Wnt signaling in the stem cell niche

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Purpose of review

All the cells present in the blood are derived from the hematopoietic stem cell (HSC). Because mature blood cells have a limited life span, HSCs must perpetuate themselves through self-renewal to maintain a functional hematopoietic compartment for the lifetime of an organism. This review focuses on studies that identify the Wnt signaling pathway as a mediator of HSC self-renewal and maintenance and analyzes its potential influence in context of the HSC niche.

Recent findings

The Wnt signaling pathway has emerged as a potential regulator of self-renewal for HSCs. Recent reports have demonstrated that Wnt signaling can directly promote HSC self-renewal and ability to reconstitute the hematopoietic system of lethally irradiated mice. The recent findings that osteoblasts are an important regulatory component of the HSC microenvironment, and that elements of the Wnt signaling pathway can influence osteoblast frequency, raise the possibility that Wnt signaling may influence HSC function indirectly through the niche as well.

Summary

In this review, the authors evaluate the experimental evidence for a direct role of Wnt signaling HSCs as well as an indirect role through its influence on the HSC niche. Defining the mechanism of action of Wnt signaling in HSC maintenance in context of the surrounding microenvironment and determining how this signal may integrate with other niche derived signals represents the next challenge in HSC biology.

Keywords

hematopoietic stem cell, self-renewal, Wnt signaling, stem cell niche

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Abbreviations

APC	adenomatous polyposis coli
BMP	bone morphogenic protein
HSC	hematopoietic stem cell
LRP	lipoprotein receptor-related protein

Introduction

Hematopoietic stem cells (HSCs), which have the capacity to self-renew as well as to differentiate into committed cells, were originally functionally identified by Till and McCulloch [1] more than 40 years ago. Since then, a number of methods have been used to isolate HSCs from the bone marrow [2–7]. One approach involves identification of HSCs based on the presence of cell surface molecules. In mice, HSCs from the bone marrow express low or undetectable levels of lineage markers ($Lin^{neg/lo}$), low levels of Thy1.1, and high levels of c-Kit and Sca-1 ($Lin^{neg/lo}$, $Sca-1^+$, $c-Kit^+$, $Thy1.1^{lo}$) [8]. When murine HSCs isolated by this method are transplanted into lethally irradiated mice, they initially provide radioprotection by generating erythroid and myeloid cells that are necessary for survival. Later, these cells balance self-renewal and differentiation to maintain the hematopoietic system for the life of the animal [9,10]. HSCs are present in the bone marrow at a frequency of 1/2000 cells, and their activity is characterized by two subpopulations: one with short-term repopulation ability (<10 weeks) and the other with long-term repopulating ability that lasts the lifetime of an organism [9].

Human HSCs can also be identified and isolated though the expression of cell surface antigens, such as CD34, CD38, and the absence of lineage markers. Xenotransplantation of human HSCs into nonobese diabetic/severe combined immunodeficient mice has proved to be a reliable model for the detection of primitive human blood cells with repopulation capacity, classifying these cells as severe combined immunodeficient repopulating cells [11]. Phenotypically, human severe combined immunodeficient repopulating cells are highly enriched among $CD34^+/CD38^-/Lin^-$ cells, whereas hematopoietic progenitors devoid of repopulating function present a more mature phenotype ($CD34^+/CD38^+$) [12,13]. The frequency of severe combined immunodeficient repopulating cells is low (less than 1/1,000,000) and differs depending on the source [14]. Emerging evidence suggests that human HSCs may also exist in the $CD34^-$ population [15–18].

