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Notch signaling in mammalian hematopoietic stem cells

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Abstract

Notch is a crucial cell signaling pathway in metazoan development. By means of cell-cell interactions, Notch signaling regulates cellular identity, proliferation, differentiation and apoptosis. Within the last decade, numerous studies have demonstrated an important role for this pathway in the development and homeostasis of mammalian stem cell populations. Hematopoietic stem cells are a well-defined population that display self-renewal and multi-lineage differentiation potential with the clinically relevant capacity to repopulate the hematopoietic system of an adult organism. Here, we review the emergence, development and maintenance of hematopoietic stem cells (HSCs) during mammalian embryogenesis and adulthood with respect to the role of Notch signaling in hematopoietic biology.

The hematopoietic system is one of the most well defined tissues for understanding and studying stem cells. Numerous studies and reviews have dealt in depth with its development, differentiation and involvement in multiple activities ranging from oncogenesis to therapy (1–5). Blood cells rapidly turn over, especially when responding to stress; thus, successful hematopoiesis requires the ability to continually replenish itself. This is accomplished by maintaining a pool of hematopoietic stem cells (HSCs) that have the ability to both self-renew and give rise to all differentiated blood lineages. Defects, either inborn or acquired, that inhibit HSC function can have disastrous consequences for the adult organism. HSCs have been successfully used in the clinic in diverse applications that include bone marrow transplant and gene therapy, and these successes have spurred investigations to understand the mechanisms that control HSC self-renewal and differentiation. Recent interest has focused on several evolutionarily conserved pathways that include Notch, Wnt and Hedgehog, which regulate self-renewal and differentiation in organisms ranging from *Drosophila* to humans. This review will focus on the role of Notch signaling in HSCs. Although it is known that Notch is required to establish the earliest embryonic HSCs, other functions for Notch in HSC biology are less well understood and controversial. This review

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will summarize what is known about the functions of Notch signaling in mammalian HSCs and attempt to shed some light on the areas of controversy.

Notch signaling

In its most simplistic view, Notch signaling delivers paracrine signals between neighboring cells. The components of the Notch signaling network, which are highly conserved from *Drosophila* to mammals, consist of four classes of molecules: Notch receptors, ligands, positive and negative regulators, and transcription factors. However, as is the case with the majority of highly conserved signaling pathways, simply cataloguing the pathway components tells a very small part of the story. Widespread expression in numerous tissues at diverse developmental times renders any broad functional generalizations for Notch signaling moot as the specific outcomes of Notch signaling depend on context, timing and dose. Thus, in some contexts Notch promotes differentiation, proliferation or survival, whereas in other contexts, Notch inhibits these processes. As a result, attempts to surmise a single authoritative role for Notch in mammalian hematopoietic development and maturation, at best, has created a lot of food for thought, and at worst, ample fodder for controversy.

Notch was discovered and named in 1917 for its famous phenotype in *Drosophila* (6, 7), and the earliest paradigms of Notch signaling were laid out in invertebrates. These studies suggest that the interplay between receptors and ligands influences lineage determination by two mechanisms: lateral inhibition, in which Notch signaling in one cell inhibits Notch signaling in its neighbor leading to diverse cell fates, and boundary induction, in which Notch signaling in one layer of cells induces signaling in its neighbor layer leading to similar cell fates as the signaling cell, such as in the Drosophila wing primordium where signaling is detected at the boundary between stromal Serrate-expressing cells (8, 9). Vertebrate Notch homologs were first described in *Xenopus* (10) and then in humans (11). In mammals, four Notch receptors (Notch1-4) have been characterized and exhibit both unique and overlapping patterns of expression. The receptors are comprised of a series of iterated structural elements. In the case of Notch1, these include the epidermal growth factor like repeats (EGFR), Lin12 Notch repeats (LNR), a RBP-Jκ-associated module (RAM) domain, seven Ankyrin repeats (ANK), two nuclear localization signals (NLS), a transactivation domain (TAD) and PEST sequences, which are important in protein turnover. Notch is most divergent in its C-terminus, where Notch3 and 4 lack the strong TAD that is present in Notch1 and 2. More detailed information on Notch receptor structure can be found in other reviews on the topic (12).

