



Wnt Signaling in Hematological Malignancies

Stephanie Grainger^{*,†}, David Traver^{*}, Karl Willert^{*,†,1}

^{*} University of California, La Jolla, San Diego, CA, United States

[†] Sanford Consortium for Regenerative Medicine, La Jolla, San Diego, CA, United States

¹ Corresponding author. E-mail address: kwillert@ucsd.edu

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Abstract

Leukemia and lymphoma are a wide encompassing term for a diverse set of blood malignancies that affect people of all ages and result in approximately 23,000 deaths in the United States per year (Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin.* 2016;66(1):7–30.). Hematopoietic stem cells (HSCs) are tissue-specific stem cells at the apex of the hierarchy that gives rise to all of the terminally differentiated blood cells, through progressively restricted progenitor populations, a process that is known to be Wnt-responsive. In particular, the progenitor populations are subject to uncontrolled expansion during oncogenic processes, namely the common myeloid progenitor and common lymphoid progenitor, as well as the myeloblast and lymphoblast. Unregulated growth of these cell-types leads to mainly three types of blood cancers (i.e., leukemia, lymphoma, and myeloma), which frequently exhibit deregulation of the Wnt signaling pathway. Generally, leukemia is caused by the expansion of myeloid progenitors, leading to an overproduction of white blood cells; as such, patients are unable to make sufficient numbers of red blood cells and platelets. Likewise, an overproduction of lymphocytes leads to clogging of the lymph

system and impairment of the immune system in lymphomas. Finally, cancer of the plasma cells in the blood is called myeloma, which also leads to immune system failure. Within each of these three types of blood cancers, there are multiple subtypes, usually characterized by their timeline of onset and their cell type of origin. Of these, 85% of leukemias are encompassed by the four most common diseases, that is, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), and chronic lymphocytic leukemia (CLL); AML accounts for the majority of leukemia-related deaths (Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin.* 2016;66(1):7–30.). Through understanding how HSCs are normally developed and maintained, we can understand how the normal functions of these pathways are disrupted during blood cancer progression; the Wnt pathway is important in regulation of both normal and malignant hematopoiesis. In this chapter, we will discuss the role of Wnt signaling in normal and aberrant hematopoiesis. Our understanding the relationship between Wnt and HSCs will provide novel insights into therapeutic targets.



1. HEMATOPOIETIC STEM CELL (HSC) DEVELOPMENT

The development of hematopoietic stem cells (HSCs) is a dynamic process, involving migration through several niches in distinct anatomical locations. The first long-term bona fide HSCs arise directly from specialized hemogenic endothelium in all species examined to date, in a process known as the endothelial-to-hematopoietic transition.¹ As this transition occurs, HSCs begin to express markers of hematopoietic fate, such as the transcriptional regulator *Runx1*.^{2,3} In mammals, the first HSCs arise from the aorta, umbilical artery, and vitelline artery in the midgestation embryo^{1,4,5} (around embryonic day 10 in mouse). These later migrate to the fetal liver, and finally the bone marrow, where HSCs are maintained throughout the lifespan of the animal.⁶ It is thought that at each of these progressive locations, the HSCs receive induction cues, such as FGF, Notch, and Wnt, which are important for their maturation and function.⁷ During this dynamic developmental process, these HSCs are subject to inputs from various surrounding niches and a variety of developmental signaling pathways, reviewed elsewhere.^{8–10} These early inputs give rise to later HSC function, and disruption of these cues fundamentally alter the identity and behavior of these cells.^{11,12} The final result of these developmental cues are long-term HSCs (LT-HSCs), housed in the bone marrow; these lie at the apex of the hematopoietic hierarchy, seeding the entire blood system for the duration of the organism's life.



2. HSC HOMEOSTASIS

LT-HSCs are defined by their ability to repopulate the hematopoietic system of an organism for a long duration.¹³ In the bone marrow, these are largely quiescent, but give rise to a subpopulation of short-term HSCs (ST-HSCs), which are limited in their capacity for self-renewal and are able to repopulate an organism only for a few weeks.¹⁴ HSCs in turn give rise to the lineage restricted common progenitors of the myeloid and lymphoid fates, which together can differentiate into all mature blood cells.^{15,16} (Fig. 1). Recent single-cell analyses have indicated that all of these progenitor types have low levels of coexpression of multiple lineages, suggesting overlapping cell fates at the transcriptional level, and that these fate decisions are rather plastic.¹⁷ Additionally, HSCs with long-term single lineage reconstitution fate have been identified, suggesting that the binary lineage tree of hematopoietic fate is more flexible than previously established.¹⁸ It is through careful regulation of inductive cues that HSCs are able to self-renew and differentiate into the full complement of blood. Disruption of these regulatory cues can accordingly result in cancers of the blood, and our understanding of these cues during normal hematopoiesis will be critical to determining how they are coopted during oncogenesis.

Our understanding of the native processes governing hematopoietic homeostasis has been hindered by technical difficulties, including the relative rarity of these cells in vivo, where it is estimated that HSCs form approximately 1/20,000 nucleated hematopoietic cells.¹⁴ As a result, it has been difficult to elucidate the molecular mechanisms governing their homeostasis, as purification of a large enough number of cells for analysis is challenging. It is apparent that HSCs receive both cell-intrinsic and -extrinsic cues to govern their maintenance reviewed in Ref. 19. In the bone marrow, HSCs reside in close proximity to endothelial cells, osteoblasts, and mesenchymal stem cells, which are proposed to supply the niche cues governing self-renewal and differentiation²⁰ (Fig. 1), though this is incompletely understood. Among others, the Wnt signaling cascade is known to play important roles in these processes.



3. WNT SIGNALING

Wnt genes encode lipid-modified, secreted signaling molecules, which regulate a diverse set of developmental and homeostatic programs,

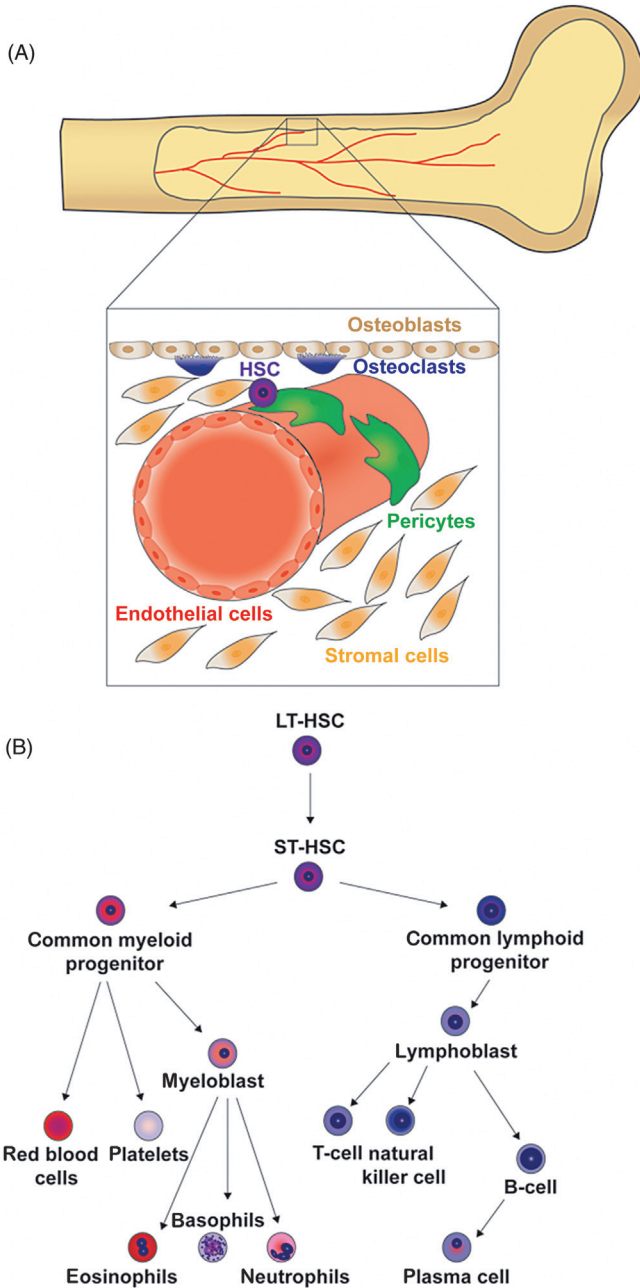


Fig. 1 Adults hematopoietic stem cells (HSCs) reside in the bone marrow. (A) The marrow of long bones is the niche for homeostasis of HSCs, where they reside in close proximity to many cell types. For example, the blood vessels, which carry