Although much is known about the identity and physiologic function of HSCs, less is known about the molecular mechanisms that regulate self-renewal. Elucidating the molecular cues that regulate self-renewal has been extraordinarily challenging, because most factors that induce HSC proliferation are accompanied by differentiation and loss of HSC function [19,20]. However,

recent studies have begun to identify signals such as sonic hedgehog [21], Notch1 [22] [23], the transcription factor HoxB4 [24–27], and the cyclin-dependent kinase inhibitor p21/cipwaf1 [28] as novel regulators of hematopoietic progenitor self-renewal (reviewed by Pazianos *et al.* [29]). In addition, recent studies have also identified the Wnt signaling pathway as a regulator of HSC homeostasis [29]. In this review we focus on studies that analyze the role of the Wnt signaling pathway in HSC homeostasis and provide an analysis of its influence in the context of its surrounding niche.

Wnt signaling

Wnts are a large family of secreted lipid-modified glyco-proteins that are expressed in a wide variety of tissues and have been shown to influence multiple processes in animal development [30,31••]. In addition, deregulation of Wnt signaling has been linked to tumorigenesis in different tissues [32–34]. In the absence of Wnt signaling, the level of β-catenin is kept low through degradation through its association with a large multiprotein complex that includes glycogen synthase kinase–3β (GSK-3β) bound to the scaffolding proteins Axin and adenomatous polyposis coli (APC; Fig. 1). In this complex, β-catenin is phosphorylated and thereby targeted for ubiquitination and degradation in the proteosome [30].

Wnt signaling is initiated by Wnt binding a membrane receptor complex composed of a Frizzled receptor and a low-density lipoprotein receptor-related protein (LRP). This binding can activate one of the several intracellular signaling pathways depending on the Wnt, Frizzled receptor, or the cell type involved (reviewed by Miller [35]). The canonical Wnt signaling pathway is thought to require activation through both Frizzled and LRP (reviewed by Pandur and Kuhl [36]). Binding of Wnt to its receptor leads to activation of Disheveled, which in turn inhibits GSK-3β mediated phosphorylation of β-catenin. Subsequently β-catenin stabilizes, accumulates in the cytosol, and translocates to the nucleus, where it interacts with members of the LEF-1/TCF family of transcription factors and activates expression of target genes [37]. Thus, signaling through the Wnt canonical pathway is centered on β-catenin activity (Fig. 1). In contrast, noncanonical Wnt signaling has been proposed to be mediated through intracellular calcium release and kinase activation (CamKII and PKC; reviewed by Kuhl *et al.* [38]).

Dual role of β-catenin in Wnt signaling and cell adhesion

The key component of the Wnt pathway, β-catenin, also plays an essential role in structural organization and cell–cell adhesion (Fig. 1). In this context it interacts with the intracellular domain of E-cadherin and connects the adherens junction complex with the actin cytoskeleton through α-catenin [39–41]. This functional complex is

