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Wnt regulation of hematopoietic stem cell development and disease

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Abstract

Hematopoietic stem cells (HSCs) are multipotent stem cells that give rise to all cells of the blood and most immune cells. Due to their capacity for unlimited self-renewal, long-term HSCs replenish the blood and immune cells of an organism throughout its life. HSC development, maintenance, and differentiation are all tightly regulated by cell signaling pathways, including the Wnt pathway. Wnt signaling is initiated extracellularly by secreted ligands which bind to cell surface receptors and give rise to several different downstream signaling cascades. These are classically categorized either β-catenin dependent (BCD) or β-catenin independent (BCI) signaling, depending on their reliance on the β-catenin transcriptional activator. HSC development, homeostasis, and differentiation is influenced by both BCD and BCI, with a high degree of sensitivity to the timing and dosage of Wnt signaling. Importantly, dysregulated Wnt signals can result in hematological malignancies such as leukemia, lymphoma, and myeloma. Here, we review how Wnt signaling impacts HSCs during development and in disease.

Keywords

Wnt; hematopoietic stem cells; leukemia; lymphoma; myeloma

Hematopoietic stem cells - the source of our blood and immune cell pool

Hematopoietic development, or the process of making blood, occurs in two waves, referred to as primitive and definitive (Galloway & Zon, 2003). During primitive hematopoiesis, blood and immune cells needed during early embryogenesis arise; these are essential for oxygenation and fighting infection in the developing conceptus. These blood cells are temporary and will not sustain the organism later in life. It is not until definitive hematopoiesis, during later development, that hematopoietic stem cells (HSCs) are born (see Figure 1). As blood cells turn over during the lifespan of an organism, these HSCs are capable of unlimited self-renewal and give rise to the blood system that will sustain the organism throughout its life. The remainder of this review will primarily focus on definitive hematopoiesis and Wnt regulation of this process, but we point our readers to reviews of primitive hematopoiesis (Bigas et al., 2013; Boyd & Bolon, 2022; Yamane, 2020).

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Long-term (LT−)HSCs, which are at the apex of the blood differentiation tree (Figure 1), have unlimited HSC repopulation potential, yet remain quiescent until they are needed. LT-HSCs give rise to short-term HSCs (ST-HSCs), which have a limited capacity for self-renewal, and these are thought to differentiate to either the common myeloid progenitor cells (CMP), or the common lymphoid progenitor (CLP). CMPs then go on to become red blood cells, platelets, or myeloblasts. A myeloblast can, in turn, become eosinophils, basophils, or neutrophils. On the other hand, CLP cells differentiate into a lymphoblasts, which become T-cells, natural killer cells, or B-cells. Finally, B-cells give rise to plasma cells. The mechanisms governing blood cell development and differentiation is actively evolving and is reviewed elsewhere (Dzierzak & Bigas, 2018; Laurenti & Gottgens, 2018). Unfortunately, when this process is dysregulated, diseases of the blood, such as leukemia, lymphoma, and anemia, arise.

Healthy HSCs collected from donors can be used to replace a patient's diseased HSC supply in a process called HSC transplant (HSCT). However, current HSCT suffers from two major limitations: First, donor-patient matching is complex, leading to insufficient suitable matches for all patients who could benefit from HSCT. Second, because most of the immune system is derived from HSCs, patients can be afflicted with long-term complications such as graft versus host disease, where the donor (graft) immune cells attack the recipient tissues. Therefore, deriving HSCs *in vitro* from pluripotent precursor cells would enable patient-specific matching, overcoming these difficulties and generating an unlimited supply of HSCs for transplant. Unfortunately, despite recent advances (Batta et al., 2014; Doulatov et al., 2013; Elcheva et al., 2014; Lis et al., 2017; Pereira et al., 2013; Pulecio et al., 2014; Sandler et al., 2014; Sugimura et al., 2017), producing therapeutic grade, long-term HSCs capable of unlimited self-renewal has challenged the field for close to 40 years, pointing to our incomplete understanding of how HSC development and homeostasis are regulated. We therefore require a better understanding of how HSCs respond to signaling cues such as the Wnt pathway.

In vivo models for hematopoietic stem cell development

Mice, zebrafish, fruit flies, and chicks, are some examples of useful animal models for studying HSC development. Each of these models has its own advantages and uses conserved genetic mechanisms for generating HSCs. This review will focus on mice, zebrafish, and fruit flies but readers are directed to an in-depth review on HSC development in chickens (Mahony & Bertrand, 2019). Studies in mice are advantageous because, like humans, they are mammals and share many attributes of their developmental biology. In addition, there are a wealth of genetic models that are already established in mice, and establishing novel genetic manipulations is standard at this point (Yoshimoto, 2018).