ranging from axial patterning to stem cell identity and self-renewal reviewed in Ref. 21.^{22–24} Their broad range of effects are in part due to the complex genetic nature of these factors with 19 independent Wnt genes in the mammalian genome and multiple genes encoding structurally and functionally distinct receptors, including Frizzled (Fzd), lipoprotein related protein (Lrp), receptor tyrosine kinase-like orphan receptor (Ror), and receptor-like tyrosine kinase (Ryk). Activation of the Wnt signaling cascade has been loosely grouped into either the canonical (β -catenin dependent) or noncanonical (β -catenin independent) pathways, though it is important to note that these pathways do not necessarily work independently or in opposition to each other. Furthermore, a given Wnt may activate distinct signaling cascades depending on expression of downstream signaling components.^{21,25–29}

In the absence of Wnt signals (Fig. 2), the DNA binding proteins of the lymphoid enhancing factor (LEF)/T-cell factor (TCF) family recruit corepressors, such as Groucho/transducing-like enhancer (GRG/TLE) and CtBP1, to silence expression of Wnt target genes. The transcriptional activator β -catenin is constitutively tagged for proteasomal degradation by the so-called destruction complex, consisting in part of GSK3 β , APC, and Axin. In the presence of a Wnt ligand, Fzd receptors and Lrp coreceptors oligomerize at the membrane, leading to dissociation/relocation of the destruction complex, thus releasing β -catenin from proteasomal targeting and allowing it to enter the nucleus where it displaces GRG/TLE from LEF/TCF and activates expression of Wnt target genes.³⁰ Activation of this arm of the Wnt signal often leads to maintenance or specification of cell fate (e.g., stem cell self-renewal or differentiation).²¹ Wnt signaling can be modulated with secreted negative regulators; such as secreted frizzled-related proteins (SFRP),³¹ which are hypothesized to sequester the Wnt ligand; Notum,^{32–34} which inactivates Wnt through deacylation; and Dickkopf (Dkk), which bind Lrp to prevent oligomerization with Fzd.³⁵

The β -catenin independent pathways remain poorly understood. In its downstream signal, there are a variety of effectors, including RhoA, JNK, and calcium influx pathways^{21,36–38} (Fig. 2). Different arms of this cascade

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- ◀ circulating HSCs and their progeny; the bone-building osteoblasts; the bone-degrading osteoclasts; the supportive pericytes, and other bone marrow stromal cells. (B) In the niche, the quiescent long-term (LT) HSCs give rise to short-term (ST) HSCs, which in turn can give rise to the common myeloid or lymphoid progenitors, which can differentiate into all cell types of the blood, and many cells from the immune system.

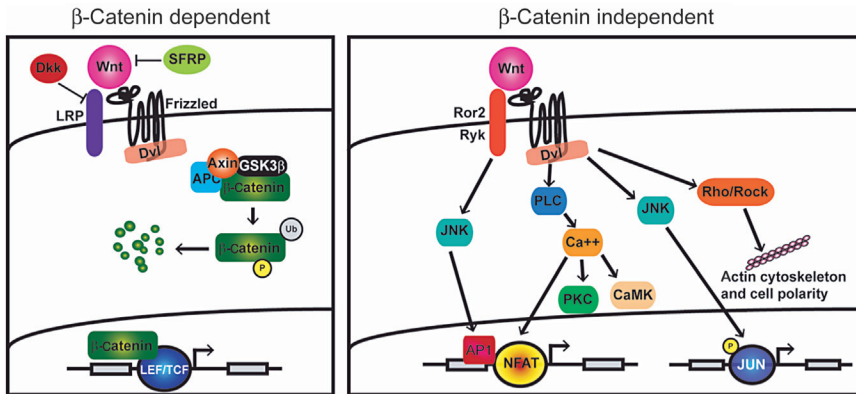


Fig. 2 Wnt signaling pathways. Schematic representations of the β -catenin dependent and independent Wnt signaling cascades. In the β -catenin dependent cascade, Wnt-Fzd-LRP interaction culminates in the nuclear translocation of β -catenin to bind lymphoid enhancing factor (LEF)/T-cell factor (TCF) transcription factors and drive gene expression. In the β -catenin independent pathway, there are several arms of signaling. For instance, Wnt-Fzd interaction leads to either Rho/Rock or Rac/JNK signaling, culminating in changes in the actin cytoskeleton or gene expression, respectively. Alternatively, WNT-Fzd-Ror2/Ryk interaction lead to changes in intracellular Ca^{++} levels, which affect protein kinase C (PKC), calmodullin-dependent protein kinase (CaMK) signaling cascades, or NFAT/AP1 driven gene expression changes. *Dkk*, Dickkopf; *Fzd*, Frizzled; *LRP*, lipoprotein related protein; *Ryk*; receptor-like tyrosine kinase; *SFRP*, secreted frizzled-related proteins.

can proceed with or without a Fzd receptor, and use different coreceptors, such as Ror and Ryk.^{27,39} The result of activation of these pathways is often polarization of the cell for division, migration or extension, or even the polarization of whole tissues (i.e., planar cell polarity).²¹ However, there is also evidence for β -catenin independent Wnt signaling in cell fate specification,⁴⁰ indicating that these seemingly distinct pathways have overlapping functions and are possibly tightly integrated.



4. WNT IN HSC DEVELOPMENT AND HOMEOSTASIS

The role of Wnt in HSC development and homeostasis has been intensely debated by the field, in part due to conflicting data in the literature.⁴¹ Furthermore, the genetic complexity of the ligands and receptors, as well as the dynamic nature of HSC development, including the multiple cell types involved in self-renewal and differentiation lends itself to a complex

series of inputs. In particular, harnessing the particular intricacies of timing and signal dosage may be the key to unlocking our understanding of Wnt in HSC ontogeny and maintenance.