Figure 1. Alternative roles of β-catenin in Wnt signaling and adhesion

In the absence of Wnt signaling (left panel) β-catenin is complexed with Axin, APC, and GSK-3β, and gets phosphorylated and targeted for degradation. β-catenin also exists in cadherin-bound form and regulates cell–cell adhesion. In the presence of Wnt signaling (right panel), β-catenin is uncoupled from the degradation complex and translocates to the nucleus, where it binds LEF-1/TCF transcription factors, thus activating target genes.
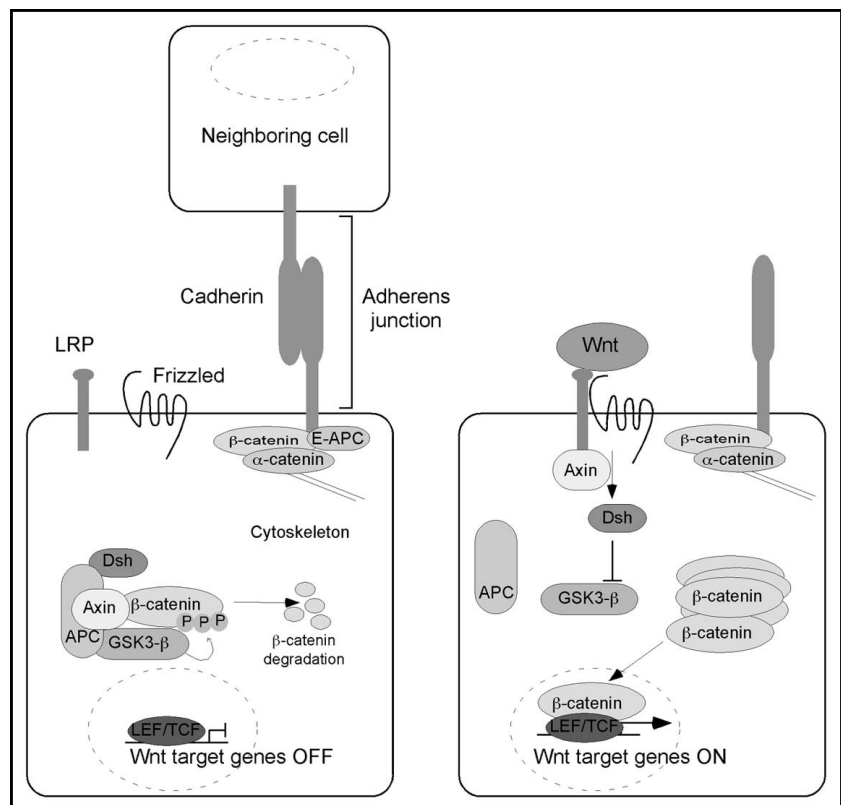
necessary for maintenance of cell layers and can influence cell migration as well. β-catenin interactions with cadherin and LEF-TCF are mediated by a common domain (arm repeat), implying that these interactions are mutually exclusive. Therefore, cadherin-mediated adhesion may act as a negative regulator of Wnt signaling because it can sequester β-catenin at the cell surface and thereby deplete it from the cytosolic pool. Similarly, components of canonical Wnt signaling have also been shown to downregulate E-cadherin expression directly [42,43••]. These data support the idea that repression of cadherin can induce the release of β-catenin from the membrane into the cytoplasm, which might then be used to amplify or sustain Wnt signaling.

Other components of Wnt signaling such as APC are also involved in cell polarity and migration (Fig. 1). In addition to its function in targeting β-catenin for degradation, APC has been described as a microtubule capping protein involved in the formation of cellular extension and polarized migration. In the absence of Wnt signaling, no β-catenin is detected at the APC cluster at the tip of the microtubules. However, activation of Wnt signaling through overexpression of a stable β-catenin (N-terminally truncated) leads to its accumulation in the microtubule APC clusters, impairing the migratory properties of some cell lines [44,45]. Moreover an APC homolog has been described at the adherens junction associated with cadherin and β-catenin, and mutations in this homolog disrupt cell–cell adhesion and increase the level of cytoplasmic β-catenin [46,47]. Thus, Wnt and cadherin pathways may intersect at multiple levels in the signaling cascade (reviewed by Nelson and Nusse [48]).
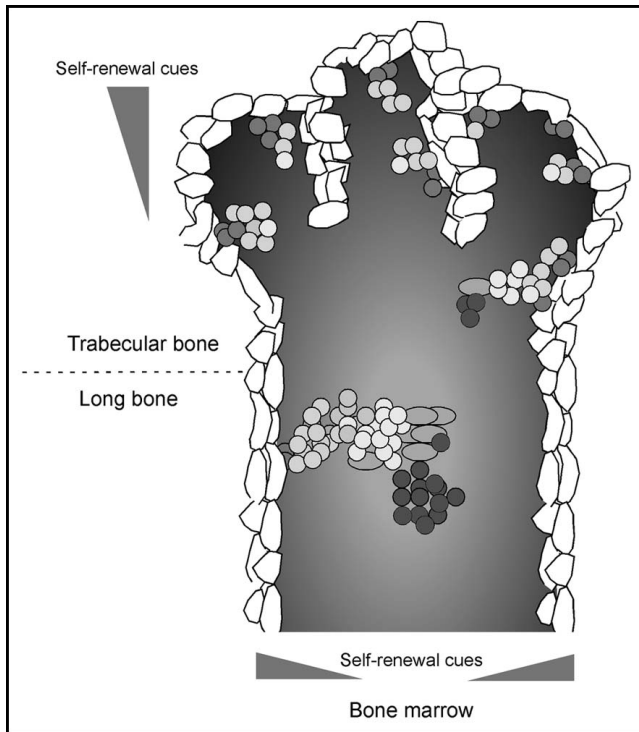
Wnt and β-catenin in hematopoietic stem cell regulation