However, HSCs are specified only during development, complicating the study of HSCs in mice due to their *in utero* development. HSC development is a dynamic process, as described below, and it can be difficult to capture the correct timepoints when these cells are born within an embryo, within a pregnant dam. Conversely, zebrafish are externally fertilized, are transparent as they develop, and HSCs begin emerging roughly 26 hours post fertilization (hpf) (Bertrand et al., 2010; Kissa & Herbomel, 2010), a process that takes

about 10 days in mice. In addition, fluorescent transgenes paired with translucent larvae enables the direct visualization of cell populations over time. Furthermore, technological advances have now made genome editing and high-resolution imaging streamlined in zebrafish (Otterstrom et al., 2022). Fruit flies, on the other hand are advantageous because they have simple genetics, very conserved transcriptional regulators and signaling pathways to humans; they are also inexpensive and have a short developmental window (Bier, 2005). Despite the differences in these three models, all species develop HSCs through a similar trajectory of specification, emergence, and expansion. These processes occur in slightly different anatomical locations but display conserved molecular mechanisms.

HSCs are derived from the mesoderm, which is pushed toward endothelial cells that are destined to either line our blood vessels or become hemogenic endothelium. Hematopoietic specification is the process by which these developing endothelial cells receive cues that direct their identity toward hemogenic endothelium (Maximow, 1924; Murray, 1932). In mice and zebrafish, hemogenic endothelium is found in the major arteries; in fruit fly, the larva lymph gland acts as the hemogenic endothelium (Figure 2).

HSC emergence occurs when specified cells of the hemogenic endothelium undergo a process called the endothelial to hemogenic transition (EHT) (Bertrand et al., 2010; Boisset et al., 2010; Kissa & Herbomel, 2010; Mizuochi et al., 2012; Rafii et al., 2013). In this process, the cells of the hemogenic endothelium undergo a trans differentiation where the endothelial program is turned off, and a hematopoietic program is initiated. These cells bud out from the endothelium to enter circulation. In different species, this happens in different tissues, and at different developmental time points, but the cues and cellular processes are highly conserved. In mice, emergence occurs in the aorta-gonad-mesonephros (AGM) region around E10.5; in zebrafish, emergence takes place in the dorsal aorta around 26 hpf; and in fruit fly, emergence occurs throughout the pupa during the pupal stage (Figure 2).

After emergence, HSCs circulate to seed the site of secondary hematopoiesis, where they undergo expansion and maturation. Expansion occurs in mice, in the fetal liver around E12.5; in zebrafish, in the caudal hematopoietic tissue (CHT) around 48 hpf; and in fruit fly, in hematopoietic pockets widely distributed in the adult fly (Figure 2). Finally, following expansion, HSCs migrate to their final sites of adult hematopoiesis: in mouse, bone marrow; in zebrafish, kidney marrow; and in fruit fly, dorsal abdominal hemocyte clusters (Ghosh et al., 2015) (Figure 2). There are roles for Wnt signaling in all these hematopoietic sites, as detailed below. There are also many other complex signaling requirements for this process, described elsewhere (Drevon & Jaffredo, 2014).

The use of *in vitro* models has been another source for studying the development of HSCs and has been of particular interest in the field of regenerative medicine. Human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have been used to gain insights into how human HSCs develop. Though we cannot yet make a bona fide HSC suitable for therapeutic use, this model has been instrumental in our understanding of HSC ontogeny in humans and is reviewed elsewhere (Demirci et al., 2020; Hyslop et al., 2005).

Wnt Signaling

Wnt signaling is highly conserved and essential for development and homeostasis in all metazoans. Wnt proteins are lipid-modified secreted proteins that bind to cell surface receptors including those encoded by the *Frizzled (Fzd*) gene family to transduce intracellular signals (Albrecht et al., 2021; Rim et al., 2022). These downstream intracellular pathways act both on transcriptional regulation of target genes in the nucleus and on processes such as actin cytoskeletal formation in the cytoplasm and are generally referred to as β-catenin dependent (BCD) or β-catenin independent (BCI).

In the BCD cascade, Wnt binding to cell surface receptors results in dissociation of β-catenin from the destruction complex which includes adenomatous polyposis coli (APC), glycogen synthase kinase 3 (GSK3) and axin, among others (Rim et al., 2022). This allows β-catenin to accumulate in the cytoplasm, and translocate into the nucleus, where it binds directly to transcription factors of the LEF/TCF family to initiate transcription of target genes. Expression of these target genes have been associated with a variety of biological outcomes including the proliferation and maintenance of stem cell niches.

The BCI pathways have multiple downstream effectors including RhoA, JNK, and calcium influx pathways, which employ different cytoplasmic proteins including Vangl, Cesr and Prickle (Menck et al., 2021). These pathways are incompletely understood but are believed to cause an array of biological effects including changes in cell adhesion and in cell membrane polarization, and movement of tissues along a plane. These changes are primarily thought to occur through changes in protein levels and localization rather than transcriptional regulation (Adler, 2012; Schlessinger et al., 2009). Additionally, BCI receptors have been implicated in HSC repopulation (Famili, Perez, et al., 2016).