Almost all *Wnts* and *Fzds* are expressed, at least to some degree, in the various hematopoietic sites during development,⁴² indicating a potential role for these during HSC ontogeny; however, the specific requirements, if any, for these are only beginning to be elucidated. For example, a role for Wnt16 in the specification of HSCs in zebrafish has been established,⁴⁰ whereas Wnt5a seems to regulate lymphopoiesis.^{43,44} In addition, a requirement for Wnt9a in the intraortic amplification of HSCs was recently shown.⁴⁵ The specific requirements for Fzd receptors is less clear, potentially due to functional redundancy, or our incomplete understanding of Wnt-Fzd specificity requirements. It is known that Fzd9 is required for lymphoid development and maturation, for example, but its potential role in the HSC itself is unclear.⁴⁶

The expression of Wnt components in the adult bone marrow are only slightly more refined, with a handful of these being expressed in putative HSC niche cells: osteoblasts, vascular cells, and mesenchymal stem cells.^{47–51} Wnt function dependent on β -catenin in HSCs is driven through LEF/TCF DNA elements, as in other cells.⁵² Accordingly, conditional deletion of β -catenin in the hematopoietic population impairs the long-term self-renewal⁵³ and regenerative capacities of HSCs.⁵⁴ Amplification of the Wnt signal, through viral expression of a constitutively active β -catenin results in increased numbers of HSCs in vitro and conversely, inhibition of Wnt signaling through viral expression of *Axin2* or addition of a soluble form of a Fzd ligand binding domain results in loss of HSC reconstitution in vivo,^{52,55} potentially due to premature differentiation. Early work showed contradictory evidence for the role of Wnt/ β -catenin signaling in the HSC system: loss of Wnt function through Wnt3a deletion,⁵⁶ β -catenin mutation,⁵³ or overexpression of the antagonist Dkk1⁵⁷ depleted the HSC pool in vivo, whereas activation of Wnt signaling through stabilized forms of β -catenin or the Wnt target prostaglandin E2, resulted in increased HSC number.^{58,59} However, others observed a depletion of the progenitor pool upon β -catenin overexpression.^{60,61} These contradictions are likely explained by dosage-dependent effects of Wnt signaling on different populations of blood cells⁶²; using an allelic series of APC mutants, it was possible to show that a low level of Wnt signaling maintains a proliferative HSC phenotype, whereas a high level of Wnt activation resulted in total impairment of repopulation capacity and exhaustion of the stem cell pool.⁶²

There is also evidence for specific function of Wnt ligands in adult HSCs. For example, *Wnt3a*, *Wnt5a*, and *Wnt10b* are all expressed in the bone marrow niche; interestingly, expression of *Wnt10b* is upregulated in response to injury to drive proliferation of bone marrow hematopoietic cells.⁶³ Fzd receptors likely play specific roles in the adult niche. For example, Fzd8 regulates the long-term quiescence of HSCs by regulating the noncanonical downstream calcium pathway.⁶⁴ HSCs from mice deficient in the β -catenin independent receptor *Ryk* have reduced quiescence, decreased self-renewal, and increased apoptosis.⁶⁵ During HSC ageing, there is a shift from β -catenin dependent to independent Wnt signaling, which seems to underlie the loss of self-renewal and lineage skewing seen in aged HSCs.⁶⁶ Altogether, these studies indicate that a careful balance of Wnt signals is required to regulate HSC specification, amplification, homeostasis and ageing. These effects of Wnt on normal HSCs are often hijacked in cancer processes, including the establishment and progression of blood cancers (Table 1).



5. WNT SIGNALING IN LEUKEMIA

The balance of self-renewal and differentiation in HSCs is critical to survival of an organism. For a cancer to progress, there must be a sustained increase in proliferation and also a block in differentiation. As such, mutations affecting cell identity or proliferation, such as *FLT3*, *TP53*, *RUNX1*,

Table 1 Known Wnt and Fzd Knockdown and Knockout (KO) Phenotypes in HSCs.

Genes	Knockdown or Knockout Phenotypes	References
<i>Wnt1</i>	Decreased number of thymocytes; some functional redundancy with <i>Wnt4</i> (mouse KO)	67
<i>Wnt3a</i>	Homozygous mutants are lethal at E12.5; loss of HSCs and progenitors and impairment of long-term engraftment (mouse KO)	56
<i>Wnt4</i>	Decreased number of thymocytes; some functional redundancy with <i>Wnt1</i> (mouse KO)	67
<i>Wnt5a</i>	Increased B-cells (mouse KO, cell culture model)	43,44
<i>Wnt9a</i>	Decreased intraortic expansion of HSCs and progenitors (zebrafish knockdown and KO)	45
<i>Wnt16</i>	Loss of HSC specification (zebrafish knockdown)	40
<i>Fzd5</i>	Extraembryonic vascular development is abnormal (mouse KO)	68
<i>Fzd9</i>	Decreased B-cells (mouse KO)	46

HSC, Hematopoietic stem cell.

and *KMT2A* are commonly found in leukemias.⁶⁹ In addition to these mutations, chromosomal translocations, especially affecting chromatin modulators, such as *MLLT3-KMT2A*, *BCR-ABL*, and *RUNX1-MECOM* are associated with, and are sometimes sufficient to cause leukemia.^{69,70} Differences in genetic causes for leukemias also give rise to cancers with different mechanisms of action, prognostic outcomes, and treatment regimes, making it crucial for us to understand the molecular cues driving these events to derive effective treatments.

In different subsets of leukemias, *WNT* expression and signaling in the bone marrow microenvironment are perturbed, often in the absence of a direct mutation in *WNT* component genes. For example, in chronic lymphocytic leukemia (CLL) B-cells, the WNT signal and expression of *WNT3* and the transcription factor *LEF1* are amplified compared to normal B-cells.⁷¹ In addition, *WNT3*, *WNT4*, *WNT5B*, *WNT6*, *WNT7B*, *WNT9A*, *WNT10A*, *WNT14*, and *WNT16* are all highly expressed in CLL B-cells.^{72,73} Similarly, in E2A-Pbx1 acute lymphoblastic leukemia (ALL) cells, *WNT16* is robustly expressed, although it can be scarcely detected in normal pre-B cells; this expression is dependent on E2A-Pbx1, suggesting that Wnt activation occurs downstream of this translocation.⁷⁴ Expression of other leukemic translocation products also induces the expression of WNT components in hematopoietic cell lines.⁷⁵

There is potential for therapeutic targeting of WNT in leukemias. The β -catenin independent coreceptor ROR1 is expressed in CLL leukemic cells, but not nonleukemic leukocytes, allowing for targeting of these cells with an anti-ROR1 monoclonal antibody (mAb, also known as cirmtuzumab).⁷⁶ High levels of ROR1 are associated with an accelerated form of CLL.⁷⁷ Addition of Wnt5a enhances the proliferation and migration of CLL cells through RhoA/Rac1; this effect can be blocked with cirmtuzumab,⁷⁸ and cannot be achieved in non-ROR1 expressing leukemic cells.⁷⁷ Clinically, combining cirmtuzumab with a B-cell receptor blocking agent is significantly more effective at clearing leukemic cells *in vivo* than either drug in isolation.⁷⁹ Taken together, these studies indicate how WNT targeting can be used to kill leukemic cells.

On the other hand, not all WNT activation is detrimental. For example, mice hemizygous for *Wnt5a*, which regulates the calcium signaling cascade, have enhanced B-cell proliferation and develop spontaneous myeloid leukemia and B-cell lymphomas, possibly because *Wnt5a* antagonizes the β -catenin signal.^{44,80} In fact, *WNT5A* has tumor suppressing activity as loss of function mutations and/or loss of expression are found in a majority of

primary human leukemia cells,⁴⁴ suggesting that this relief of β -catenin signaling repression may be necessary for some leukemias to progress. In another instance, exogenous Wnt3a reduced the proliferative capacity of B-cells in vitro⁸¹; however, this may be due to the dosage-dependent effects of Wnt on HSCs.

Many upstream regulators of Wnt signaling, such as members of the *Dickkopf* (*Dkk*) or *SFRP* families have been reported to be transcriptionally silenced due to promoter hypermethylation in human samples,^{82–86} thereby presumably elevating endogenous Wnt signaling activity, which may promote proliferation and oncogenic transformation. In RUNX1-ETO mediated (acute myeloid leukemia) AML, decreased expression of *SFRP1* results from direct transcriptional repression from the *RUNX1-ETO* gene product, and leads to an increase in Wnt activation.⁸⁷ Although these are classically thought to be negative regulators of the Wnt signal, this is not always the case. This decrease in antagonist expression actually seems to have a positive effect on patient survival in *BCR-ABL* ALL,⁸⁸ suggesting that SFRPs may act to activate the WNT signal in leukemic cells. SFRPs have been suggested to act as carrier proteins for WNTs in other systems, acting to expand the range of the WNT signal, which may also play a role in leukemia.⁸⁹ Taken together, these studies exemplify that Wnt signaling is ectopically activated in many subsets of leukemia, leading to increased proliferative and self-renewal capacities of the leukemic stem cells.

