epidermis leads to hair germ-like invaginations in the epidermis²⁸. A much more dramatic phenotype is obtained upon overexpression of a constitutively stable form of β -catenin²⁹, which induces pro-

Table 1 Summary of selected studies demonstrating the effects of Wnt signalling on stem cells from varied tissues

| Stimulus | Cell type | Species | Effect | Assay | Reference |
|--|---------------------------------|-------------|--|---------------------------------|-----------|
| Epidermal stem and progenitor cells | | | | | |
| Activated β -catenin | Keratinocytes | Mouse | Inhibition of differentiation | <i>In vitro</i> | 83 |
| Activated β -catenin transgenic | Follicular stem cells | Mouse | Hair follicle and tumour formation | <i>In vivo</i> | 29 |
| <i>Lef1</i> ^{-/-} | Hair follicles | Mouse | Loss of hair formation | <i>In vivo</i> | 27 |
| <i>Lef1</i> transgenic | Hair follicles | Mouse | Increased hair follicle formation | <i>In vivo</i> | 28 |
| Conditional deletion of β -catenin | Follicular stem cells | Mouse | Cell fate alteration | <i>In vivo</i> | 31 |
| Intestinal stem and progenitor cells | | | | | |
| <i>Tcf4</i> ^{-/-} | Intestinal stem cells | Mouse | Depletion of crypt stem cells | <i>In vivo</i> | 20 |
| <i>Dkk1</i> transgenic | Epithelial cells in intestine | Mouse | Loss of crypts; reduced epithelial proliferation | <i>In vivo</i> | 15 |
| Adenoviral <i>Dkk1</i> | Epithelial cells in intestine | Mouse | Reduced epithelial proliferation | <i>In vivo</i> | 16 |
| Haematopoietic stem and progenitor cells | | | | | |
| <i>Tcf1</i> ^{-/-} | T-cell progenitors | Mouse | Loss of early proliferative progenitors | <i>In vivo</i> | 84 |
| <i>Lef1</i> ^{-/-} <i>Tcf1</i> ^{-/-} | T-cell progenitors | Mouse | Loss of early proliferative progenitors; block in T-cell differentiation | <i>In vivo</i> | 85 |
| <i>Wnt1</i> ^{-/-} <i>Wnt4</i> ^{-/-} | T-cell progenitors | Mouse | Reduced thymic proliferation and cellularity | <i>In vivo</i> | 86 |
| Axin inducible transgenic | T-cell progenitors | Mouse | Reduced thymic cellularity and increased apoptosis | <i>In vivo</i> | 87 |
| Conditional deletion of β -catenin (lck-driven) | T-cell progenitors | Mouse | Block in differentiation, reduced proliferation | <i>In vivo</i> | 51 |
| <i>Lef1</i> ^{-/-} | B-cell progenitors | Mouse | Reduced survival and proliferation | <i>In vivo</i> | 52 |
| <i>Fzd9</i> ^{-/-} | B-cell progenitors | Mouse | Loss of early B-cell progenitors | <i>In vivo</i> | 62 |
| <i>Wnt5A</i> + SLF | Fetal liver enriched precursors | Mouse | Increased progenitors | <i>In vitro</i> | 46 |
| <i>Wnt5A</i> or <i>Wnt11A</i> | Bone marrow, non-adherent | Quail | Increased differentiation | <i>In vitro</i> | 82 |
| <i>Wnt3A</i> + stromal cells | Bone marrow, unenriched | Mouse | Inhibition of haematopoiesis | <i>In vitro</i> | 88 |
| <i>Wnt3A</i> /activated β -catenin | Purified HSCs | Mouse | Increased self renewal | <i>In vitro/in vivo</i> | 45, 47 |
| Axin | Purified HSCs | Mouse | Inhibition of proliferation/reconstitution | <i>In vitro/in vivo</i> | 45 |
| Conditional deletion of β -catenin | Bone marrow | Mouse | Normal self renewal/ reconstitution | <i>In vivo</i> | 50 |
| <i>Wnt5A</i> + SLF + feeder cells | Bone marrow enriched precursors | Human | Increased progenitors | <i>In vitro</i> | 48 |
| <i>Wnt5A</i> | Bone marrow enriched precursors | Human | Increased reconstitution | <i>In vivo</i> (xenotransplant) | 49 |
| Neural stem and progenitors | | | | | |
| Activated β -catenin transgenic | Neural progenitors | Mouse | Increased expansion | <i>In vivo</i> | 67 |
| Conditional deletion of β -catenin | Neural progenitors | Mouse | Decreased expansion | <i>In vivo</i> | 68 |
| Conditional activation of β -catenin | Neural progenitors | Mouse | Increased expansion | <i>In vivo</i> | 68 |
| Embryonic stem cells | | | | | |
| GSK3-specific inhibitor 6-bromoindirubin-3'-oxime (BIO) | Embryonic stem cells | Mouse/human | Maintenance of pluripotency | <i>In vitro</i> | 74 |
| <i>APC</i> mutations | Embryonic stem cells | Mouse | Increased inhibition of differentiation | <i>In vivo/in vitro</i> | 75 |