Given the broad implications and roles for the Wnt pathway during development and homeostasis, regulation of the pathway is critical. One of the major ways Wnt signaling is regulated *in vivo* is through modification of the Wnt ligand through addition of the monounsaturated lipid, palmitoleic acid (PA) (Takada et al., 2006). This occurs early during Wnt processing and is essential for its function. In addition, this acylation renders the Wnt protein highly hydrophobic and poorly soluble. As such, the secreted Wnt ligand is thought to have a short signaling range, making Wnt activity very spatially restricted. In addition to regulation of the Wnt ligand, Fzd-specific transmembrane ubiquitin E3 ligases, Rnf43 and Znrf3, inhibit Fzd expression on the cell surface by targeting it for ubiquitin-mediated endocytosis (Zebisch & Jones, 2015). Rnf43 and Znrf3 are both downstream targets of BCD Wnt signaling and, thus, act as intrinsic negative feedback within the pathway to limit Wnt signaling temporally (Hao et al., 2012; Koo et al., 2012). Loss of these types of control mechanisms can also lead to dysregulation of stem cell niches.

Wnt signaling in HSC development and homeostasis

Until relatively recently, data has been inconsistent surrounding the role of Wnt signaling in HSC development and maintenance (Staal, Chhatta, et al., 2016). But it is now clear that regulation of the spatial extent and timing of Wnt signaling impacts HSC development and

homeostasis in various ways at different stages of hematopoiesis (Table 1). For example, Wnt signaling acts as a rheostat, with varying levels controlling HSC destiny: low Wnt signaling maintains HSC proliferation, higher levels enhance HSC function, intermediate signal promotes myeloid differentiation, even more signaling enhances T-cell differentiation, while high Wnt signaling impairs hematopoiesis and repopulation capacity (Luis et al., 2011).

Studies of distinct components of the BCD Wnt signaling cascade have ultimately converged on similar themes for Wnt function in HSC development and maintenance. In one example, mutating GSK3β constitutively activates β-catenin and increases HSC numbers which leads to eventual HSC apoptosis (Kirstetter et al., 2006; Scheller et al., 2006). Likewise, APC mutation can increase the number of HSCs (Li et al., 2013). Interestingly, APC mutations that produce varying levels of BCD signaling activation have differential effects on HSCs (Luis et al., 2011). One study used these differentially BCD Wnt activating APC mutations to monitor HSC transplant capacity in irradiated mice. They found that when BCD signaling is slightly increased over baseline, replacement of HSCs through reconstitution improves; however, when BCD is increased even further the HSCs do not successfully reconstitute (Luis et al., 2011), suggesting the requirement for a “Goldilocks” level of Wnt signaling. Moreover, gene expression analyses of APC have found that failed HSC reconstitution is due to increased HSC differentiation and loss of stemness (Famili, Brugman, et al., 2016). Together, these studies demonstrate that the BCD Wnt cascade is critical for HSC numbers, and that the dosage of the Wnt signal is important.

Several studies have demonstrated a requirement for Wnt signaling in HSC development by using chemical or genetic inhibitors of Wnt signaling. IWR-1-endo, which is a Wnt inhibiting drug through stabilization of Axin, increases total HSCs numbers during proliferation in adult zebrafish (Kimura et al., 2022). Conversely, blocking the β-catenin/TCF binding complex with PKF-115 inhibits Wnt and decreases HSC numbers during development (Ruiz-Herguido et al., 2012). This differing effect on Wnt inhibition on HSCs is likely attributable to adult versus developing HSCs. In zebrafish, expression of dominant-negative TCF (dntcf, which downregulates Wnt signaling) throughout the developmental periods of HSC specification and emergences results in fewer HSCs (Goessling et al., 2009; Grainger et al., 2016). Notably, dntcf does not have this effect if induced after HSC emergence, highlighting that this signal occurs earlier in development, even though the phenotypic effect is seen later in development (Grainger et al., 2016),. Taken together with the data above, these studies support that more BCD Wnt signaling can lead to more HSCs, but that too high of a Wnt dosage can be detrimental to HSC development.

One of the bottlenecks in the Wnt field continues to be how a Wnt ligand chooses and signals through its cognate Fzd receptor. Speaking to this, several Wnts and Fzds are expressed in hematopoietic sites across species. For example, murine *Wnt5a*, *Wnt10b*, *Fzd3*, and *Fzd7* are expressed in E11 yolk sac and E14 fetal liver (Austin et al., 1997), while *Wnt3a*, *Wnt5a*, *Wnt10b*, *Fzd1*, *Fzd3*, *Fzd4*, *Fzd5*, *Fzd6*, *Fzd7* and *Fzd8* are expressed in the bone marrow niche (Yamane et al., 2001). The differences in complements of Wnt/Fzds

in these developmental and homeostatic niches, respectively, suggest that there may be specificity of function with different pairings.

The specificity of Wnt signaling events has also been shown during HSC development in zebrafish, where somitically expressed Wnt ligands are important for two different stages of HSC development. *Wnt9a* is required for intra-aortic amplification of HSCs, together with its cognate receptor Fzd9b (Grainger et al., 2019; Grainger et al., 2016), while Wnt16 is necessary for HSC specification (Clements et al., 2011; Grainger et al., 2016). Though these ligands are both expressed in the somite, resulting in a convergence of signaling in the hemogenic endothelium, they drive separate developmental processes. These studies underline how specific Wnt/Fzd pairings lead to distinct events during HSC development.