Some leukemic stem cells are dependent on Wnt for self-renewal and/or survival. For example, β -catenin enhances the self-renewal and proliferation capacity of *BCR-ABL* chronic myeloid leukemia (CML) cells, which also show an increase in β -catenin signaling during their expansion; this effect is lost when the Wnt signal is dampened with AXIN overexpression.⁹⁰ This Wnt-mediated self-renewal is due to a splicing defect in *GSK3 β* , which impairs its ability to bind AXIN, and target β -catenin for degradation.^{90,91} Furthermore, mouse *BCR-ABL* CML cells refractory to standard treatment are dependent on β -catenin, indicating a potential role for Wnt in the evolution of these types of hematological malignancies.⁹² This dependence on β -catenin for cancer progression is not limited to CML; it is also seen in subsets of AML,⁹³ CLL,⁷¹ and ALL.^{94,95} In addition, Wnt is also known to synergize with other leukemic mutations in mouse models, immortalized cells, and patient samples,^{96–100} likely due to the increased WNT signal resulting from aberrant transgenes. Finally, the dependence of leukemic stem cells on Wnt is not limited to the β -catenin pathway, as *BCR-ABL* CML cells also rely on the Wnt-mediated calcium pathway for survival¹⁰¹; inhibition of

numerous components of Wnt/Ca²⁺/NFAT sensitizes cells to BCR-ABL inhibition. Altogether, these studies point to WNT as a potential therapeutic target; however, consideration of the effective dosage of the WNT signal and the particular cascade affected must be carefully considered.



6. WNT SIGNALING IN LYMPHOMA

Lymphomas are blood cancers that arise in the lymph nodes, as opposed to leukemias that mainly arise in the bone marrow. There are many diseases that fall under the umbrella term of lymphoma, including Hodgkin's Lymphoma, Mantle Cell Lymphoma (MCL), and Burkitt's lymphoma, to name a few. As these cancers arise in a plethora of anatomical locations, it has been difficult to discern the molecular cues regulating their initiation and progression. It is known that lymphomas differ in the genetic causes and also their activation of, and reliance on, WNT signaling. For example, patient samples of many different types of lymphoma have differentially increased levels of nuclear (active) β -catenin, but the majority of these do not contain mutations in APC or β -catenin,^{102–105} suggesting that activation of the pathway is induced by autocrine or paracrine cues. β -Catenin independent signaling has also been proposed to play a role in anaplastic large cell lymphoma, although the extent to which this applies to other lymphomas is unclear.¹⁰⁶

Many WNT components are found to be dysregulated in various types of lymphoma. For example, MCL is associated with a chromosomal translocation, which leads to an increase in expression of the context-dependent Wnt target gene *cyclinD1*, which is not sufficient to drive lymphoma on its own.^{107,108} A microarray study compared gene expression in MCL cells and naïve B-cells (their wild-type counterparts), and found that several WNT pathway components, including *TCF7*, *FZD7*, *LRP5*, *AXIN1*, *APC*, *DVL3* were upregulated in MCL cells, indicating a potential requirement for WNT signaling in the disease progression.¹⁰⁹ Furthermore, MCL cells frequently show inactivation of GSK3 β , which leads to an increase in nuclear β -catenin.¹¹⁰ These studies fall in line with the general paradigm that an increase in the canonical arm of the Wnt signal leads to an increase in tumorigenicity.

Other lymphomas also have dysregulated Wnt; for example, there is evidence for overexpression of the transcription factors TCF1 and LEF1 in subset of T-cell lymphomas and small B-cell lymphomas.^{111,112} This

upregulation also occurs in lymphomas that are chemoresistant,¹¹³ suggesting that WNT could be involved in the evolution of these cancer stem cells (the slow-dividing cells that initiate tumors) as well. It is worthy of note that TCF1 has also been implicated as a tumor suppressor since 50% of *Tcf1*^{-/-} mice spontaneously develop thymic tumors.¹¹⁴ The authors suggest that this may be due to TCF/LEF1 specific functions in this process, mainly TCF1 directly inhibiting *LEF1* transcription and leading to an overall increase in the Wnt signal. Like in leukemia, the absence of Wnt5a, which is thought to antagonize the β -catenin signal, can lead to cancer. Speaking to this, loss of heterozygosity in *Wnt5a*^{+/-} mice develop B-cell lymphomas that are clonal in origin.⁴⁴ These studies also highlight the importance of Wnt signaling as an initiating event in lymphoma, which has also been proposed based on expression studies in humans.¹¹⁵ However, the reliance of cancer stem cells on Wnt in lymphomas is less clear than that of leukemic stem cells. In a mouse model of lymphoma, which develops to 100% penetrance in all lymphoid organs, the disease is reliant on upregulation of the Wnt signal,¹¹⁶ demonstrating that at least some lymphoma cancer cells are reliant on Wnt for survival. Finally, different WNTs also differentially affect these cancer cells. For example, a recent study highlights that WNT5A is a motility factor in Hodgkin's lymphoma; addition of WNT5A increases the motility of Hodgkin's lymphoma cells in culture, however, neither WNT10A and WNT10B nor WNT16 are able to elicit the same response.¹¹⁷ Developing a clear understanding of how different Wnts affect downstream pathways in these diseases will be crucial toward developing therapeutic interventions for patients.



7. WNT SIGNALING IN MYELOMA

Myeloma encompasses cancers of the plasma cells, of which multiple myeloma (MM) is the most common and is characterized by an accumulation of plasma cells in the bone marrow. WNT activation in the absence of β -catenin or APC mutation has been observed in multiple myelomas cells, which were dependent on this signal for continued growth.¹¹⁸ In fact, treatment of these with the compound PKF115-584 (which targets the β -catenin/TCF complex) results in cytotoxicity of primary patient MM cells and established cell lines, without effect on normal plasma cells.¹¹⁹ *Wnt2b*, *Wnt5a*, *Wnt7a*, *Wnt10b*, *Wnt11*, and *Wnt16* are all expressed in MM cell lines¹²⁰; however, it is worthy of noting that cell-autonomous Wnts are

not necessarily involved in MM and the effects may not be limited to these particular Wnts. There is some evidence for sensitizing MM cells to autocrine and paracrine Wnt signals from the bone marrow niche.¹²¹ In addition to the β -catenin dependent signal, Wnt3a is able to induce the RhoA signal in MM cells, including changes in cytoskeleton and morphology, indicative of an effect on planar cell polarity.¹²² These alterations lead to an increase in the migration and invasion ability of these cells, and similar effects can be achieved using Wnt1 or Wnt4; these effects are dependent on the RhoA arm of Wnt signaling.¹²⁰ These data support roles for both β -catenin dependent and independent WNT signaling in MM cells.