nounced hair follicle morphogenesis in interfollicular epidermis. These observations were extended using a tamoxifen-activatable β -catenin transgene, which could induce *de novo* hair follicle formation in adult interfollicular epidermis³⁰. The findings that β -catenin and *Lef1* mRNA accumulate in embryonic skin placodes—the structural precursors to hair follicles—and that conditional deletion of β -catenin leads to loss of these placodes³¹, imply that the Wnt pathway contributes to the complex, incompletely elucidated signalling network that leads to placode induction and ultimately the establishment of hair follicles during embryonic development.

Independently of the events described above, the Wnt cascade controls the fate of cells that derive from the bulge stem cells in established hair follicles. Two of the four Tcf/Lef family members are expressed in the hair follicle. One of these, Tcf3, is expressed specifically in bulge stem cells³². Transgenic expression of Tcf3 in interfollicular epidermis inhibits terminal differentiation of keratinocytes and promotes features of bulge cells. The effects of Tcf3 were independent of β -catenin, yet dependent on its ability to repress target gene transcription³². Although not yet tested in a loss-of-function experiment, it is strongly suggested that the presence of Tcf3 in the bulge maintains stem cell features through repression of specific Tcf target genes. Indeed, a Tcf reporter transgene (TOPGAL) was generally silent in the Tcf3-positive bulge region, yet was stimulated at the start of the hair cycle; that is, when the formation of a new hair is initiated³². The second Tcf/Lef family member, *Lef1*, is primarily expressed in the matrix and precortex of the hair³². Of interest, a number of hair keratin genes carry Tcf-binding sites in their promoters²⁸. This implies the counterintuitive notion that Wnt signalling in the matrix, relayed through β -catenin and *Lef1*, drives terminal differentiation of the hair lineage. The TOPGAL

reporter transgene demonstrated strong activity in the precortex of the hair shaft, coinciding with the induction of hair-specific keratin expression³². In accordance with a specific function of Wnt signalling in the hair lineages, conditional deletion of the β -catenin gene after hair follicle formation led to the complete loss of hair, possibly due to the fact that bulge stem cells failed to provide hair lineage precursors³¹. Rather, the cells adopted an epidermal fate. A similar fate change was observed upon transgenic expression of a dominant-negative version of *Lef1* in the hair follicle, which led to the formation of cysts filled with interfollicular epidermal cells and sebocytes at the base of hair follicles^{33,34}. In another study, the same keratin14-driven Δ N*Lef* transgene suppressed hair differentiation, but skewed the cells towards a sebaceous fate³⁵. In sum, Wnt signalling in hair follicles, relayed through *Lef1*, promotes entry into the post-mitotic hair lineage, whereas specific inhibition of Wnt target genes through Tcf3 may maintain bulge stem cells.

Although not formally proved, strong expression of *Lef1*, itself a Wnt target gene, in the transit amplifying cells of the matrix suggests that maintenance of these proliferative cells requires canonical Wnt signalling. In concordance, the matrix cells appear to be the substrate for malignant transformation by mutational activation of the Wnt cascade. One study²⁹ reported that a constitutively active β -catenin transgene induces pilomatricoma-like lesions. Pilomatricomas are tumours characterized by an exterior zone of densely packed cells, resembling the hair follicle matrix. The tumours also contain a transitional zone of cells displaying a gradual loss of nuclei and an inner zone of 'shadow' cells consisting of enucleated cellular 'ghosts'. In another study, activation of a tamoxifen-activatable β -catenin transgene led to hair follicle tumours resembling trichofolliculomas³⁰. Moreover, most spontaneous pilomatricomas in humans carry activating mutations in β -catenin³⁶. Like adenomas