As with HSC development, Wnts and Fzds have important roles in HSC maintenance and differentiation. For example, purified murine Wnt3a protein has been found to induce HSC self-renewal *in vitro* (Willert et al., 2003). Fzd6 is expressed in human LT-HSCs, supporting that HSC self-renewal is dependent on differential expression of Wnt-related receptors as well as ligands (Wagner et al., 2004). Additionally, murine HSC growth is inhibited when treated with the soluble cystine rich domain (CRD) of the frizzled receptor, which antagonizes Wnt signaling (Reya et al., 2003). In human and murine HSCs, β-catenin and purified Wnt proteins stimulate HSC self-renewal (Reya et al., 2003; Ruiz-Herguido et al., 2012; Willert et al., 2003), and in mice lethally irradiated, β-catenin and purified Wnt proteins increase HSC reconstitution (Reya et al., 2003) but deletion of β-catenin diminishes LT-HSC self-renewal (Zhao et al., 2007). APC inactivation in mice increases HSC cell cycle entry and exhaustion, subsequent defects of the CMP and CLP progenitor pool, suggesting that APC is needed for HSC maintenance and survival (Qian et al., 2008). Li et al (2013) found that inactivation of β-catenin rescues HSC exhaustion caused by APC inactivation. In addition, loss of β-catenin also prevented extreme HSC proliferation and apoptosis and CMP/CLP defects in APC-deficient mice. In culmination, this research suggests that APC is a regulator of HSC maintenance and differentiation through the BCD pathway (Li et al., 2013). LEF/TCF also has a role in HSC maintenance. Irradiated mice with transplanted HSCs containing mutated LEF/TCF binding sites driving GFP expression did not have GFP fluorescence in the bone marrow after 14-weeks whereas irradiated mice with transplanted HSCs containing wildtype LEF/TCF binding sites driving GFP expression did have GFP expression in the bone marrow (Reya et al., 2003). These studies support the importance of BCD in HSC maintenance.

BCI Wnt signaling also plays a role in HSC homeostasis. For example, N-cadherin-expressing osteoblasts primarily express BCI Wnt ligands and BCD inhibitors during quiescence (Sugimura et al., 2012). An example of this is Flamingo, which has homology to cadherins and regulates expression of Fzd8 at the interface between HSCs and N-cadherin-expressing osteoblasts (Sugimura et al., 2012). Through this mode of regulation, Flamingo downregulates BCD signaling and activates BCI signaling to maintain quiescence in LT-HSCs (Akashi et al., 2003; Sugimura et al., 2012). In another example, mice lacking receptor Ryk involved in the BCI Wnt pathway have fewer quiescent HSCs, decreased self-renewal capabilities, and increased apoptosis (Famili, Perez, et al., 2016). These studies generally support that BCI Wnt signaling is important for HSC maintenance.

Research thus far supports that both BCI and BCD have important roles in the development, maintenance, and differentiation of HSCs. One area that requires more investigation is the pairings of Wnts with Fzds to activate BCI and BCD Wnt signaling, which has only recently begun to be investigated in HSCs and other contexts (Cho et al., 2017; Eubelen et al., 2018; Grainger et al., 2019; Grainger et al., 2016; Vanhollebeke et al., 2015; Zhou & Nathans, 2014). Parsing out these potential pairings as they relate to HSCs may aid in making HSCs *in vitro* and eventually treating disease.

Wnt signaling and hematological malignancies

The discovery of *Wnt1* in 1982 revealed that activation of the gene lead to an oncogenic phenotype in mice (Nusse et al., 1984; Nusse & Varmus, 1982; Tsukamoto et al., 1988). Since then, a substantial amount of research has been focused on the relationship between the Wnt pathway and cancer, and cancers of the blood are no exception. Maintaining a careful balance of Wnt signaling is critical for HSC development and homeostasis. Disruption of Wnt signaling can lead to uncontrolled expansion of blood progenitor cells and leads to cancers of the blood, which are classified as leukemia, lymphoma, and myeloma. Many of these blood cancers are dependent on β-catenin for survival and progression (Gutierrez et al., 2010; Hu et al., 2009; Khan et al., 2007; Mazieres et al., 2005; Siapati et al., 2011). Studies of leukemia, lymphoma and myeloma in patient cells and animal models have provided some insights into how Wnt regulates cancer, which we overview briefly below, but has also been reviewed extensively elsewhere (Grainger et al., 2018; Janovska & Bryja, 2017; Staal, Famili, et al., 2016; van Andel et al., 2019).