Although many studies have shown that Wnt signaling is involved in the development of many organs and tissues, its role in the hematopoietic system has only recently begun to be explored. The decision of HSCs to self-renew or to differentiate is governed by a complex interplay between both intrinsic signals (HSC cell autonomous) and stimuli from the surrounding microenvironment (non cell autonomous components). Recent evidence demonstrates that Wnt proteins can be produced by HSCs themselves as well as by the microenvironment. Studies carried out on human bone marrow suggest that Wnt 2B, Wnt 5A, and Wnt 10B are expressed in unfractionated bone marrow cells, whereas Wnt 5A (but not Wnt 2B or Wnt 10B) is expressed in the CD34+/Lineage− cell, a population that is enriched for progenitors and HSCs [49]. Wnt 3A, Wnt 5A, and Wnt 10B are also found to be expressed in various cell populations in mouse bone marrow [50], whereas Wnt 5A and Wnt 10B are expressed in the murine fetal liver [51]. Additionally, stromal cell clones that can support HSC growth express Wnt 5A [52••,52b]. Taken together these data suggest that members of the Wnt family can be produced by both hematopoietic cells and their stromal cell environment. Although examining Wnt expression on isolated cells allows the identification of the precise source of Wnts, one disadvantage is that it is difficult to gauge the spatial relation between the cells producing Wnts and the HSCs themselves. Thus to understand truly which Wnts may have the most influence on HSCs, it will be important in the future to determine which Wnts are made in the microenvironment immediately adjacent to the HSCs. Expression of Wnt receptors can be found on both HSCs and niche cells. Long-term human HSCs express Frizzled 6 [53], and supportive stromal cell lines express some member of the Frizzled family and LRP-6 [52••,52b]. These phenotypic data suggest that HSCs as well as stromal cells in their environment can receive and probably respond to Wnt signaling.

Several reports suggest that Wnt signaling directly promotes hematopoietic progenitors/stem cell self-renewal. In one study, soluble Wnt proteins were shown to synergize with SLF to promote the growth and inhibit the differentiation of murine hematopoietic progenitors *in vitro* [51]. In addition, we recently showed that β-catenin, a mediator of the Wnt signaling pathway, as well as purified Wnt proteins can promote self-renewal of murine and human HSCs *in vitro,* and increase the ability of murine HSCs to reconstitute the hematopoietic system of lethally irradiated mice [31••,54••]. Conversely, overexpression of Axin, which enhances β-catenin degradation and thereby inhibits Wnt signaling, causes inhibition of HSC growth *in vitro* and decreased reconstitution of irradiated mice [54••]. Furthermore, HSCs *in vivo* activate the Wnt reporter and respond to an endogenous Wnt signal [54••]. In addition, identification of HoxB4, Notch1 [54••], as well as Hes-1 (Duncan AW, Reya T, unpublished observation) as being upregulated by components of Wnt signaling in HSC suggests that there may be a molecular hierarchy of control of HSC self-renewal. Wnt signaling may have an effect on enhancing self-renewal of human HSCs and progenitors as well. This is supported by the finding that Wnt 5A treatment of human hematopoietic progenitors in the presence of stromal cell contact promoted the expansion of undifferentiated progenitors *in vitro* [49]. Furthermore treatment of mice with Wnt 5A-conditioned medium resulted in increased human HSC repopulation in a nonobese diabetic–severe combined immunodeficient xenotransplant model [55••]. Cumulatively these studies suggest that Wnt signaling plays a role in regulating human HSC self-renewal.