In mammals, there are five Notch ligands of the Delta (Dll1, Dll3 and Dll4) and Jagged (Jag1 and Jag2) families, which interact with Notch receptors on neighboring cells. Similar to the receptors, Notch ligands also contain multiple EGF-like repeats as well as DSL domains, which together are responsible for ligand-receptor binding and the subsequent initiation of signaling. While both Jagged and Delta initiate signaling via their DSL domains, they differ in the number of EGF repeats (13); however, the mechanism by which EGF repeat number influences function is poorly understood. As discussed below, different Notch ligands appear to have specificity for different Notch receptors. For example, Dll1 is

the critical ligand for Notch2 in murine marginal zone B cell development and Dll4 binds Notch1 to efficiently initiate T cell development (14). Diversity in Notch signaling is not only restricted to the expression of different ligands in neighboring cells; post-translational modification of the Notch receptor by glycosyltransferases of the Fringe family also influences ligand specificity (15). In addition, protein turnover, specifically ubiquitination of the Delta ligand, regulates its endocytosis, thus affecting signaling near cells where Delta is presented (16). These examples illustrate the complexity of Notch receptor-ligand interactions, which are discussed in detail in recent reviews (17, 18)

Initiation of Notch signaling occurs when the Notch receptor is activated by ligand-induced proteolysis. Two successive proteolytic events occur subsequent to binding of the ligand DSL domain to the EGF-repeats of the receptor: 1) ADAM10, a metalloprotease, first cleaves the extracellular domain of the Notch receptor, then 2) the intracellular notch (ICN) domain is released from the membrane by cleavage via the intramembrane aspartyl protease complex γ–secretase (19, 20). γ–secretase cleavage releases ICN, permitting it to translocate into the nucleus where it associates with CSL (acronym for RBP-J; CBF, Suppressor of Hairless, Lag1) (21, 22). Nuclear translocation of ICN leads to the formation of a transcriptionally active complex comprised of ICN, CSL, and a member of the Mastermind-like (MamL) family, which are large scaffolding proteins that recruit co-activators with histone acetyl transferase activity, including p300 (23–25). While there are many Notch target genes, some of which will be further discussed below in the hematopoietic context, the Hes and Hrt proteins are two of the best characterized targets with roles in multiple developmental programs including neurogenesis, myogenesis, and intestinal differentiation (13, 26). Hes genes generally function to repress transcription of other genes by forming complexes with co-repressors, such as TLE/Groucho. It is important to note that many Notch transcriptional targets, including Hes, are not exclusively activated by Notch; thus, target gene expression, by itself, is not indicative of Notch signaling. The Notch transcriptional activation complex is short-lived. MamL likely recruits kinases, such as Cdk8, that lead to ICN phosphorylation and ubiquitination (27). An important E3 ligase in this process is FBW7 (27, 28). The physiological rate of ICN turnover is of great importance, and is best exhibited by the pro-oncogenic consequences of PEST mutations, which decrease turnover (29, 30)

Notch and angiogenic development

The initial functions of Notch in embryonic HSC development are intimately tied to its activities in vasculogenesis; thus before discussing Notch and HSCs, it is important to review the role of Notch in vascular development and angiogenesis. The first embryonic HSCs are observed in the dorsal aorta, vitelline artery and umbilical artery (31, 32) but not in venous vessels, suggesting that arterial specification is an important prerequisite for HSC emergence. Notch is essential for mammalian vascular morphogenesis and artery specification; Notch1-deficient embryos have widespread vascular defects leading to death at embryonic day (e) 9.5. Although Notch4 deficiency by itself does not perturb embryonic development, it exacerbates Notch1 deficiency by producing embryos with more severe defects in angiogenic organization (33). Targeted deletions of other components of the Notch signaling pathway, such as CSL (33, 34) Mib1 (35), or Hey1 and Hey2 (36), also caused loss

of arterial specification. Loss of just a single copy of the ligand, Delta-like-4 (Dll4), led to aortic constriction with reduced vitelline circulation and an increased pericardial space (37). The severe arterial phenotype caused by decreased Dll4 copy number suggests that this is the most important Notch ligand in vascular development. Although some defects are observed in Jagged1 mutants, additional work is needed to determine its role in angiogenesis.