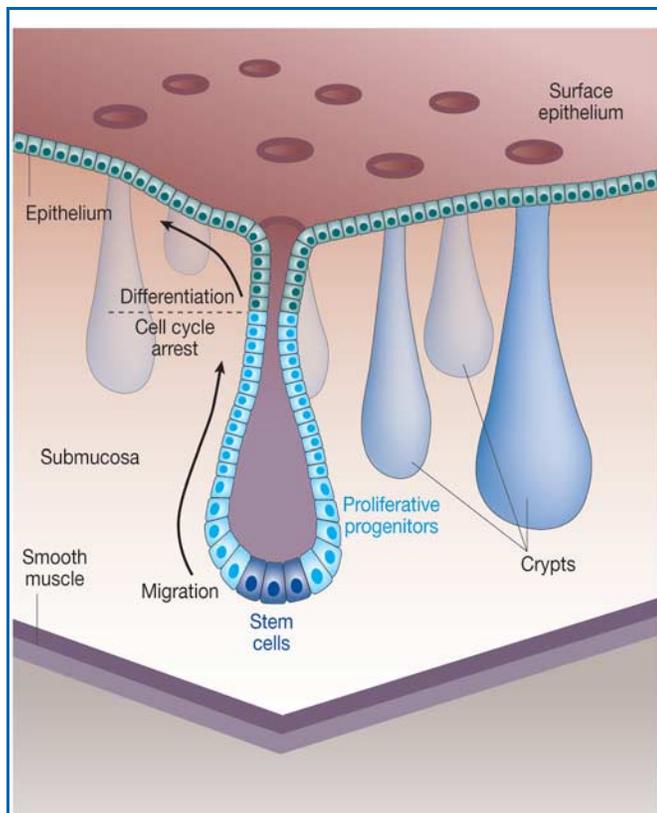


Figure 3 Tissue anatomy of the colonic epithelium. Putative stem cells (dark blue) reside at the crypt bottom. Proliferating progenitor cells occupy two-thirds of the crypt. Differentiated cells (green) populate the remainder of the crypt and the flat surface epithelium. (Adapted from ref. 89.)

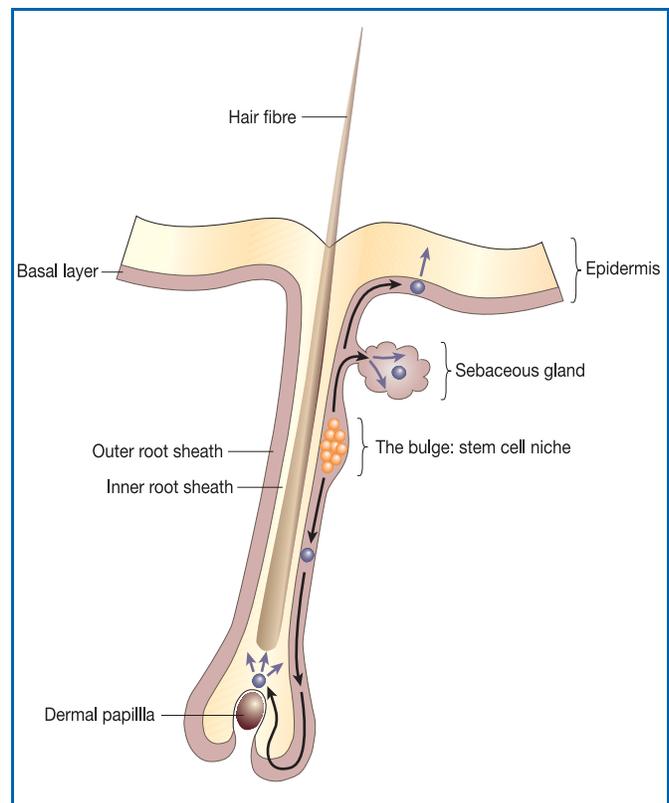


Figure 4 The hair follicle. Stem cells reside in the bulge niche. Cells can migrate upwards from here to populate the sebaceous gland and the interfollicular epidermis. Cells that migrate downwards enter the matrix where they rapidly proliferate and then differentiate to form the hair. (Adapted from ref. 90.)

in the gut, pilomatricomas may have hijacked the Wnt-driven expansion of hair precursors in the matrix/precortex region of the hair. Whereas several aspects of hair follicle biology are thus controlled by the Wnt cascade, it remains undetermined whether the Wnt pathway is also required in the interfollicular epidermis, or is mutated in tumours derived thereof.