MM leads to the osteolytic bone disease, resulting from the destruction of bone (osteoclast induction) and repression of its repair (osteoblast suppression). These in turn lead to further bone marrow niche dysfunction and these effects are also in part due to loss of Wnt regulation. For example, the WNT inhibitors SFRP2 and DKK1 are secreted by several MM cell lines, as well as primary patient cells.¹²³ SFRP2 inhibits bone mineralization in vitro,¹²⁴ and both *Dkk1* and *SFRP2* expression are associated with advanced destructive bone lesions in patient samples.^{123,124} In addition, soluble Dkk1 can inhibit the differentiation of osteoblasts in vitro, suggesting that inhibition of Wnt through these soluble factors can inhibit bone formation and repair.^{123,125} Severe combined immunodeficient (SCID) mice implanted with MM tumor cells spontaneously develop a loss in bone mineral density, which can be overcome with anti-Dkk1 antibody treatment; this is due to increased levels of osteoblasts and decreased osteoclasts, leading to an overall induction in bone density.^{126–128} In addition to its role in MM initiation, MM tumor progression during chemoresistance involves the adhesion of MM cells to the bone marrow stroma, where expression of Wnt3 correlates with increased adhesion, likely progressing through the RhoA signaling cascade.¹²⁹ Taken altogether, these data point to the importance in Wnt regulating the interplay between MM and bone homeostasis, which when disrupted leads to osteolytic bone disease.



8. CONCLUSIONS AND PERSPECTIVES

The link between aberrant WNT signaling and many cancers is well established. However, in the case of blood malignancies, this requirement has been less clear, possibly due to the dosage-dependent effects of WNT on the stem cell niche and the dynamic nature of blood development and

homeostasis. These studies together show that WNT plays a role in leukemias and lymphomas. Of note, these observations indicate a potential role for planar cell polarity in the pathogenesis of MM. Planar cell polarity has largely been studied in the context of whole tissue polarity, in contrast to the single cell nature of the blood; however, the involvement of the RhoA arm of Wnt signaling in MM suggests that this may be a mechanism exploited by tumorigenic single cells as well. One study has also pointed to a role for planar cell polarity in CLL.¹³⁰ Further studies will reveal the involvement of Wnt/planar cell polarity in tumorigenesis of hematopoietic cells. In addition to the roles described earlier, aberrant WNT seems to be a common mechanism for drug resistance in leukemia and lymphoma,^{131–133} suggesting that these tumors may be “WNT addicted.” In one example, pretreatment of purified patient ALL blasts to suppress the WNT signal was sufficient to restore chemosensitivity in relapsed patient cell lines,¹³⁴ an indication of one potential therapeutic avenue targeting WNT. A thorough understanding of how WNT regulates normal and malignant hematopoiesis will be key to future therapeutic interventions.

REFERENCES

1. Kissa K, Herbomel P. Blood stem cells emerge from aortic endothelium by a novel type of cell transition. *Nature*. 2010;464:112–115.
2. Burns CE, Traver D, Mayhall E, Shepard JL, Zon LI. Hematopoietic stem cell fate is established by the Notch-Runx pathway. *Genes Dev*. 2005;19(19):2331–2342.
3. North TE, Stacy T, Matheny CJ, Speck NA, de Bruijn MF. Runx1 is expressed in adult mouse hematopoietic stem cells and differentiating myeloid and lymphoid cells, but not in maturing erythroid cells. *Stem Cells*. 2004;22:158.
4. Boisset JC, van Cappellen W, Andrieu-Soler C, Galjart N, Dzierzak E, Robin C. In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium. *Nature*. 2010;464(7285):116–120.
5. Bertrand JY, Chi NC, Santoso B, Teng S, Stainier DY, Traver D. Haematopoietic stem cells derive directly from aortic endothelium during development. *Nature*. 2010;464(7285):108–111.
6. Murayama E, Kissa K, Zapata A, et al. Tracing hematopoietic precursor migration to successive hematopoietic organs during zebrafish development. *Immunity*. 2006;25:963–975.
7. Ciriza J, Thompson H, Petrosian R, Manilay JO, Garcia-Ojeda ME. The migration of hematopoietic progenitors from the fetal liver to the fetal bone marrow: lessons learned and possible clinical applications. *Exp Hematol*. 2013;41(5):411–423.
8. Kim AD, Stachura DL, Traver D. Cell signaling pathways involved in hematopoietic stem cell specification. *Exp Cell Res*. 2014;329(2):227–233.
9. Robertson AL, Avagyan S, Gansner JM, Zon LI. Understanding the regulation of vertebrate hematopoiesis and blood disorders—big lessons from a small fish. *FEBS Lett*. 2016;590(22):4016–4033.
10. Kauts ML, Vink CS, Dzierzak E. Hematopoietic (stem) cell development: how divergent are the roads taken? *FEBS Lett*. 2016;590(22):3975–3986.

11. Butko E, Pouget C, Traver D. Complex regulation of HSC emergence by the Notch signaling pathway. *Dev Biol.* 2016;409(1):129–138.
12. Lee Y, Decker M, Lee H, Ding L. Extrinsic regulation of hematopoietic stem cells in development, homeostasis and diseases. *Wiley Interdiscip Rev Dev Biol.* 2017.
13. Spangrude GJ, Heimfield DS, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. *Science.* 1988;241:58.
14. Morrison SJ, Weissman IL. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity.* 1994;1(8):661–673.
15. Akashi K, Traver D, Miyamoto T, Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature.* 2000;404(6774):193–197.
16. Kondo M, Weissman IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell.* 1997;91(5):661–672.
17. Olsson A, Venkatasubramanian M, Chaudhri VK, et al. Single-cell analysis of mixed-lineage states leading to a binary cell fate choice. *Nature.* 2016;537(7622):698–702.
18. Notta F, Zandi S, Takayama N, et al. Distinct routes of lineage development reshape the human blood hierarchy across ontogeny. *Science.* 2016;351(6269):aab2116.
19. Mendelson A, Frenette PS. Hematopoietic stem cell niche maintenance during homeostasis and regeneration. *Nat Med.* 2014;20(8):833–846.
20. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. *Nature.* 2014;505(7483):327–334.
21. Loh KM, van Amerongen R, Nusse R. Generating cellular diversity and spatial form: Wnt signaling and the evolution of multicellular animals. *Dev Cell.* 2016;38(6):643–655.
22. Niehrs C. The complex world of WNT receptor signalling. *Nat Rev Mol Cell Biol.* 2012;13(12):767–779.
23. Acebron SP, Niehrs C. β -Catenin-independent roles of Wnt/LRP6 signaling. *Trends Cell Biol.* 2016;26(12):956–967.
24. Yang Y, Mlodzik M. Wnt-Frizzled/planar cell polarity signaling: cellular orientation by facing the wind (Wnt). *Annu Rev Cell Dev Biol.* 2015;31:623–646.
25. Rocheleau CE, Downs WD, Lin R, et al. Wnt signaling and an APC-related gene specify endoderm in early *C. elegans* embryos. *Cell.* 1997;90(4):707–716.
26. Habib SJ, Chen BC, Tsai FC, et al. A localized Wnt signal orients asymmetric stem cell division in vitro. *Science.* 2013;339(6126):1445–1448.
27. Mikels AJ, Nusse R. Purified Wnt5a protein activates or inhibits beta-catenin-TCF signaling depending on receptor context. *PLoS Biol.* 2006;4(4):e115.
28. He X, Saint-Jeannet J-P, Wang Y, Nathans J, Dawid I, Varmus H. A member of the Frizzled protein family mediating axis induction by Wnt-5A. *Science.* 1997;275:1652–1654.
29. Tao Q, Yokota C, Puck H, et al. Maternal wnt11 activates the canonical wnt signaling pathway required for axis formation in *Xenopus* embryos. *Cell.* 2005;120(6):857–871.
30. Li VS, Ng SS, Boerema PJ, et al. Wnt signaling through inhibition of beta-catenin degradation in an intact axin1 complex. *Cell.* 2012;149(6):1245–1256.
31. Rattner A, Hsieh JC, Smallwood PM, et al. A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. *Proc Natl Acad Sci USA.* 1997;94(7):2859–2863.
32. Kakugawa S, Langton PF, Zebisch M, et al. Notum deacylates Wnt proteins to suppress signalling activity. *Nature.* 2015;519(7542):187–192.
33. Zhang X, Cheong SM, Amado NG, et al. Notum is required for neural and head induction via Wnt deacylation, oxidation, and inactivation. *Dev Cell.* 2015;32(6):719–730.
34. Giraldez AJ, Copley RR, Cohen SM. HSPG modification by the secreted enzyme Notum shapes the wingless morphogen gradient. *Dev Cell.* 2002;2(5):667–676.

35. Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature*. 1998;391(6665):357–362.
36. Boutros M, Paricio N, Strutt DI, Mlodzik M. Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and wingless signaling. *Cell*. 1998;94(1):109–118.
37. Angers S, Moon RT. Proximal events in Wnt signal transduction. *Nat Rev Mol Cell Biol*. 2009;10(7):468–477.
38. Habas R, Kato Y, He X. Wnt/Frizzled activation of Rho regulates vertebrate gastrulation and requires a novel Formin homology protein Daam1. *Cell*. 2001;107(7):843–854.
39. Yoshikawa S, McKinnon RD, Kokel M, Thomas JB. Wnt-mediated axon guidance via the Drosophila Derailed receptor. *Nature*. 2003;422(6932):583–588.
40. Clements WK, Kim AD, Ong KG, Moore JC, Lawson ND, Traver D. A somitic Wnt16/Notch pathway specifies haematopoietic stem cells. *Nature*. 2011;474:220–224.
41. Staal FJ, Chhatta A, Mikkers H. Caught in a Wnt storm: complexities of Wnt signaling in hematopoiesis. *Exp Hematol*. 2016;44(6):451–457.
42. Corrigan PM, Dobbin E, Freeburn RW, Wheadon H. Patterns of Wnt/Fzd/LRP gene expression during embryonic hematopoiesis. *Stem Cells Dev*. 2009;18:759–772.
43. Malhotra S, Baba Y, Garrett KP, Staal FJ, Gerstein R, Kincade PW. Contrasting responses of lymphoid progenitors to canonical and noncanonical Wnt signals. *J Immunol*. 2008;181(6):3955–3964.
44. Liang H, Chen Q, Coles AH, et al. Wnt5a inhibits B cell proliferation and functions as a tumor suppressor in hematopoietic tissue. *Cancer Cell*. 2003;4(5):349–360.
45. Grainger S, Richter J, Palazon RE, et al. Wnt9a is required for the aortic amplification of nascent hematopoietic stem cells. *Cell Rep*. 2016;17(6):1595–1606.
46. Ranheim EA, Kwan HC, Reya T, Wang YK, Weissman IL, Francke U. Frizzled 9 knock-out mice have abnormal B-cell development. *Blood*. 2005;105(6):2487–2494.
47. Spencer GJ, Utting JC, Etheridge SL, Arnett TR, Genever PG. Wnt signalling in osteoblasts regulates expression of the receptor activator of NF- κ B ligand and inhibits osteoclastogenesis in vitro. *J Cell Sci*. 2006;119(Pt 7):1283–1296.
48. Dufourcq P, Descamps B, Tojais NF, et al. Secreted frizzled-related protein-1 enhances mesenchymal stem cell function in angiogenesis and contributes to neovessel maturation. *Stem Cells*. 2008;26(11):2991–3001.
49. Li H, Daculsi R, Grellier M, Bareille R, Bourget C, Amedee J. Role of neural-cadherin in early osteoblastic differentiation of human bone marrow stromal cells cocultured with human umbilical vein endothelial cells. *Am J Physiol*. 2010;299(2):C422–C430.
50. Qiu W, Chen L, Kassem M. Activation of non-canonical Wnt/JNK pathway by Wnt3a is associated with differentiation fate determination of human bone marrow stromal (mesenchymal) stem cells. *Biochem Biophys Res Commun*. 2011;413(1):98–104.
51. Planutiene M, Planutis K, Holcombe RE. Lymphoid enhancer-binding factor 1, a representative of vertebrate-specific Lef1/Tcf1 sub-family, is a Wnt-beta-catenin pathway target gene in human endothelial cells which regulates matrix metalloproteinase-2 expression and promotes endothelial cell invasion. *Vasc Cell*. 2011;3:28.
52. Reya T, Duncan AW, Ailles L, et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature*. 2003;423:409–414.
53. Zhao C, Blum J, Chen A, et al. Loss of β -catenin impairs the renewal of normal and CML stem cells in vivo. *Cancer Cell*. 2007;12:528–541.
54. Lento W, Ito T, Zhao C, et al. Loss of beta-catenin triggers oxidative stress and impairs hematopoietic regeneration. *Genes Dev*. 2014;28(9):995–1004.
55. Ruiz-Herguido C, Guiu J, D'Altri T, et al. Hematopoietic stem cell development requires transient Wnt/ β -catenin activity. *J Exp Med*. 2012;209:1457–1468.

56. Luis TC, Weerkamp F, Naber BAE, et al. Wnt3a deficiency irreversibly impairs hematopoietic stem cell self-renewal and leads to defects in progenitor cell differentiation. *Blood*. 2009;113:546–554.
57. Fleming HE, Janzen V, Lo Celso C, et al. Wnt signaling in the niche enforces hematopoietic stem cell quiescence and is necessary to preserve self-renewal in vivo. *Cell Stem Cell*. 2008;2:274–283.
58. North TE, Goessling W, Walkley CR, et al. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature*. 2007;447:1007–1011.
59. Goessling W, North TE, Loewer S, et al. Genetic interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration. *Cell*. 2009;136:1136–1147.
60. Scheller M, Huelsken J, Rosenbauer F, et al. Hematopoietic stem cell and multilineage defects generated by constitutive β -catenin activation. *Nat Immunol*. 2006;7:1037–1047.
61. Kirstetter P, Anderson K, Porse BT, Jacobsen SE, Nerlov C. Activation of the canonical Wnt pathway leads to loss of hematopoietic stem cell repopulation and multilineage differentiation block. *Nat Immunol*. 2006;7(10):1048–1056.
62. Luis TC, Naber BAE, Roozen PPC, et al. Canonical wnt signaling regulates hematopoiesis in a dosage-dependent fashion. *Cell Stem Cell*. 2011;9:345–356.
63. Congdon KL, Voermans C, Ferguson EC, et al. Activation of Wnt signaling in hematopoietic regeneration. *Stem Cells*. 2008;26:1202–1210.
64. Sugimura R, He Xi C, Venkatraman A, et al. Noncanonical Wnt signaling maintains hematopoietic stem cells in the niche. *Cell*. 2012;150:351–365.
65. Famili F, Perez LG, Naber BA, Noordermeer JN, Fradkin LG, Staal FJ. The non-canonical Wnt receptor Ryk regulates hematopoietic stem cell repopulation in part by controlling proliferation and apoptosis. *Cell Death Dis*. 2016;7(11):e2479.
66. Florian MC, Nattamai KJ, Dorr K, et al. A canonical to non-canonical Wnt signalling switch in haematopoietic stem-cell ageing. *Nature*. 2013;503(7476):392–396.
67. Mulroy T, McMahan JA, Burakoff SJ, McMahan AP, Sen J. Wnt-1 and Wnt-4 regulate thymic cellularity. *Eur J Immunol*. 2002;32(4):967–971.
68. Ishikawa T, Tamai Y, Zorn AM, et al. Mouse Wnt receptor gene Fzd5 is essential for yolk sac and placental angiogenesis. *Development*. 2001;128(1):25–33.
69. Dohner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. *N Engl J Med*. 2015;373(12):1136–1152.
70. Shtivelman E, Lifshitz B, Gale RP, Canaani E. Fused transcript of abl and bcr genes in chronic myelogenous leukaemia. *Nature*. 1985;315(6020):550–554.
71. Gutierrez Jr A, Tschumper RC, Wu X, et al. LEF-1 is a prosurvival factor in chronic lymphocytic leukemia and is expressed in the preleukemic state of monoclonal B-cell lymphocytosis. *Blood*. 2010;116(16):2975–2983.
72. Lu D, Zhao Y, Tawatao R, et al. Activation of the Wnt signaling pathway in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA*. 2004;101(9):3118–3123.
73. Memarian A, Hojjat-Farsangi M, Asgarian-Omran H, et al. Variation in WNT genes expression in different subtypes of chronic lymphocytic leukemia. *Leuk Lymphoma*. 2009;50(12):2061–2070.
74. McWhirter JR, Neuteboom ST, Wancewicz EV, Monia BP, Downing JR, Murre C. Oncogenic homeodomain transcription factor E2A-Pbx1 activates a novel WNT gene in pre-B acute lymphoblastoid leukemia. *Proc Natl Acad Sci USA*. 1999;96(20):11464–11469.
75. Muller-Tidow C, Steffen B, Cauvet T, et al. Translocation products in acute myeloid leukemia activate the Wnt signaling pathway in hematopoietic cells. *Mol Cell Biol*. 2004;24(7):2890–2904.
76. Fukuda T, Chen L, Endo T, et al. Antisera induced by infusions of autologous Ad-CD154-leukemia B cells identify ROR1 as an oncofetal antigen and receptor for Wnt5a. *Proc Natl Acad Sci USA*. 2008;105(8):3047–3052.

