Notch in the niche: new insights into the role of Notch signaling in the bone marrow

Ashley N. Vanderbeck[1-3] and Ivan Maillard[2-4]

[1]VMD-PhD program at University of Pennsylvania School of Veterinary Medicine; [2]Immunology Graduate Group, University of Pennsylvania; [3]Abramson Family Cancer Research Institute, University of Pennsylvania and [4]Division of Hematology-Oncology, Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA

E-mail: IVAN MAILLARD - imaillar@pennmedicine.upenn.edu

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In the bone marrow, specialized non-hematopoietic cells form unique microenvironmental niches that support and regulate the functions of hematopoietic stem and progenitor cells (HSPC).[1] Although many niche factors are well defined, the role of Notch signaling remains controversial (see Figure 1). Notch signaling in HSPC has been reported to regulate hematopoietic stem cell maintenance, suppress myelopoiesis, and promote megakaryocyte/erythroid cell development.[2-7] Mechanistically, most previous reports have been built on the concept that Notch receptors in HSPC interact with Notch ligands expressed in niche endothelial cells, or alternatively in other components of the bone marrow (including other non-hematopoietic and hematopoietic cells) (Figure 1, ① and ③). In contrast, several genetic models that inhibit all transcriptional effects of Notch signaling only in HSPC indicated that canonical Notch signaling is dispensable for HSPC maintenance, as well as myelo-erythropoiesis, under both homeostatic and stress conditions.[8,9] In this issue of *Haematologica*, Shao *et al.* bring a new perspective to this debate: perhaps Notch signaling is critical for stress hematopoiesis, but indirectly so by promoting niche cell regeneration through Notch ligand-receptor interactions that remain confined to the bone marrow endothelium[10] (Figure 1, ②).

Unlike secreted niche factors, Notch signaling is a juxtacrine communication pathway between signal-sending cells expressing agonistic Notch ligands (Dll1, Dll4, Jagged1, or Jagged2), and signal-receiving cells expressing Notch receptors (Notch1-4).[11] Ligand-receptor interactions induce regulated proteolytic cleavage of the Notch receptor, releasing the Notch intracellular domain which is then free to translocate to the nucleus and alter gene transcription in signal-receiving cells. Notch receptor and ligand expression has been reported in HSPC, osteoblasts, as well as key constituents of the perivascular niche, such as bone marrow endothelial cells.[2,3,5,6,12,13] Because Notch ligands and receptors are expressed by a variety of hematopoietic and non-hematopoietic cells, defining specific interactions that are biologically and functionally relevant for the HSPC microenvironment is a difficult task. For example, Notch signaling could be an important aspect of either endothelial-hematopoietic cell cross-talk (Figure 1, ①), or communication directly between endothelial niche cells (Figure 1, ②). Likewise, tight control of Notch signaling between hematopoietic cells is essential, as de-repression of *Dll4* in erythroblasts leads to premature differentiation of HSPC into T cells (Figure 1, ③).[14]

Shao *et al.* provide compelling new data indicating that activation of Notch signaling between endothelial cells is a key component of HSPC niche restoration after bone marrow injury. Hematopoietic cell recovery after chemotherapy or radiation-induced myelosuppression relies heavily on regeneration of the endothelial cell network in order to support the hematopoietic compartment.[6,15,16] By examining the role of Notch signaling after injury using bone marrow chimeras and genetic models of cell type-specific Notch inactivation, Shao *et al.* dissected the functional importance of two possible routes of communication: cross-talk between endothelial cells and HSPC (Figure 1, ①), as well as Notch signaling between endothelial cells (Figure 1, ②) that indirectly affects HSPC. First, the authors demonstrated that endothelial restoration after bone marrow injury relied on activation of Notch signaling through the Notch1 receptor. Myeloablative stress induced by chemotherapy or irradiation caused lethal pancytopenia in mice harboring a hypomorphic *Notch1* allele. This phenotype was linked to a reduction in the number and frequency of HSPC after injury. Depletion of lymphoid-primed progenitors was also apparent. However, transplantation of *Notch1* hypomorphic HSPC into wildtype hosts revealed that the hematopoietic recovery defect was extrinsic to HSPC. Moreover, ablation of the *Notch1* receptor gene specifically in bone marrow endothelial cells using a tamoxifen-inducible VE-cadherin Cre^{ERT2} transgene recapitulated the pancytopenia, morbidity and hematopoietic failure observed after injury in *Notch1* hypomorphic mice. Together, these data suggest a role for Notch signaling during endothelial cell recovery. To further investigate this hypothesis, the authors found that Notch signaling was promptly activated in bone marrow endothelial cells after injury. Tie2 activation, which is critical for endothelial cell regeneration, appeared to enhance Notch signaling by inducing expression of both Notch receptors and ligands in bone marrow endothelial cells.[16] Thus, Notch signals could be induced in bone marrow endothelial cells via a cross-talk involving expression of both Notch ligands and receptors in the endothelial compartment, with subsets of cells functioning as signal-sending and others as signal-receiving cells (Figure 1, ②) .