Wnt signalling in haematopoietic stem cells

Haematopoietic stem cells (HSCs), the cells that give rise to the blood throughout our lifetime, are perhaps the best understood stem cells in our body. These cells were originally identified functionally by Till and McCulloch^{37,38}. Since then, great progress has been made in understanding the biology of HSCs, in large part because they have been successfully isolated and the stages through which they progress during development delineated (reviewed in ref. 39). HSC activity in the adult bone marrow has been found to lie within a very rare population of cells characterized by their expression of undetectable or low levels of haematopoietic lineage markers, high levels of c-kit and Sea-1 and low levels of Thy-1.1³⁹. These cells probably reside in the context of a stromal cell niche, which, at least in part, consists of cells of the osteoblast lineage (Fig. 5)^{57,58}. The ability to isolate HSCs and progenitors has also allowed recent advancement in the understanding of the molecular control of their function, particularly that of self-renewal and maintenance. There is growing evidence that signals such as Notch, Hedgehog and Wnt—pathways that control many developmental processes and are dysregulated in cancer—might regulate self-renewal of haematopoietic progenitors and stem cells^{40–43}. In the case of Wnt signalling, the ligands (that is, Wnt proteins) are produced by HSCs themselves as well as by the microenvironment, suggesting that HSCs may use them in an autocrine or paracrine manner (reviewed in ref. 44). Furthermore, the observation of Wnt reporter activity in HSCs in the context of their native

microenvironment suggests that HSCs directly respond to Wnt signalling *in vivo*⁴⁵. Functional studies show that *in vitro*, soluble Wnt proteins synergize with Steel factor (also known as stem cell factor or SLF) to promote the growth and inhibit the differentiation of murine haematopoietic progenitors⁴⁶ (Table 1). In addition, β -catenin as well as purified Wnt3A protein can promote self renewal of murine HSCs *in vitro* and enhance their ability to reconstitute the haematopoietic system of lethally irradiated mice *in vivo*^{45,47}. The effects of Wnt signalling on HSCs in mice are recapitulated in human HSCs and progenitors as well. Wnt5A treatment of human haematopoietic progenitors in the presence of stromal cell contact promotes the expansion of undifferentiated progenitors *in vitro*⁴⁸, and treatment of mice with Wnt5A-conditioned medium results in increased human HSC re-population in a NOD-SCID xenotransplant model⁴⁹. Cumulatively, these studies suggest that Wnt signalling can contribute to HSC and progenitor cell self-renewal. Because Wnt proteins that act through the canonical pathway (such as Wnt3A) and those that can act through non-canonical pathways (for example, Wnt5A) seem to influence HSCs and progenitors equivalently, the pathway dominantly used during HSC function *in vivo* remains a question for further study.

That Wnt signalling may also be required for normal growth of HSCs *in vitro* and *in vivo* is suggested by inhibition of HSC growth and decreased reconstitution after ectopic expression of axin⁴⁵; however, mice in which β -catenin has been conditionally deleted revealed no haematolymphoid defects, raising questions about the role of β -catenin in HSCs and in haematopoietic progenitor development⁵⁰. These data are difficult to reconcile with the previous literature that suggests that β -catenin and other elements of the Wnt signalling pathway are required for T-cell development⁵¹, B-cell survival^{52,53}, and haematopoietic stem and progenitor function (reviewed in ref. 54). One possibility is that the interferon-induced deletion of β -catenin sets up a significantly different context for study of the requirement of specific genes in HSC development⁵¹; alternatively, compensation by γ -catenin, a close relative of β -catenin, may allow β -catenin mutant HSCs to function normally⁵⁵. This is supported by the recent finding that γ -catenin overexpression leads to enhanced re-plating efficiency of HSCs, selectively causing expansion of small, uncommitted, blast-like colonies *in vitro*⁵⁶, and the fact that axin can enhance degradation not only of β -catenin but of γ -catenin as well. Analyses of mice mutant for other components of the Wnt pathway are clearly needed to elucidate the precise nature of its influence on HSC maintenance and self renewal *in vivo*. Understanding the influence of Wnt signalling on HSCs in the context of the native microenvironment will also be enhanced by identifying the function of the specific Wnt proteins produced at the HSC niche^{57,58}. Because the niche is also likely to provide other signals that have been shown to influence HSCs (such as Shh and Notch ligands⁵⁹), it will be critical to determine the roles that these signals have relative to Wnts and whether they control distinct elements of self renewal.