Leukemia is caused by the uncontrolled expansion of myeloid progenitors, leading to an overabundance of white blood cells and platelets; leukemias are categorized into four subtypes: acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), and chronic lymphocytic leukemia (CLL) (Siegel et al., 2016). BCD Wnt signaling is a driving force in leukemic progression using *in vitro* and *in vivo* models. For example, in AML mouse models, HSCs and myeloid progenitor cells can derive pre-leukemia initiating cells (pre-LIC) and evidence suggests that β-catenin is necessary for pre-LICs to progress into mature, self-renewing LICs (Lane et al., 2011; Wang et al., 2010; Yeung et al., 2010), which has also been shown for CML cells (Nagao et al., 2008), CLL (Franiak-Pietryga et al., 2015) and T-ALL (Giambra et al., 2015). Additionally, there are several oncogenic chimeric fusion proteins associated with leukemias, such as AML1-ETO, PML-RARα, or PLZF-RARα, which activate Wnt signaling in hematopoietic cell lines (Cheng et al., 2011; Muller-Tidow et al., 2004). This activation of signaling leads to enhanced proliferation, which has been shown for example in the AML cell lines, CD82 and CD70/CD27 (Ji et al., 2019; Riether et al., 2017). In AML cells *in vitro*, loss of key components of the Wnt pathway can in turn dampen this proliferative effect. Knockdown of Wnt receptor, Fzd1, inhibits AML proliferation, while cells with increased Fzd1 expression are chemoresistant (Wang et al., 2018). BCD Wnt signaling also contributes to CML in mouse models as evidenced by elevated nuclear β-catenin levels which drove the self-renewal capacity of the CML cells (Jamieson et al., 2004). Furthermore, granulocyte macrophage progenitor cells arise from CML progenitor cells due to an in-frame splice deletion in GSK3β leading to increased β-catenin expression (Abrahamsson et al., 2009).

Reduction of BCD Wnt signaling via inhibition of the BCR-ABL-PI3K-AKT pathway reduces the tumor forming ability of the LICs (Hu et al., 2016). This evidence supports a role for BCD Wnt signaling in leukemia however, it's important to note that others have found evidence that BCI signaling is active in leukemias as well. For example, BCR-ABL CML cell survival is dependent on Wnt-mediated calcium signaling (Gregory et al., 2010). Additionally, BCI signaling is implicated in ALL where upregulated E2A-Pbx1 fusion protein activates the expression of WNT16 (McWhirter et al., 1999).

Given its requirement in leukemia, Wnt signaling has naturally been investigated as a therapeutic target. For instance, the Wnt co-receptor, ROR1, is expressed in human CLL cells and even higher levels have been found in an accelerated form of CLL (Cui et al., 2016; Fukuda et al., 2008). For this reason, research has set out to target these cells with anti-ROR1 monoclonal antibody (Cirmtuzumab). For example, by blocking ROR-1, Cirmtuzumab inhibits Wnt5a signaling and prevents RhoA/Rac activation, thus reducing proliferation and migration of CLL cells, but it has no effect on non-leukemic cells (Cui et al., 2016; Yu et al., 2016). In fact, co-treatment of leukemic cells with Cirmtuzumab and a B-cell receptor blocking drug is more effective at clearing leukemic cells than using the B-cell blocking drug on its own (Yu et al., 2017).

An important consideration when therapeutically targeting the Wnt pathway is that loss of Wnt signaling in leukemia cells is not always detrimental to their persistence. For example, Wnt5a+/− mice have enhanced B-cell proliferation and develop spontaneous myeloid leukemia compared to Wnt5a homozygous mice. This may be because Wnt5a regulates the calcium cascade which antagonizes the β-catenin pathway (Liang et al., 2003; Ying et al., 2007) thereby suggesting that leukemia is more likely to progress with active β-catenin signaling. B-cells *in vitro* had reduced proliferation capacity when treated with Wnt3a, indicating that Wnt3a may also antagonize Wnt signaling in leukemia cells (Nygren et al., 2007). These differing effects of Wnt5 and Wnt3a on leukemia cells further supports the need for ligand/receptor specificity research to understand the role of Wnt signaling in leukemia.

Much like leukemias, lymphomas have been shown to have increased levels of Wnt pathway activation (Ge et al., 2012; Gelebart et al., 2008; Groen et al., 2008; Zhang et al., 2010). Regulators of the Wnt pathway include *TCF7*, *FZD7*, *LRP5*, *AXIN1*, *APC*, and *DVL3* in mantle cell lymphoma (Rizzatti et al., 2005) and TCF1 and LEF1 in some T-cell and small B-cell lymphomas (Dorfman et al., 2003; Tandon et al., 2011). Lymphomas harboring fusion oncoproteins can also lead to increased expression of Wnt target genes such as *cyclinD1*, but this translocation does not drive lymphoma on its own (Bodrug et al., 1994; Bosch et al., 1994). Altogether, these studies suggest that increased canonical Wnt signaling leads to disease progression in lymphomas. Similar to leukemia, Wnt5a is suspected to antagonize canonical Wnt in lymphoma as loss of heterozygosity in Wnt5a+/− mice leads to B-cell lymphoma (Liang et al., 2003).