77. Cui B, Ghia EM, Chen L, et al. High-level ROR1 associates with accelerated disease progression in chronic lymphocytic leukemia. *Blood*. 2016;128(25):2931–2940.
78. Yu J, Chen L, Cui B, et al. Wnt5a induces ROR1/ROR2 heterooligomerization to enhance leukemia chemotaxis and proliferation. *J Clin Invest*. 2016;126(2):585–598.
79. Yu J, Chen L, Cui B, et al. Cirmtuzumab inhibits Wnt5a-induced Rac1 activation in chronic lymphocytic leukemia treated with ibrutinib. *Leukemia*. 2017;31(6):1333–1339.
80. Ying J, Li H, Chen YW, Srivastava G, Gao Z, Tao Q. WNT5A is epigenetically silenced in hematologic malignancies and inhibits leukemia cell growth as a tumor suppressor. *Blood*. 2007;110(12):4130–4132.
81. Nygren MK, Dosen G, Hystad ME, Stubberud H, Funderud S, Rian E. Wnt3A activates canonical Wnt signalling in acute lymphoblastic leukaemia (ALL) cells and inhibits the proliferation of B-ALL cell lines. *Br J Haematol*. 2007;136(3):400–413.
82. Liu TH, Raval A, Chen SS, Matkovic JJ, Byrd JC, Plass C. CpG island methylation and expression of the secreted frizzled-related protein gene family in chronic lymphocytic leukemia. *Cancer Res*. 2006;66(2):653–658.
83. Roman-Gomez J, Jimenez-Velasco A, Agirre X, et al. Transcriptional silencing of the Dickkopf-3 (Dkk-3) gene by CpG hypermethylation in acute lymphoblastic leukaemia. *Br J Cancer*. 2004;91(4):707–713.
84. Roman-Gomez J, Jimenez-Velasco A, Cordeu L, et al. WNT5A, a putative tumour suppressor of lymphoid malignancies, is inactivated by aberrant methylation in acute lymphoblastic leukaemia. *Eur J Cancer*. 2007;43(18):2736–2746.
85. Pehlivan M, Sercan Z, Sercan HO. sFRP1 promoter methylation is associated with persistent Philadelphia chromosome in chronic myeloid leukemia. *Leuk Res*. 2009;33(8):1062–1067.
86. Reins J, Mossner M, Neumann M, et al. Transcriptional down-regulation of the Wnt antagonist SFRP1 in haematopoietic cells of patients with different risk types of MDS. *Leuk Res*. 2010;34(12):1610–1616.
87. Cheng CK, Li L, Cheng SH, et al. Secreted-frizzled related protein 1 is a transcriptional repression target of the t(8;21) fusion protein in acute myeloid leukemia. *Blood*. 2011;118(25):6638–6648.
88. Martin V, Agirre X, Jimenez-Velasco A, et al. Methylation status of Wnt signaling pathway genes affects the clinical outcome of Philadelphia-positive acute lymphoblastic leukemia. *Cancer Sci*. 2008;99(9):1865–1868.
89. Mii Y, Taira M. Secreted Frizzled-related proteins enhance the diffusion of Wnt ligands and expand their signalling range. *Development*. 2009;136(24):4083–4088.
90. Jamieson CH, Ailles LE, Dylla SJ, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N Engl J Med*. 2004;351(7):657–667.
91. Abrahamsson AE, Geron I, Gotlib J, et al. Glycogen synthase kinase 3beta missplicing contributes to leukemia stem cell generation. *Proc Natl Acad Sci USA*. 2009;106(10):3925–3929.
92. Hu Y, Chen Y, Douglas L, Li S. β -Catenin is essential for survival of leukemic stem cells insensitive to kinase inhibition in mice with BCR-ABL-induced chronic myeloid leukemia. *Leukemia*. 2009;23(1):109–116.
93. Siapati EK, Papadaki M, Kozaou Z, et al. Proliferation and bone marrow engraftment of AML blasts is dependent on beta-catenin signalling. *Br J Haematol*. 2011;152(2):164–174.
94. Mazieres J, You L, He B, et al. Inhibition of Wnt16 in human acute lymphoblastoid leukemia cells containing the t(1;19) translocation induces apoptosis. *Oncogene*. 2005;24(34):5396–5400.