Aberrant Wnt signalling in leukaemia

Much like the parallels between adenomas and progenitor cells in the intestinal crypt, the influence of Wnt pathway components on haematopoietic stem and progenitor cells suggests that the same pathway may be dysregulated to cause leukaemia. Although this possibility has been speculated on for some time, it has only recently been supported by experimental evidence demonstrating that oncogenic growth in leukaemias of both myeloid and lymphoid lineages is dependent on Wnt signalling.

Myeloid leukaemias can be broadly classified as chronic or acute, and Wnt signalling has been implicated in regulating the growth of cells from both subtypes of leukaemia. Chronic myelogenous leukaemia results predominantly from a BCR-Abl translocation that originates in HSCs but exhibits its consequences in more committed myeloid precursors. Granulocyte-macrophage progeni-

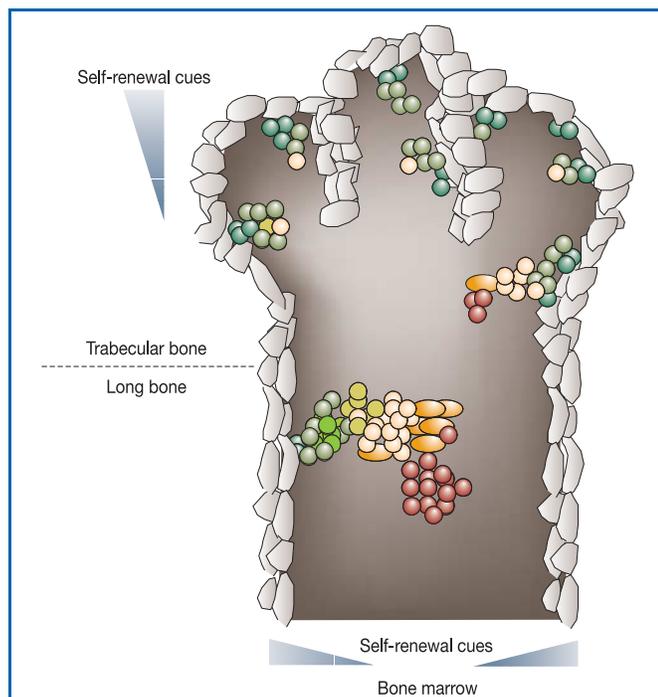


Figure 5 Proposed model of HSC development in the niche. HSCs are shown (dark green) at the endosteal marrow adjacent to the bone's surface mainly at the trabecular bone, and are postulated to migrate inward in the central marrow as they differentiate (precursors in light green; differentiated cells in yellow, orange and red) away from a possible gradient of self-renewal cues. (Adapted from ref. 44.)