Myeloma is cancer of plasma cells, with multiple myeloma (MM) being the most common type of myeloma. Wnt2b, Wnt5a, Wnt7a, Wnt10b, Wnt11, and Wnt16 are expressed in MM patient cells and MM cell lines (Qiang et al., 2005). Like LICs and lymphoma cells, MM

cell growth is dependent on Wnt signaling (Derksen et al., 2004). Primary MM cells are killed following treatment with an inactivator of the β-catenin/TCF complex (PKF115-584), without affecting normal plasma cells, indicating that Wnt signaling may be necessary for MM cell viability but not healthy plasma (Sukhdeo et al., 2007).

Blood cancer cells are housed in the bone marrow, and unsurprisingly have a large impact on the bone microenvironment. For example, MM cells secrete the Wnt antagonists SFRP2 and DKK1, both of which inhibit bone mineralization and are associated with destructive bone lesions in patients (Oshima et al., 2005; Tian et al., 2003), and *in vitro*(Gunn et al., 2006; Tian et al., 2003). As a result, osteolytic bone disease manifests in patients with MM due to absorption of bone by osteoclasts and repression of new bone formation by osteoblasts. DKK1 antibody treatment can reverse decreased bone mineral density in severe combined immunodeficient mice with MM tumor cells (Fulciniti et al., 2009; Heath et al., 2009; Yaccoby et al., 2007), providing a possible treatment option for MM patients. These studies demonstrate the importance of Wnt signaling to skeletal health and cancer progression, and how these converge to devastating impacts.

Epigenetic Regulation in HSCs and Wnt Signaling

Epigenetic modulations can lead to heritable differential gene expression without alteration to DNA and include alterations such as DNA methylation, and histone posttranslational modifications including methylation and acetylation (Wright & Beato, 2012). These mechanisms act on chromatin organization to either increase (euchromatin) or decrease (heterochromatin) accessibility of DNA to transcription and consequently, control gene expression. This pattern of methylation and acetylation markers is known as the Histone Code (Strahl & Allis, 2000). Like other cell types, HSCs are impacted by modifications in the histone code.

Methylation of DNA is catalyzed by DNA Methyl transferases (DNMTs) and the removal of methyl groups is performed by demethylases such as LSD1 (Lysine specific demethylase 1) (Jones & Liang, 2009). LSD1 is a critical regulator of HSC differentiation and proliferation (Kerenyi et al., 2013; Sprussel et al., 2012), and cooperates with BCD Wnt signaling through the transcriptional repressor Gfi1b to repress hyperproliferation of HSCs (Shooshtarizadeh et al., 2019). Methylated DNA is recognized by methyl-CpG binding domains (MBD) or C2H2 zinc finger proteins, which are a mechanism of epigenetic regulation beyond histones, and can lead to increased or decreased gene expression (Fitz-James & Cavalli, 2022). For example, the methyltransferases DNMT3a/b, have been found to be both essential for HSC self-renewal and to carry out *de novo* methylation patterns which silence self-renewal genes within HSCs (Challen et al., 2014; Jeong et al., 2018; Trowbridge & Orkin, 2011). Epigenetic regulation of Wnt signaling likely plays an important role in the maintenance of HSCs, CMPs and CLPs. For instance, CML and CLL cells exhibit aberrant hypermethylation of sFRPs, which are thought to act as Wnt antagonists (Liu et al., 2006; Pehlivan et al., 2009); silencing of another Wnt antagonist, Dkk3 is associated with ALL (Roman-Gomez et al., 2004). Finally, Wnt5a, which seems to play a tumor suppressor role in hematological malignancies is silenced in several blood cancer (Roman-Gomez et al., 2007; Ying et al., 2007).

Given the unsuccessful attempts to generate LT-HSCs *in vitro*, it is possible that during culture the epigenetic signals of cells is altered. For example, long term culture of mesenchymal stem cells (MSCs) shows both hypo- and hypermethylation with additional complication of neighboring CpGs as well as alterations in nuclear organization, resembling epigenetic drift (Franzen et al., 2021). There are large gaps in knowledge within the epigenetics field generally, but specifically within Wnt signaling. Additionally, conditions surrounding epigenetic modulations *in vivo* are not yet clear, thus there is likely a large amount still to be discovered about how HSCs and Wnt signaling are regulated epigenetically.

Conclusion

Understanding the cellular signals occurring during hematopoiesis will enable advancement in treatment for hematological malignancies through improvement of HSCT, and more specific targeting of cancer cells. Wnt signaling is critical during hematopoiesis and members of both β-catenin dependent (BCD) and independent (BCI) pathways are dysregulated in cancer indicating potential therapeutic targets. Additional research into specific requirements for Wnt/Fzd pairings in HSC development and homeostasis will fill gaps in knowledge in this field and be key to future therapeutic interventions and our understanding of how our blood is made and replenished.