In terms of the angiogenic signal transduction pathways, Notch lies downstream of VEGF and upstream of EphrinB2. Targeted mutagenesis of VegfA identified an essential role in vascular development as mouse embryos heterozygous for a *VegfA* mutation have constricted blood vessels (38, 39). Conversely, expression of individual VegfA isoforms in transgenic mouse models demonstrated that VegfA regulates arterial endothelial cell fates by allowing different populations of endothelial cells to migrate, proliferate and form new blood vessels in microenvironments of various tissues (40). When mammalian cell cultures are supplemented with VEGFA, they induce Notch1 and Dll4 expression in arterial endothelial cells, but not in venous endothelial cells (41). Furthermore, the VegfA-Notch signaling axis is also prominent during the emergence of tip cells and formation of angiogenic sprouts. Tip cells and the stalk cells that follow them are responsible for the generation of new blood vessels while maintaining contact with the original vessel from which the sprout formed. Tip cells are induced by a VEGF gradient, and express Dll4, thus activating the Notch1 receptor on the stalk cells (42). The interplay between tip and stalk cells allows Notch signaling to function in the growth and maturation of newly formed arterial vessels. COUP-TFII inhibits Notch signaling in endothelial cells, allowing veins to form instead of arteries (43). *EphrinB2*, which produces a ligand for the Eph receptor, was identified as a direct target of Notch signaling in human endothelial cell lines (44). Downstream of Notch, EphrinB2 maintains arterial cell identity, prevents arteriovenous shunt formation and allows for remodeling of the vascular plexus into individual vessels and capillary beds (45). When Notch signaling is impaired, *EphrinB2* expression is lost (33, 34, 36, 37, 46, 47).

Definitive hematopoiesis initiates in several anatomical sites from hemogenic endothelium. (48–53). The dorsal aorta in the vicinity of the developing urogenital ridges (aorta/gonad/mesonephros, or AGM region), is one of the best characterized regions of hematopoietic stem cell (HSC) formation (54). In the AGM, expression of the Notch receptors, Notch1 and Notch4, and ligands, Dll4, Jagged1 and Jagged2, persists following arterial specification through the time when hematopoietic cells are forming via an endothelial to hematopoietic cell transition (55–57). Multiple cell surface markers, such as PECAM-1 (58), the angiopoietin receptor Tie-2 (59), CD34 (60), VE-cadherin and Flk1 (61, 62) are shared between endothelial and hematopoietic cells (63, 64). The close relationship between endothelial cells and HSCs is strengthened by functional studies showing that endothelial cells can generate hematopoietic cells in vivo, and that endothelial progenitors derived from embryonic stem cells can differentiate first into endothelial cells, which in turn give rise to hematopoietic cells (29, 48, 49, 51–53, 65–67). Notch is thought to function both in establishing arterial identity and generating the definitive HSC (55, 68–70). Nevertheless, it has not yet been possible to determine whether Notch is responsible for the architectural

organization of the sites of HSC emergence or whether it plays a direct mechanistic function in the formation of the definitive hematopoietic stem cell.

HSC emergence

Embryonic hematopoiesis is accomplished by the collective and overlapping specification of progenitor cells into blood cells at various embryonic sites that fluctuate during development (Fig. 1). The first organ with hematopoietic potential is the yolk sac, starting at e7.25 in the mouse (71, 72). At this time of embryonic development, a functional vascular network has not yet emerged, resulting in the *in situ* generation of hematopoietic cells in the yolk sac. The yolk sac initially produces macrophages, megakaryocytes, and nucleated red blood cells, the latter of which aid in the oxygenation of the rapidly developing embryo (72, 73). This initial wave of hematopoiesis is deemed “primitive”, as the most predominant cells produced are primitive erythrocytes, which can be distinguished from adult “definitive” erythrocytes by virtue of their large size, the presence of a nucleus in circulating cells, and the expression of embryonic hemoglobin (74).