Recently, an interferon-inducible β-catenin knockout was shown to have no defects in the hematolymphoid system [56••]. These data are difficult to reconcile with the previous literature, which suggests that elements of the Wnt signaling pathway play a vital role in maintain-

Figure 2. Proposed model of HSC development in the niche

HSCs are shown at the endosteal marrow adjacent to the bone's surface mainly at the trabecular bone and are postulated to migrate inward in the central marrow as they differentiate away from a possible gradient of self-renewal cues.

because the same loxP–β-catenin mouse crossed to mouse with Cre driven specifically in thymocytes exhibited clear defects in T-cell development [60]. Clearly, further experiments are required to reconcile these findings both in lymphocytes and in HSCs.

Hematopoietic stem cell niche

The bone marrow stroma functions to exert a regulatory effect on HSC self-renewal and differentiation. This supportive microenvironment has been defined as a niche in which the stem cells are maintained in their undifferentiated state. The HSC microenvironment is composed of a heterogeneous population of cells that includes fibroblasts, adipocytes, endothelial cells, and osteoblasts all derived from a common mesenchymal precursor [61].

Two recent studies using different genetic approaches show that increasing the number of osteoblasts in the niche resulted in a parallel increase in the number of HSCs. In one study, osteoblasts from transgenic mice expressing a constitutively active parathyroid hormone receptor displayed enhanced growth, resulting in a concomitant increase in HSCs [62••]. Further *in vitro* experiments demonstrated that stroma from these transgenic mice supported HSC function though the Notch pathway. This finding, together with the fact that elements of the Notch pathway are upregulated following Wnt activation of HSCs [54] (Duncan *et al.*, submitted), suggests the possibility that Wnt-mediated HSC homeostasis might involve increased Notch signaling via the microenvironment. That osteoblasts provide a niche for HSCs is supported by data derived from conditional deletion of bone morphogenetic protein (BMP) receptor IA in adult mice, which led to an increase in the trabecular bone area as well as an increase in the absolute number

ing T-cell development, B-cell survival, and HSC proliferation and self-renewal [29,57,58]. It is possible that β-catenin may overlap in function with another family member, γ-catenin [59], and therefore its loss is compensated effectively in the hematolymphoid compartment. Alternatively, it is possible that the absence of defects is the result of inefficient Cre-mediated deletion *in vivo*,