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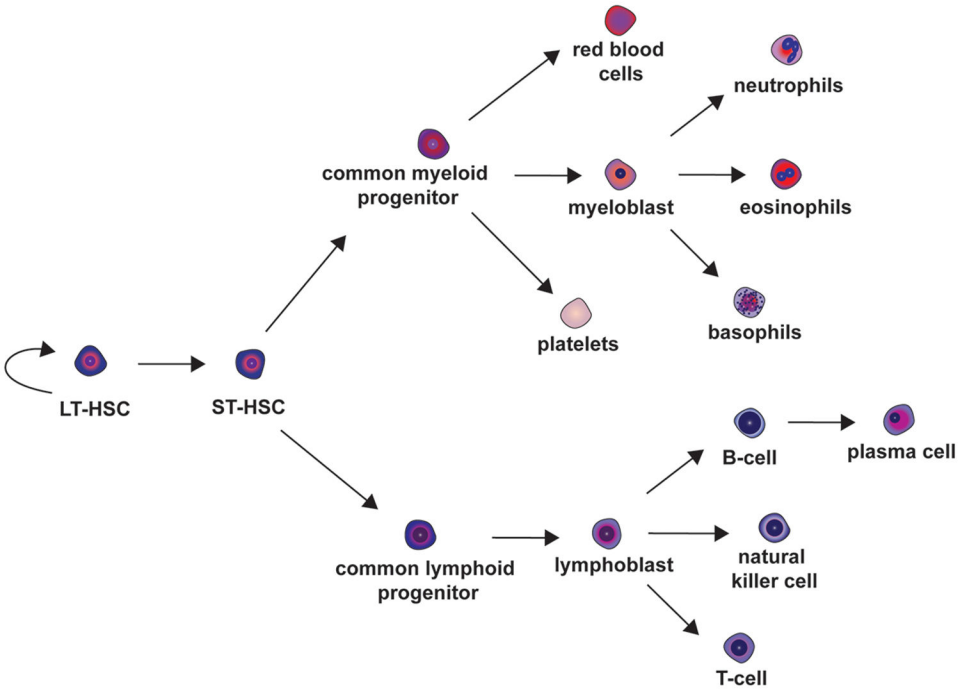

Figure 1.

Hematopoietic stem cell differentiation. Long-term (LT) HSC cells self-renew or repopulate the HSC pool. Short-term (ST) HSCs can differentiate into a common myeloid progenitor cell or a common lymphoid progenitor cell, which further differentiate into blood and immune cells.

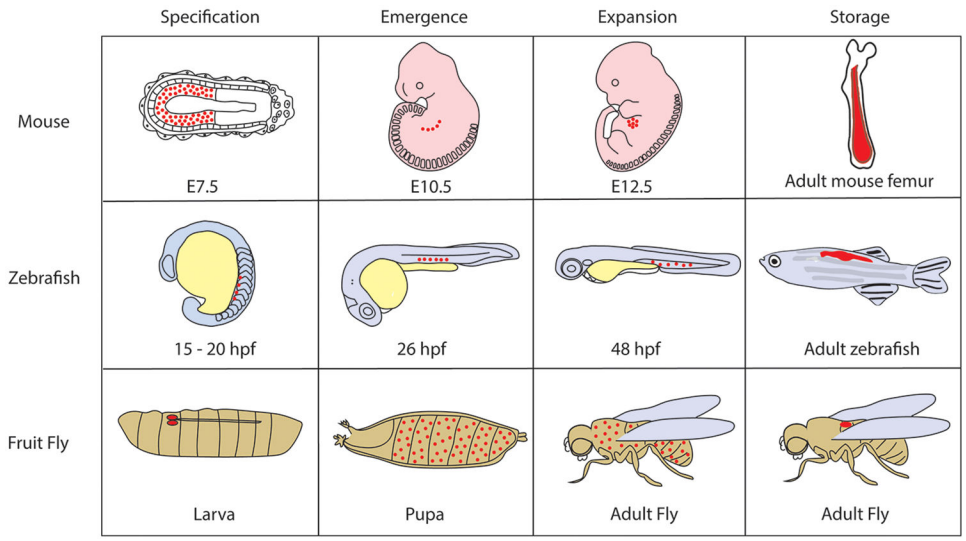

Figure 2.

Locations of hematopoietic specification, emergence, expansion, and storage in mice, zebrafish, and fruit fly. Specification, indicated as red dots, in mice and zebrafish occurs in hemogenic endothelium, whereas in fruit fly larvae, specification occurs in the lymph glands. Emergence occurs in the aorta-gonad-mesonephros of mice, in the dorsal aorta of zebrafish, and across the pupa of fruit fly. HSC expansion takes place in the fetal liver of mice, in the caudal hematopoietic tissue of zebrafish, and in hematopoietic pockets across the adult fruit fly.