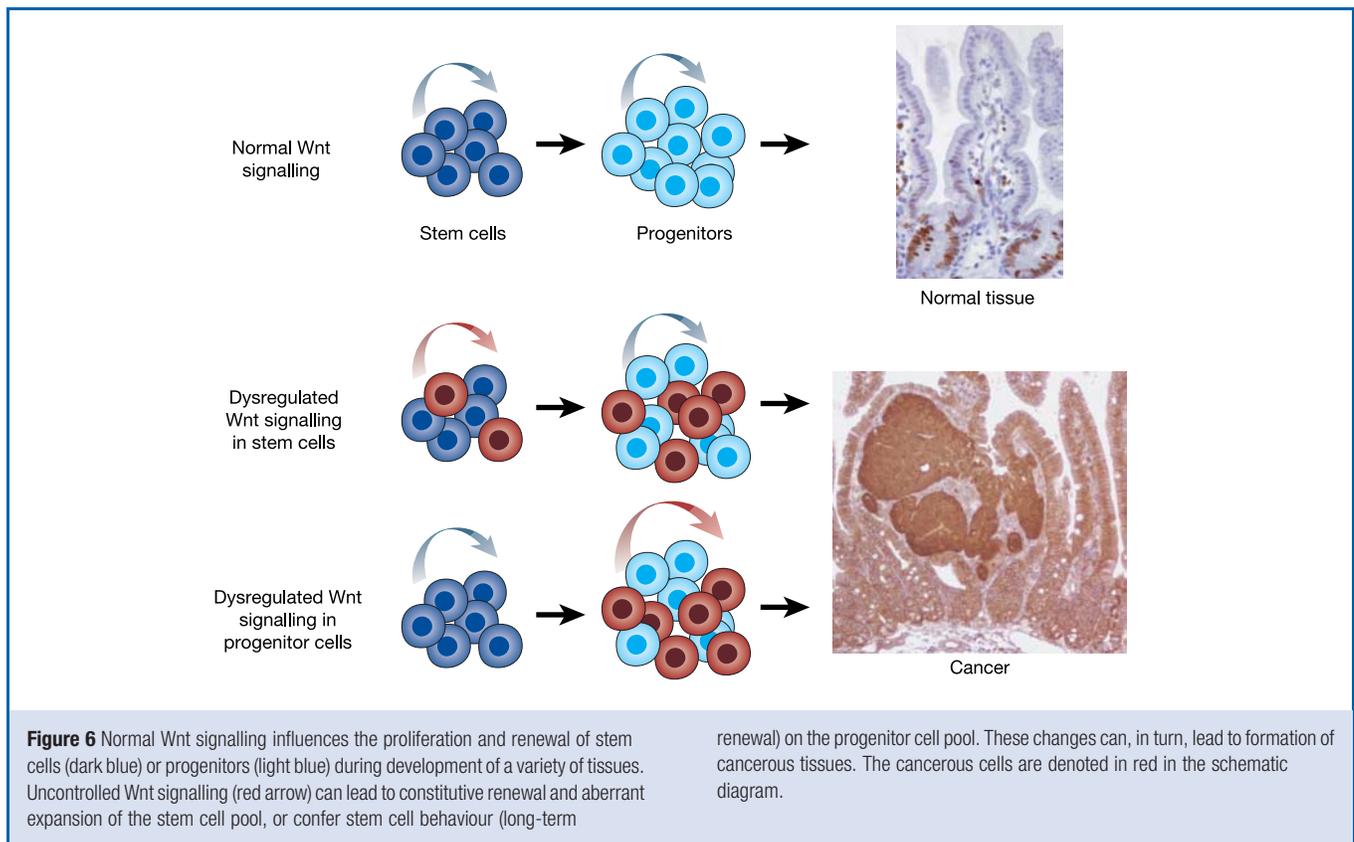
tors (GMPs) from chronic myelogenous leukaemia patients and blast crisis cells from patients resistant to therapy display activated Wnt signalling, as determined by Wnt reporter activity and accumulation of nuclear β -catenin⁶⁰. Additionally, inhibition of β -catenin through ectopic expression of axin decreases the re-plating capacity of leukaemic cells *in vitro*, suggesting that chronic myelogenous leukaemia precursors are dependent on Wnt signalling for growth and renewal. In an interesting parallel with its effects on normal HSCs, overactivation of Wnt signalling also endowed GMPs—which normally have limited self-renewal capacity—with stem-cell-like properties of long-term renewal, at least *in vitro*⁶⁰. This supports the notion that for progenitor cells to be transformed they must adopt a stem-cell-like ability to renew, and suggests that Wnt signalling is a cue that can control such a transformation event. The possibility that leukaemic cells depend on Wnt signalling is supported by studies of acute myelogenous leukaemia, which show that the re-plating efficiency of HSCs transduced with fusion proteins encoded by translocations commonly found in acute myelogenous leukaemia was abrogated upon inhibition of γ -catenin^{56,61}. Conversely, mice that were transplanted with HSCs overexpressing γ -catenin displayed acute myelogenous leukaemia-like symptoms⁵⁶. Although these emerging data suggest that Wnt activation through β - and γ -catenin may lead to myeloid leukaemias, drawing a causative link awaits generation of a mouse model where aberrant Wnt signalling can drive leukaemogenesis.