Definitive hematopoiesis begins in the yolk sac, but then shifts to the embryo proper, the vitelline and umbilical vasculature, and the placenta. (54). The first definitive cells produced are committed progenitors for definitive erythroid and myeloid lineage cells but not lymphoid lineage cells, and appear in the yolk sac starting at e8.5, and slightly later in the caudal region of the embryo itself (75). At e9.5, HSCs capable of engrafting the myeloid and erythroid lineages of busulfan-conditioned neonatal mice (neonatal repopulating HSCs), but not adult mice can be found in the yolk sac (76). Later, beginning at e10.5, HSCs that can engraft an adult mouse and give rise to all of the blood lineages, including lymphocytes, appear. (32, 77). The best-characterized location for the generation of HSCs is the para-aortic splanchnopleura aorta-gonad-mesonephros (PsP/AGM) region, which includes the dorsal aorta (32, 77). Multiple systematic approaches involving several model systems, such as quail-chick chimeras, diploid-triploid Xenopus embryos, and transplantation experiments with mice showed that the AGM-like region harbored HSCs (78, 79). The dorsal aortae in the AGM region are formed at e8 by the medial migration of a sheet of lateral mesoderm, which touches a plate of endodermal cells. The paired aortae later fuse to form a single aortic tube (80). Visible clusters of hematopoietic cells are observed to form beginning at e9.5 in the vitelline and umbilical arteries, and slightly afterwards in the ventral wall of the dorsal aorta (64, 81). The combination of HSC activity and the visualization of hematopoietic clusters makes these major arteries (dorsal aorta, vitelline and umbilical) the most credible sites for the emergence of the first definitive mammalian HSC. Recent evidence suggests that the placenta is also a site of early HSC emergence or at least a niche for HSC expansion (82–84). The placental HSCs are clearly functional, however, the onset of placental LT-HSC activity is not earlier than in the AGM, and given its high vascular content and location downstream of the dorsal aorta in the fetal circulation, it may be that the HSCs, which emerged in the AGM, migrated and colonized the placenta before moving on to their eventual embryonic depository in the fetal liver. An inherent challenge in defining the anatomic origins of HSCs is that blood is a mobile organ, thus distinguishing sites of emergence from those of colonization can be difficult. For example HSCs are found in the

yolk sac at e11.5, but they are also detectable in circulating blood, raising the possibility that they may have migrated to the yolk sac through the circulation (85, 86).

Despite its robust potency to generate de novo LT-HSCs, the AGM is not a site for HSC expansion (87). This may be due to its anatomical location in a high flow area of the circulation, however, this may not be the full explanation as large clusters are present in the umbilical artery, which connects the dorsal aorta to the placenta (64, 81). HSCs are detected in the fetal liver circa. e11 and reside there until near birth at e18. It is during their time in the fetal liver that the fetal HSCs expand and acquire the surface markers that ultimately define them in the adult bone marrow (BM) (88, 89). The fetal liver supports HSC differentiation into all of the hematopoietic lineages, with the exception of T cells, whose specification occurs in the thymus (72).

The fetal liver is the site of greatest HSC expansion during ontogeny (90–92). While it would seem that this unique feature of the fetal liver environment would be of high interest for its potential clinical relevance in understanding and harnessing HSC expansion, an absence of markers that would allow better identification of the HSC niche in the fetal liver, has made direct visualization and subsequent study of the interaction between the hematopoietic and stromal compartments in the fetal liver challenging. To date, it is not fully understood how HSCs can expand so rapidly in the fetal liver, and yet maintain their long-term reconstituting potential.

Notch and Definitive Hematopoiesis

The intimate connection between embryonic arterial and HSC development suggested that Notch signaling may have a role in this process (69, 70). The connection between Notch and definitive hematopoiesis was made by Kumano *et al.*, who showed that Notch1 signaling was required to generate neonatal-repopulating HSCs in the AGM region (55). When analyzing embryos of either Notch1-deficient or Notch2-deficient mice, they observed that neonatal repopulating HSC development and angiogenesis were severely impaired in the Notch1-null embryos but were unaffected in Notch2-null embryos. Furthermore, they found that eliminating Notch signaling had no effect on either primitive or definitive hematopoiesis in the yolk sac. To establish the timing of Notch involvement, they explanted e9 or e10 wild-type AGM regions in the presence of a γ-secretase inhibitor, and observed that elimination of Notch signaling impaired the generation of hematopoietic progenitors at e9. However, when γ-secretase inhibitor was added at e10, it did not affect the ability of hematopoietic progenitors to proliferate or differentiate in colony assays (55). These data argued that Notch signaling was required for the generation, but not for the proliferation or maintenance of hematopoietic cells.