Figure 3. Detailed view of HSC interaction with stromal cell components

Wnt signaling can influence HSCs directly as well as indirectly by expanding the niche. Notch maybe upregulated on Wnt signaling and further contribute to HSC maintenance.
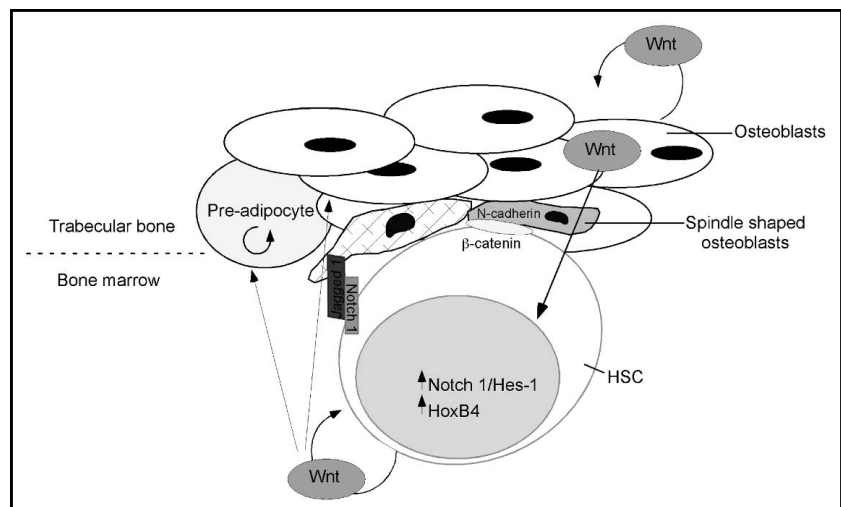
of HSCs compared with wild-type mice [63••]. These authors also showed that BMP receptor-deficient osteo-blasts represented a population of spindle-shaped cells lining the surface of the trabecular bone area and that HSCs could be found adjacent to these cells. Further-more N-cadherin and β-catenin were found to be asym-metrically localized at the osteoblast–HSC junction, suggesting that these molecules represent important components of the interaction between stem cells and their niche. These studies highlight the fact that the trabecular bone is the primary site where HSCs reside and that osteoblasts are a critical component of the stem cell niche. This finding is in accordance with the early studies that suggested that HSCs may mostly be local-ized in the endosteal marrow adjacent to the inner sur-face of bone and that more differentiated cells are found at the center of the marrow [64,65]. Together, these data lead to a model (Fig. 2) in which HSCs that lie at the interface between the trabecular bone and the bone mar-row may receive a high concentration of self-renewal cues, and may then migrate inward away from self-renewal cues as they differentiate, as demonstrated for Drosophila germ cells [66]. These data also suggest that Wnt signaling and cadherin-mediated cell adhesion may be interconnected mechanisms regulating HSC mainte-nance within the niche itself (Fig. 3). Because it seems that maintenance of HSC at the niche requires immobi-lization of β-catenin with cadherin at the junction with osteoblasts, it is possible that Wnt signaling induces HSC activation for self-renewal, and that this leads to relocalization of β-catenin to the cytosol pool, which can then be used to sustain Wnt signaling.

The influence of Wnt signaling on the hematopoietic stem cell niche

In addition to its role in regulating HSC self-renewal, growing lines of evidence suggest that the Wnt signaling pathway could indirectly modulate HSCs by influencing the niche as well. Wnt signaling plays an important role in bone formation, as evidenced by the reduced bone mass observed in loss-of-function LRP-5 mutations in both humans and mice [67,68]. Consistent with these findings, overexpression of an activated form of human LRP-5 leads to endosteal hyperostosis and increased tra-becular bone volume [69], suggesting that enhanced Wnt signaling increases the frequency of osteoblasts. Wheth-er this expansion of osteoblasts influences HSC fre-quency remains unknown, because HSC frequency and function were not specifically examined in these trans-genic mice. The observed bone phenotype resembles the one observed in the BMP receptor IA mutant [63••]. The fact that Wnt and BMP synergize to regulate N-cadherin expression in chondrocytes [70,71], a lineage related closely to osteoblasts, suggests that the same sig-nals may also play a role in generating the N-cadherin-expressing osteoblast population that supports HSCs in the niche.

Bone marrow stroma also contains cells of the adipocyte lineage, and Wnt proteins have been shown to maintain preadipocytes in an undifferentiated state [72]. Because increased numbers of mature adipocytes can inhibit he-matopoiesis [73], it is possible that Wnt also contributes to the maintenance of the HSC niche by reducing the frequency of differentiated adipocytes. Because osteo-blasts and adipocytes are derived from a common mes-enchymal precursor and loss of bone is accompanied by an increase in adipose tissue in the bone marrow com-partment [73], regulation of the ratio of adipocytes to osteoblasts may be crucial for the development and maintenance of the HSC niche. Together these *in vivo* and *in vitro* observations suggest that in addition to in-fluencing HSCs directly, Wnt signaling might also be involved in the maintenance of the cellular elements of the stem cell niche.

Conclusion

The Wnt signaling pathway plays an active role in the maintenance of both HSCs and their niche. Because Wnt involvement in self-renewal may be a general feature of stem cells from other systems as well [74,75], it is pos-sible that Wnt may also influence these lineages through both direct and indirect mechanisms. In the future, to understand truly the regulation of stem cell renewal, it will be critical to elucidate how the varied signals derived from the niche are integrated to maintain the stem cell state.

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