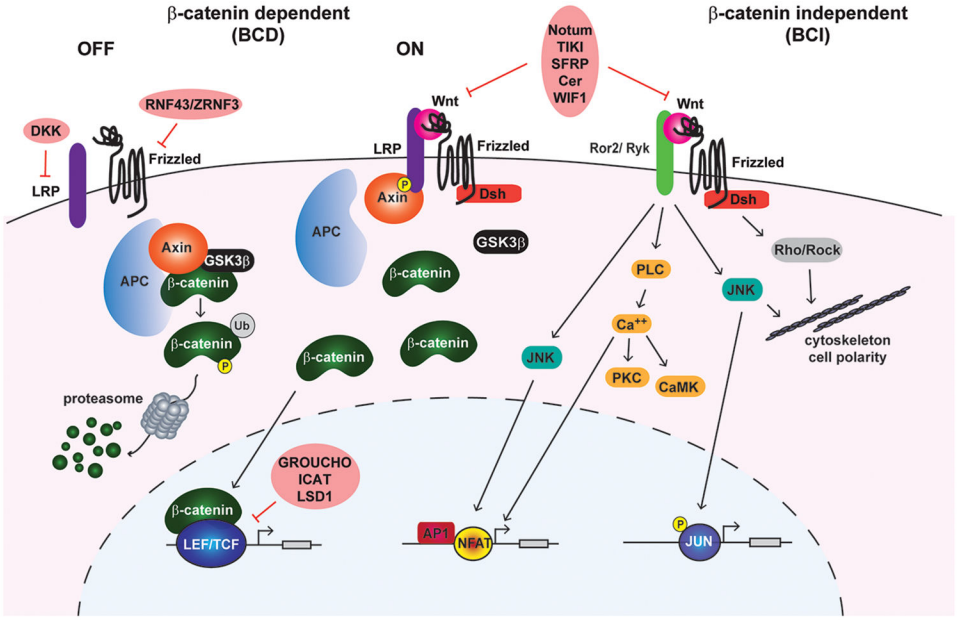

Figure 3:

Schematic of Wnt signaling pathways with and without the presence of a Wnt ligand. BCD pathway is activated by interaction of Frizzled (Fzd) receptor, lipoprotein related protein (LRP) and the Wnt ligand. The signal leads to nuclear translocation of β-catenin into the nucleus where it binds to lymphoid enhancing factor (LEF)/ T cell factor (TCF) transcription factors, thereby initiating transcription of Wnt target genes. One proposed β-catenin independent (BCI) pathway is initiated by the formation of a Ror2/Ryk-Wnt-Fzd complex. This leads to alterations in Ca^{2+} intracellular levels, impacting the protein kinase C (PKC), calmodullin-dependent protein kinase (CaMK) cascades, or NFAT/AP1 driven gene expression. *DKK,* Dickkopf; *Dsh,* Disevelled; *GSK3β,* Glycogen Synthase Kinase 3β; *LSD1,* Lysine Specific Demethylase 1*; Ryk,* receptor-like tyrosine kinase; *Sfrp,* Secreted frizzled-related protein.

Table 1.

The impact of alterations and mutations in Wnt signaling on HSC development.

Developmental duration of Wnt disruption	Disruption(s) used	Species [reference]	Anticipated impact on Wnt signal	Observed Impact on HSPCs
mesoderm - endpoint	Wnt9a loss of function, Fzd9b loss of function, EGFR loss of function, Wnt16 loss of function, Exogenous DKK1	Fruit fly (Sinenko et al., 2009), zebrafish (Clements et al., 2011; Grainger et al., 2019; Grainger et al., 2016); mouse (Luis et al., 2009); human ESC differentiation (Woll et al., 2008)	Decreased	Decreased
	Exogenous Wnt1 or Wnt3a/WNT3A	Fruit fly (Sinenko et al., 2009), human ESC differentiation (Gertow et al., 2013; Wang & Nakayama, 2009; Woll et al., 2008)	Increased	Increased
	Wnt2 knockout cells	mouse ESC differentiation (Wang et al., 2007)	Decreased	Increased
specification - endpoint	dntcf expression, Porcupine inhibition, dkk1 overexpression, Tankyrase inhibition, Axin1 overexpression, Wnt3a loss of function, WNT9A loss of function	zebrafish(Goessling et al., 2009; Grainger et al., 2016; Wang et al., 2007); mouse (Luis et al., 2009); human (Richter et al., 2018)	Decreased	Decreased
	wnt8 overexpression, GSK3β inhibition, constitutively active β-catenin, increased Axin-LRP6 interaction, exogenous Wnt3a, exogenous WNT9A, GSK3β inhibition	zebrafish (Goessling et al., 2009; Grainger et al., 2016; Wang et al., 2013); mouse (Frame et al., 2016; Goessling et al., 2009); human (Richter et al., 2018)	Increased	Increased
specification only	Porcupine inhibition	human ESC differentiation(Sturgeon et al., 2014)	Decreased	Decreased
	GSK3β inhibition	human ESC differentiation (Kitajima et al., 2016; Sturgeon et al., 2014)	Increased	Decreased
emergence - endpoint	GSK3β inhibition	mouse (Ruiz-Herguido et al., 2012)	Increased	Increased
	β-catenin inhibition	mouse (Frame et al., 2016; Ruiz-Herguido et al., 2012)	Decreased	Decreased
	β-catenin	mouse (Ruiz-Herguido et al., 2012)	Decreased	No effect on HSCs
	β-catenin loss of function	mouse (Zhao et al., 2007)	Decreased	Decreased
	Axin stabilization	Zebrafish (Kimura et al., 2022)	Decreased	Increased