The essential role of Notch1 signaling in generating HSCs has been confirmed by multiple approaches. Like the Notch1-deficient mice, RBP-J/CSL and mindbomb (*Mib1*)-deficient embryos lacked AGM hematopoiesis, but contained primitive progenitors in the yolk sac (56, 93) some of which, such as the erythroid linneage, showed an increase in cell number (94). A cell autonomous function for Notch1 was demonstrated in studies by Hadland *et al.,* who investigated the developmental potential of Notch1-deficient embryonic stem cells

using *in vitro* differentiation and *in vivo* chimera generation (68). They found that despite the capacity to maintain a progenitor pool in the yolk sac, the Notch1-deficient embryonic stem cells contributed poorly to the fetal liver and failed to contribute to bone marrow. In contrast, Notch2-4 deficiency does not inhibit hematopoiesis, even though Notch2 and Notch3-deficient mice exhibit defects in vasculogenesis (33, 93, 95, 96). It is not clear whether the essential function for Notch1 in embryonic HSC generation results from the unique expression of this receptor in the HSC progenitor cells, expression of ligands which favor Notch1 signaling or specific aspects of the Notch1 receptor itself. With regards to the latter, Notch1 is the most potent of the four receptors in activating transcription. Studies using knockin mice containing chimeric Notch receptors will help to elucidate the role of specific Notch receptors in HSC generation.

From the ligand perspective, animals deficient in Dll4 die at e9.5 from severe vascular defects primarily due to the lack of arterial organization (37). In contrast, loss of Jagged1 allows for the establishment of the arterial program and expression of *EphrinB2*, but impairs hematopoietic potential of the AGM (57); whereas, Jagged2 knockout mice exhibit no differences in vasculogenesis or hematopoiesis when compared to wildtype mice. Taken together, these results begin to attribute certain developmental roles to specific ligands, with Dll4 being responsible for the initiation of the Notch-mediated vascular program and Jagged1 coupling with Notch1 to initiate definitive hematopoiesis. As the HSC defect is not absolute in the Jagged1-deficient mice (57), other ligands are likely to be involved. The precise way that Notch ligands orchestrate vasculogenesis and hematopoiesis is currently unknown.

The interaction occurring on the surface of two neighboring cells between the Notch receptor and its ligand is just the initial step in a process that ultimately leads to the transcriptional regulation of Notch target genes and definitive HSC emergence. Tissue specific regulation of Notch targets is a common theme in Notch signaling; some targets, such as Hes and Hrt, play roles in numerous tissues ranging from the nervous system to muscle (26), while other targets such as GATA3, CD25 and pre-Tα have specific and temporally regulated functions in T-cell development (97–99). In the hematopoietic program, identifying the pertinent target genes has not been easy, mainly due to the challenge of distinguishing between a Notch target involved in determination of the aortic cell fate as opposed to a target specifically required for HSC generation. To date, the list of proposed candidates is neither long nor definitive. GATA2 expression is lost in the aortic endothelium of Jagged1-null embryos, where it is directly regulated by Notch1 (57). Evidence of the role of Hes1 in maintenance of HSC self-renewal has been observed *in vitro* (100) yet its importance in HSCs emergence from the AGM is not known. Runx1 is required for HSC emergence (62), where its function is primarily restricted to the endothelial to hematopoietic cell fate conversion and does not extend to other aspects of HSC biology, although it loss contributes to leukemia (101). Direct regulation of Runx1 by Notch has not yet been described, however, the observation that ectopic Runx expression partially rescues the hematopoietic defects observed in the Notch1-deficient AGM suggests that these two pathways are linked (102). In addition, mutations that affect Notch signaling have been shown to decrease Runx1 expression in both mice and zebrafish (55, 57, 93, 103). In zebrafish, BMP4 is required for Runx1-dependent HSC generation (104), and its expression

has been reported in the mesenchyme tissue below the dorsal aorta, adjacent to Runx1 expressing cells (105, 106). Synergy between Notch and BMP signaling may be required for GATA2 and Runx1 expression, which in turn, drives the endothelial to hematopoietic cell fate conversion and ultimately the generation of HSCs.

Notch in adult hematopoietic stem cells

Although it is clear that Notch1 is essential to produce definitive HSCs in the embryo, the requirement for Notch signaling at subsequent stages of mammalian HSC development has generated significant controversy. Some of this controversy is driven by gain of function studies in which Notch signaling was induced *in vitro* by various experimental techniques; whereas other aspects of the controversy are driven by disparate results utilizing different reagents.