Recent studies suggest that lymphoid neoplasias may also be influenced by Wnt signalling. Wnt16 was initially cloned owing to its overexpression in pre-B-cell leukaemia lines carrying the E2A-PbX translocation⁶², raising the possibility that autocrine Wnt usage may contribute to oncogenesis. This possibility is supported by the observed influence of Wnt signalling on growth and survival of normal B-cell progenitors^{52,53}. A similar autocrine loop of Wnt dependence has also been proposed for regulating the growth of multiple myeloma, a cancer of terminally differentiated B cells⁶³. A

number of primary myelomas and myeloma cell lines were found to express the stabilized form of β -catenin; although no mutations in Wnt signalling components were detected, highly increased levels of Wnt proteins including Wnt5A and Wnt10B were detected. In an interesting parallel with the influence of the Shh pathway in cancers of the lung and gastrointestinal tract⁶⁴, these data suggest that leukaemias may not harbour the type of Wnt pathway mutations found in colon cancer or epidermal tumours, but they may nonetheless be critically dependent on the autocrine/paracrine use of Wnt signalling for sustaining cancerous self-renewal. Paradoxically, although in most contexts Wnt proteins induce proliferation and are associated with oncogenesis, a small subset of Wnt5A hemizygous mice develop lymphoid and myeloid leukaemia⁶⁵. This suggests that in some contexts a Wnt5A-mediated non-canonical pathway inhibits the canonical pathway, perhaps at the level of Tcf phosphorylation and function⁶⁶, and that the loss of this suppression may lead to hyperproliferation of B-cell precursors and subsequent leukaemogenesis. Thus, antagonism between the canonical pathway and non-canonical pathways may be an essential mechanism for controlling dysregulated renewal and oncogenesis.

Perspectives

The concept that Wnt signalling may preferentially influence progenitor cell expansion in development and in cancer extends beyond the examples discussed above. Recent data show that activating Wnt signalling through β -catenin can increase cycling and expansion of neural progenitors, and that its loss causes a reduction in the progenitor compartment^{67,68}. Just as normal activation of Wnt signalling may promote self-renewal of neuronal stem cells, aberrant Wnt pathway activation may be tumorigenic in the nervous system. This is supported by the fact that medulloblastoma, a paediatric brain tumour of the cerebellum, harbours mutations in β -catenin⁶⁹ and axin^{70,71}, suggesting that some medulloblastomas may arise from a primitive progenitor that



becomes transformed in response to uncontrolled Wnt signalling. Furthermore, in mammary tissues where stem cells have yet to be definitively isolated, a controlling role for Wnt in progenitor cell fate or maintenance is suggested by studies of Wnt1 transgenic mice that develop mammary tumours. These tumours have an increased frequency of cells with stem and progenitor properties^{72,73}, in contrast to tumours from mice overexpressing other oncogenes. This suggests again that the Wnt pathway may be unique in its ability to target stem and progenitor cells for transformation, and perhaps reflects a role of the Wnt pathway in self-renewal of normal breast epithelium⁷³. Finally, the influence of Wnt signalling on stem cells appears to extend to the most primitive of all stem cells: the embryonic stem cell. Modulation of Wnt signalling by controlling the dose of APC, or by treatment with a GSK3- β inhibitor, can enhance self-renewal of both mouse and human embryonic stem cells^{74,75}.

Looking to the future, it is clear that an important area of investigation will involve analysing how the Wnt pathway exerts its effect and whether shared molecular targets are involved in influencing self-renewal in the context of stem cells and cancer¹⁷. Additionally, Wnt signalling probably integrates with other niche-derived signals such as BMP, Shh and Notch^{76–78}. In some contexts, these signals may be independently responsible for distinct aspects of tissue self-renewal, such as survival, proliferation and inhibition of differentiation. In other cases, the various signalling cascades may act in a hierarchy, and regulate each other. It will be critical to understand the coordinated activity of these pathways in the *in vivo* control of stem cell function and define whether the same patterns hold true in cancer. Finally, although much of the focus of this review is on Wnt-controlled cell renewal, there is no doubt that, depending on the context, Wnt signalling can influence commitment and differentiation as well^{79–82}. A clearer view of the different cellular and molecular contexts that elicit differential outcomes of Wnt signalling will be essential to understand more completely the basis of Wnt's effects on renewal in stem cells and cancer.

This review highlights how the Wnt pathway may be a common element in regulating stem cell/progenitor cell renewal and maintenance in a variety of systems, and how this ability is exploited by cancer (Fig. 6). Because Wnt signalling can act to maintain stem cells as well as cancer cells, the ability to modulate the Wnt pathway either positively or negatively may be of therapeutic relevance. Thus, controlled activation of Wnt signalling may allow us to enhance stem cell and progenitor cell activity, when regeneration is needed. On the other hand, inhibition of Wnt signalling may prove to be an effective road to inhibiting the uncontrolled renewal that drives cancer. □

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