When considering the impact of Notch signaling in the bone marrow, it has often been assumed that the only functionally significant signals for hematopoiesis are mediated directly between niche cells and HSPC. However, it is also possible that non-cell-autonomous signals regulate HSPC function indirectly, while cell-autonomous Notch signals are dispensable. This concept has been entertained previously, as transplantation of wildtype bone marrow cells into recipient mice lacking the capacity to undergo Notch-driven signals in the radioresistant host compartment led to altered hematopoietic differentiation, and eventually to myeloproliferative disease.[17] Likewise, Shao *et al.* highlight an often overlooked potential mechanism of HSPC regulation by showing that disruption of Notch signaling among endothe-

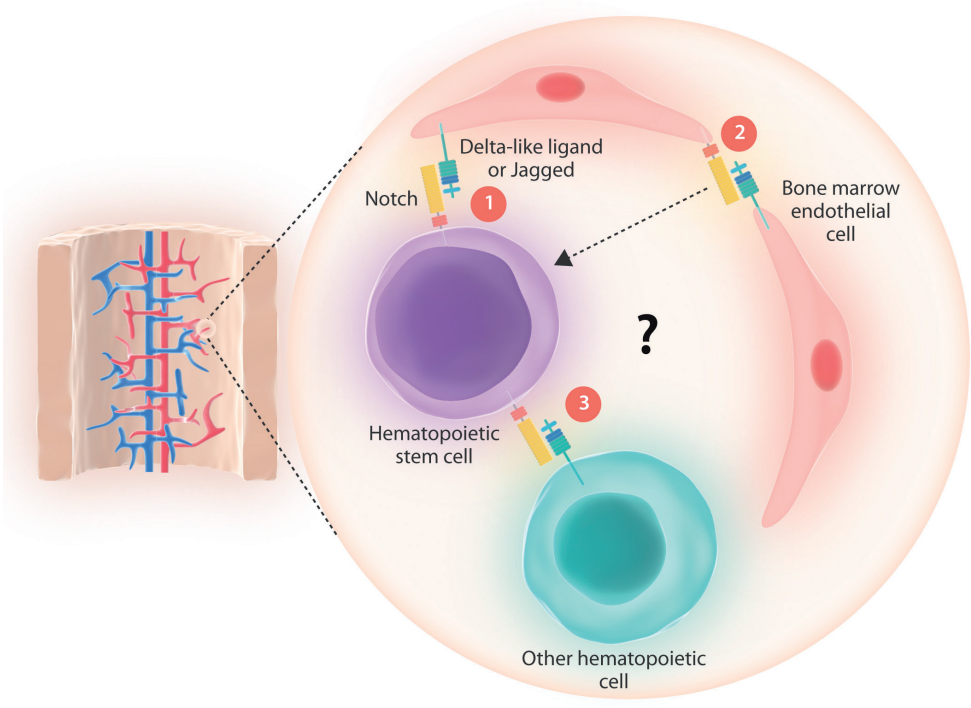

Figure 1. Potential routes of Notch signaling in the bone marrow. Notch ligands and receptors are expressed by both non-hematopoietic and hematopoietic cellular elements in the bone marrow. Potential routes of Notch signaling that influence the function of hematopoietic stem and progenitor cells (HSPC) include: (①) interaction of Notch ligands in endothelial cells with Notch receptors in HSPC; (②) Notch ligand-receptor interactions between endothelial cells, with indirect effects on HSPC; (③) interaction of Notch receptors in HSPC with Notch ligands expressed by other hematopoietic cells. In this issue, Shao *et al.* provide new data in support of Notch signaling within the endothelium (②) as a critical regulator of hematopoietic recovery after bone marrow injury.

lial cells impaired hematopoietic recovery (Figure 1, ②).