Multiple gain-of-function studies have suggested that Notch induction can expand hematopoietic progenitors, including stem cells. Adding soluble Jagged1 ligand to *ex vivo* cultures of human $CD34^{+}CD38^{-}Lin^{-}$ cord blood cells (107) or incubating murine BM $Lin^{-}Sca1^{+}cKit^{+}$ (LSK) cells with a fusion protein containing the Dll1 ligand (108) caused *in vitro* expansion of these progenitor populations and allowed for short-term hematopoietic reconstitution in mice following transplantation. In another study, osteoblastic cells engineered to express high levels of Jagged1 by constitutive expression of parathyroid hormone regulators, led to an increase in the number of hematopoietic cells in the BM of mice (109). Although this study showed that Jagged1 upregulation led to concomitant intracellular Notch1 production, the ultimate effect of constitutive parathyroid hormone activation in osteoblasts may account for alternative means of HSC expansion as parathyroid hormone activates bone remodeling and the osteoblastic secretion of multiple growth factors, including IGF-1 and BMPs, all of which could influence HSC homeostasis (110, 111). Constitutive Notch signaling via expression of the Notch1 intracellular domain expanded hematopoietic progenitors both *in vitro* and *in vivo*, however, these Notch1-expanded progenitors either showed extra-thymic T-cell development or were unable to provide serial reconstitution of lethally irradiated mice, suggesting that they were not bone fide LT-HSCs (103, 112, 113). Hes1 may be an important mediator of progenitor expansion as retroviral Hes1 expression prolongs *ex vivo* self-renewal of murine HSCs (100) Employing this well-established *ex vivo* system, recent findings have uncovered diverse roles carried out by Notch receptors, particularly Notch2, in the generation and maintenance of primitive progenitors ($sca1^{+}c\text{-}kit^{+}$) at the expense of myeloid differentiation (114). Perhaps the most compelling evidence for Notch-induced expansion of LT-HSCs comes from recent *ex vivo* co-culture experiments that rely on endothelial cell expression of Jagged1, Jagged2, Dll1 and Dll4 to stimulate Notch signaling in HSCs. These studies showed that Notch expanded the $CD34^{-}Flt3^{-}LSK$ long-term repopulating fraction, which was able to provide durable, multi-lineage hematopoietic reconstitution in lethally irradiated mice. The self-renewal capacity observed in this study required both Notch1 and Notch2 expression in the co-cultured HSCs (115)

Together, these gain-of-function studies show that Notch induction has the potential to expand multipotent hematopoietic progenitors that can generate all of the hematopoietic

lineages, and in some circumstances, produce bone fide LT-HSCs. These findings have already led to promising applications in the clinics. For example, Notch ligands were used to expand cord blood progenitors that shortened the time to engraftment in several patients that received the ex vivo expanded cells (116). As shortening the time to engraftment decreases the complications of bone marrow transplant, these results suggest that Notch-expanded HSCs have the potential for tremendous benefit in the post-BMT setting.

Although these studies demonstrate an important role for Notch in expanding multipotent progenitors, they do not show that Notch is essential for HSCs subsequent to their emergence in the early embryo. Although one study suggested that Notch signaling was high in murine HSCs and played an important role in self-renewal (117), multiple studies using different loss-of-function approaches have failed to identify an essential role for Notch signaling in adult HSCs in the mouse. Deletion of *Notch1, Notch1* and *Notch2* or *RBP-J* had no effect on adult HSCs in the mouse (118–122). Likewise, a combination of Notch1 and Jagged1 inactivation in BM progenitors or BM stromal cells produced similar results (119). In addition, transgenic expression of DNMAML, which prevents transcriptional activation by all four Notch receptors failed to influence LT-HSCs in mice (122). All four of these approaches eliminated Notch1-dependent T cell development, showing that Notch signaling had been blocked. Not only did these studies provide immunophenotypic and functional evidence that Notch did not influence adult HSC homeostasis under steady-state conditions, but they also failed to identify an important function for Notch when HSCs were exposed to proliferative stress. For example, neither 5-FU treatment or a competitive secondary transplant of DNMAML-expressing HSCs resulted in a significant difference in the reconstitution of the hematopoietic system (122). Even though both Notch1 and Notch2 were expressed on murine HSCs, the expression of several Notch transcriptional targets, such as Hes1 and Dtx1 was very low (122). One caveat to the latter point is that the identity of the direct Notch targets in HSCs is unknown. Furthermore, even though Hes1 may have a role in HSC expansion, conditional genetic inactivation of Hes1 in adult mice, had no effect on the LT-HCS and progenitor frequency in the BM (123). Together, these multiple lines of genetic data provide strong evidence that Notch signaling does not have an essential role in many aspects of adult murine HSC biology.