95. Khan NI, Bradstock KF, Bendall LJ. Activation of Wnt/beta-catenin pathway mediates growth and survival in B-cell progenitor acute lymphoblastic leukaemia. *Br J Haematol*. 2007;138(3):338–348.
96. Tickenbrock L, Schwable J, Wiedehage M, et al. Flt3 tandem duplication mutations cooperate with Wnt signaling in leukemic signal transduction. *Blood*. 2005;105(9):3699–3706.
97. Tickenbrock L, Hehn S, Sargin B, et al. Activation of Wnt signaling in cKit-ITD mediated transformation and imatinib sensitivity in acute myeloid leukemia. *Int J Hematol*. 2008;88(2):174–180.
98. Kaveri D, Kastner P, Dembele D, Nerlov C, Chan S, Kirstetter P. β -Catenin activation synergizes with Pten loss and Myc overexpression in Notch-independent T-ALL. *Blood*. 2013;122(5):694–704.
99. Sadras T, Perugini M, Kok CH, et al. Interleukin-3-mediated regulation of beta-catenin in myeloid transformation and acute myeloid leukemia. *J Leukoc Biol*. 2014;96(1):83–91.
100. Ugarte GD, Vargas MF, Medina MA, et al. Wnt signaling induces transcription, spatial proximity, and translocation of fusion gene partners in human hematopoietic cells. *Blood*. 2015;126(15):1785–1789.
101. Gregory MA, Phang TL, Neviani P, et al. Wnt/Ca2+/NFAT signaling maintains survival of Ph+ leukemia cells upon inhibition of Bcr-Abl. *Cancer Cell*. 2010;18(1):74–87.
102. Zhang D, O'Neil MF, Cunningham MT, Fan F, Olyae M, Li L. Abnormal Wnt signaling and stem cell activation in reactive lymphoid tissue and low-grade marginal zone lymphoma. *Leuk Lymphoma*. 2010;51(5):906–910.
103. Groen RW, Oud ME, Schilder-Tol EJ, et al. Illegitimate WNT pathway activation by beta-catenin mutation or autocrine stimulation in T-cell malignancies. *Cancer Res*. 2008;68(17):6969–6977.
104. Gelebart P, Anand M, Armanious H, et al. Constitutive activation of the Wnt canonical pathway in mantle cell lymphoma. *Blood*. 2008;112(13):5171–5179.
105. Ge X, Lv X, Feng L, Liu X, Wang X. High expression and nuclear localization of beta-catenin in diffuse large B-cell lymphoma. *Mol Med Rep*. 2012;5(6):1433–1437.
106. Hegazy SA, Alshareef A, Gelebart P, et al. Disheveled proteins promote cell growth and tumorigenicity in ALK-positive anaplastic large cell lymphoma. *Cell Signal*. 2013;25(1):295–307.
107. Bosch F, Jares P, Campo E, et al. PRAD-1/cyclin D1 gene overexpression in chronic lymphoproliferative disorders: a highly specific marker of mantle cell lymphoma. *Blood*. 1994;84(8):2726–2732.
108. Bodrug SE, Warner BJ, Bath ML, Lindeman GJ, Harris AW, Adams JM. Cyclin D1 transgene impedes lymphocyte maturation and collaborates in lymphomagenesis with the myc gene. *EMBO J*. 1994;13(9):2124–2130.
109. Rizzatti EG, Falcao RP, Panepucci RA, et al. Gene expression profiling of mantle cell lymphoma cells reveals aberrant expression of genes from the PI3K-AKT, WNT and TGFbeta signalling pathways. *Br J Haematol*. 2005;130(4):516–526.
110. Chung R, Peters AC, Armanious H, Anand M, Gelebart P, Lai R. Biological and clinical significance of GSK-3beta in mantle cell lymphoma—an immunohistochemical study. *Int J Clin Exp Pathol*. 2010;3(3):244–253.
111. Dorfman DM, Greisman HA, Shahsafaei A. Loss of expression of the WNT/beta-catenin-signaling pathway transcription factors lymphoid enhancer factor-1 (LEF-1) and T cell factor-1 (TCF-1) in a subset of peripheral T cell lymphomas. *Am J Pathol*. 2003;162(5):1539–1544.
112. Tandon B, Peterson L, Gao J, et al. Nuclear overexpression of lymphoid-enhancer-binding factor 1 identifies chronic lymphocytic leukemia/small lymphocytic lymphoma in small B-cell lymphomas. *Mod Pathol*. 2011;24(11):1433–1443.

113. Koivula S, Valo E, Raunio A, Hautaniemi S, Leppa S. Rituximab regulates signaling pathways and alters gene expression associated with cell death and survival in diffuse large B-cell lymphoma. *Oncol Rep.* 2011;25(4):1183–1190.
114. Tiemessen MM, Baert MR, Schonewille T, et al. The nuclear effector of Wnt-signaling, Tcf1, functions as a T-cell-specific tumor suppressor for development of lymphomas. *PLoS Biol.* 2012;10(11):e1001430.
115. Kimura Y, Arakawa F, Kiyasu J, et al. The Wnt signaling pathway and mitotic regulators in the initiation and evolution of mantle cell lymphoma: Gene expression analysis. *Int J Oncol.* 2013;43(2):457–468.
116. Wang Y, Liu Y, Wu C, et al. Epm2a suppresses tumor growth in an immunocompromised host by inhibiting Wnt signaling. *Cancer Cell.* 2006;10(3):179–190.
117. Linke F, Zaunig S, Nietert MM, et al. WNT5A: a motility-promoting factor in Hodgkin lymphoma. *Oncogene.* 2017;36(1):13–23.
118. Derksen PW, Tjin E, Meijer HP, et al. Illegitimate WNT signaling promotes proliferation of multiple myeloma cells. *Proc Natl Acad Sci USA.* 2004;101(16):6122–6127.
119. Sukhdeo K, Mani M, Zhang Y, et al. Targeting the beta-catenin/TCF transcriptional complex in the treatment of multiple myeloma. *Proc Natl Acad Sci USA.* 2007;104(18):7516–7521.
120. Qiang YW, Walsh K, Yao L, et al. Wnts induce migration and invasion of myeloma plasma cells. *Blood.* 2005;106(5):1786–1793.
121. van Andel H, Ren Z, Koopmans I, et al. Aberrantly expressed LGR4 empowers Wnt signaling in multiple myeloma by hijacking osteoblast-derived R-spondins. *Proc Natl Acad Sci USA.* 2017;114(2):376–381.
122. Qiang YW, Endo Y, Rubin JS, Rudikoff S. Wnt signaling in B-cell neoplasia. *Oncogene.* 2003;22(10):1536–1545.
123. Tian E, Zhan F, Walker R, et al. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. *N Engl J Med.* 2003;349(26):2483–2494.
124. Oshima T, Abe M, Asano J, et al. Myeloma cells suppress bone formation by secreting a soluble Wnt inhibitor, sFRP-2. *Blood.* 2005;106(9):3160–3165.
125. Gunn WG, Conley A, Deininger L, Olson SD, Prockop DJ, Gregory CA. A crosstalk between myeloma cells and marrow stromal cells stimulates production of DKK1 and interleukin-6: a potential role in the development of lytic bone disease and tumor progression in multiple myeloma. *Stem Cells.* 2006;24(4):986–991.
126. Yaccoby S, Ling W, Zhan F, Walker R, Barlogie B, Shaughnessy Jr JD. Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo. *Blood.* 2007;109(5):2106–2111.
127. Heath DJ, Chantry AD, Buckle CH, et al. Inhibiting Dickkopf-1 (Dkk1) removes suppression of bone formation and prevents the development of osteolytic bone disease in multiple myeloma. *J Bone Miner Res.* 2009;24(3):425–436.
128. Fulciniti M, Tassone P, Hideshima T, et al. Anti-DKK1 mAb (BHQ880) as a potential therapeutic agent for multiple myeloma. *Blood.* 2009;114(2):371–379.
129. Kobune M, Chiba H, Kato J, et al. Wnt3/RhoA/ROCK signaling pathway is involved in adhesion-mediated drug resistance of multiple myeloma in an autocrine mechanism. *Mol Cancer Ther.* 2007;6(6):1774–1784.
130. Kaucka M, Plevova K, Pavlova S, et al. The planar cell polarity pathway drives pathogenesis of chronic lymphocytic leukemia by the regulation of B-lymphocyte migration. *Cancer Res.* 2013;73(5):1491–1501.
131. Riether C, Schurch CM, Flury C, et al. Tyrosine kinase inhibitor-induced CD70 expression mediates drug resistance in leukemia stem cells by activating Wnt signaling. *Sci Transl Med.* 2015;7(298):298ra119.

132. Eiring AM, Khorashad JS, Anderson DJ, et al. β -Catenin is required for intrinsic but not extrinsic BCR-ABL1 kinase-independent resistance to tyrosine kinase inhibitors in chronic myeloid leukemia. *Leukemia*. 2015;29(12):2328–2337.
133. Dharmapuri G, Doneti R, Philip GH, Kalle AM. Celecoxib sensitizes imatinib-resistant K562 cells to imatinib by inhibiting MRP1-5, ABCA2 and ABCG2 transporters via Wnt and Ras signaling pathways. *Leukemia Res*. 2015;39(7):696–701.
134. Dandekar S, Romanos-Sirakis E, Pais F, et al. Wnt inhibition leads to improved chemosensitivity in paediatric acute lymphoblastic leukaemia. *Br J Haematol*. 2014;167(1):87–99.

FURTHER READING

Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016;66(1):7–30.