Concluding Remarks

While the debate over the role of Notch in post-natal hematopoiesis has persisted over a decade, the wealth of data generated by both sides of the argument has increased our understanding of Notch signaling and of the potential that this important signaling pathway could provide if properly harnessed. Though a direct role of Notch signaling in adult HCSs residing in the bone marrow microenvironment cannot be established, the capacity to expand cord blood or bone marrow stem cells and progenitors by the careful administration of Notch ligands in regulated culture conditions may prove to be of great clinical importance. Future investigation on the mechanism by which these quiescent, Notch irresponsive post-natal HCSs, are capable of self-renewal in the presence of Notch ligand should provide important insights into the developmental biology of hematopoiesis. For example, the spatiotemporal regulation of Notch signaling in hematopoiesis may not be a rigid one-way street, but may be visualized as a parabolic swing of the developmental pendulum where high expression of

Notch in HCSs indicates an earlier developmental stage, such as HCS emergence from the AGM or expansion without exhaustion exhibited in the fetal liver, low expression represents the quiescent state of HSCs in the bone marrow niche, and once again, high levels are required for T cell development in adult hematopoiesis. Though this is a very simplified view of what is likely a very complex molecular mechanism, it is possible that the gain-of-function studies have rekindled an aspect of HSC developmental memory during which hematopoietic cells were actively expanding under the influence of Notch. These speculations, when placed in the overall context of Notch function in adult hematopoiesis, are likely to be most important in *ex vivo* experimental conditions, as extraphysiological levels of Notch in BM HSCs leads to abnormal hematopoiesis and ectopic T-cell development (120, 124). Nevertheless, the possibility of Notch to expand multipotent progenitors will not only impact patient care but may also be useful in human ES and iPS technologies, where it may be possible to use these strategies to expand developing HSCs.

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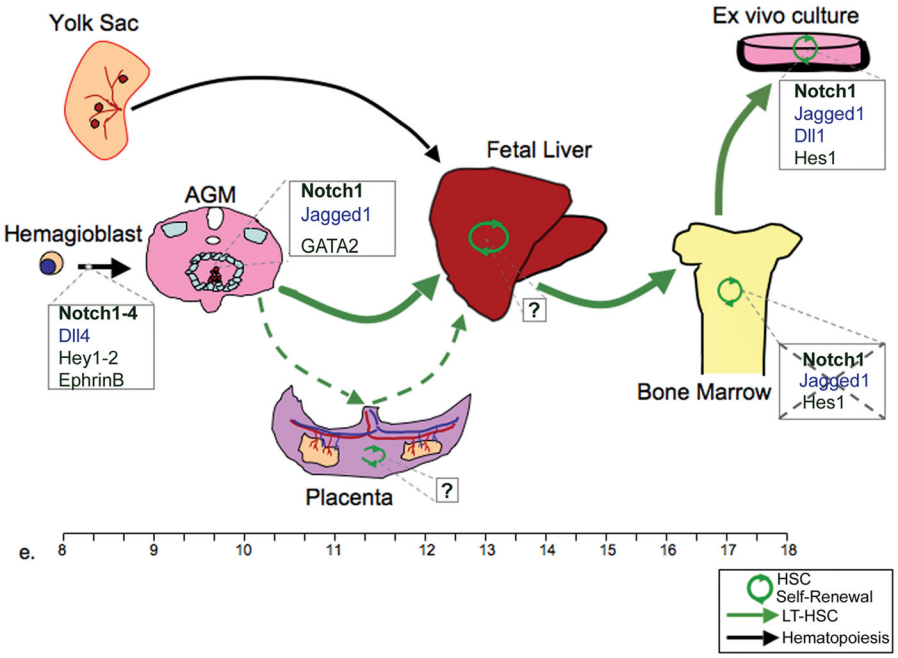

Figure 1. The role of Notch in generation and maturation of hematopoietic stem cells

Diagram shows the progression of hematopoietic stem cells (HSCs) in fetal development from the emergence of the LT-HCSs in the AGM at day e.9 and the migration to sites of self-renewal and expansion in the placenta and fetal liver (e.11) as well as final prenatal localization of HSCs in the bone marrow at e.17. Known involvement of Notch receptors (bold), ligands (in blue) and targets at various stages of HSC development are marked in boxes next to each fetal organ. Likewise, specific lack of function for Notch is displayed by strikethrough.