While the authors identified Notch signaling as an essential component of the response to injury in the bone marrow, the mechanisms underlying Notch's impact in this context remain unclear. The bone marrow injury response includes a complex interplay of signaling cues secreted from multiple cellular sources. For example, VEGF-A, as well as Angiopoietin-1, are thought to control regeneration and reassembly of the bone marrow vasculature.[15,16] Importantly, the cellular source, role and regulation of individual factors may differ markedly between steady-state conditions and after bone marrow injury.[18] Shao *et al.* found that lack of Notch activation after injury increased apoptosis among endothelial cells, suggesting that Notch functions as a pro-survival cue. During angiogenesis, Notch inhibits proliferation of endothelial cells and ultimately allows for proper formation of functional blood vessels.[19] Thus, Notch signaling may restrict bone marrow endothelial cell activation and entry into the cell cycle, ultimately protecting the endothelium from the DNA damage induced by chemotherapy and irradiation. Alternatively, Notch may have a more direct role in re-establishing the niche, analogous to its involvement in "tip/stalk" cell crosstalk during neoangiogenesis.[19] Sprouting of new vessels requires a delicate balance of tip/stalk cell differentiation in which tip endothelial cells lead new vessel sprouting for invasion and migration. Tip cells are highly responsive to VEGF, require a high glycolytic flux and, although they express Dll4, do not actively engage Notch signaling. On the other hand, stalk cells undergo high levels of Notch signaling which reduces expression of VEGFR2/3 and glycolytic enzymes, ultimately helping to repress the tip cell fate while maintaining stalk cell identity.[19] Thus, Notch activity may be important during early stages of bone marrow vasculature reassembly by regulating pathways similar to tip/stalk cell differentiation and by integrating angiogenic cues with the metabolic status of the endothelium. Finally, it is possible that Notch acts through yet to be discovered mechanisms unique to the bone marrow vasculature, whose regulation during steady-state conditions and after injury remains only partially understood.

Notch signaling may also have roles in hematopoiesis beyond its functions in the HSPC niche. *Dll4* inactivation in mesenchymal progenitor cells was reported to decrease bone marrow common lymphoid progenitor numbers and impair thymopoiesis.[12] Similarly, endothelial *Dll4* inactivation was recently linked to decreased lymphoid progenitors and enhanced myelopoiesis.[5] Consistent with these data, Shao *et al.* reported a cell-autonomous hematopoietic cell defect in T-cell production by mice that received transplanted *Notch1* hypomorphic HSPC, which was associated with decreased numbers of lymphoid progenitors in the bone marrow. Altogether, these data leave room for the possibility of a bone marrow niche that provides prethymic Notch signals during early lymphoid development, in addition to the effects of Notch signaling in niche regeneration.

As another important consideration, individual Notch ligand/receptor pairs may have unique effects on hematopoietic function. Shao *et al.* focus on signaling through the Notch1 receptor, which is the predominant receptor expressed in endothelial cells. However, both Notch1 and Notch2 are present in HSPC, and a specific role for Notch2 in HSPC differentiation following bone marrow injury has been reported.[3] Recent advances in the biophysics of Notch signaling could provide explanations as to how engagement of distinct receptor-ligand pairs can lead to divergent functions.[20] Nandagopal *et al.* showed that Dll1/Notch1 signaling induced pulsatile Notch activation whereas Dll4/Notch1 signaling resulted in sustained Notch activation during myogenesis, allowing for ligand discrimination. Additional differences in the signaling potential of specific ligand-receptor pairs may also exist.[21] Whether similar biophysical and functional differences apply to the effects of individual Notch receptors in hematopoietic progenitors remains to be investigated.

Altogether, Shao *et al.* provide compelling data indicating that activation of Notch signaling between bone marrow endothelial cells is necessary for niche regeneration, as well as efficient and timely hematopoietic recovery after bone marrow injury. With a panoply of Notch receptors and ligands expressed throughout the bone marrow, Notch has the potential to regulate a number of communication channels between and among bone marrow cellular compartments. Future research should parse these cellular conversations to fully understand how Notch signaling helps to orchestrate hematopoiesis.

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Time for revival of the red blood cell count and red cell mass in the differential diagnosis between essential thrombocythemia and polycythemia vera?

Hans Carl Hasselbalch

Department of Hematology, Zealand University Hospital, Roskilde, Denmark

E-mail: HANS CARL HASSELBALCH - hans.hasselbalch@gmail.com

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Red blood cell indices, red blood cell mass and bone marrow biopsy in the differential diagnosis between essential thrombocythemia and polycythemia vera?

The correct diagnostic classification of the Philadelphia-negative chronic myeloproliferative neoplasms (MPN) in the three subcategories, essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF), relies upon diagnostic criteria that aim at minimizing misclassification.[1] Several reports have addressed the issue that *JAK2*V617F positive "ET" patients are frequently misclassified since they actually have a diagnosis of PV.[2-8] This misclassification is partly based upon the use of the hemoglobin (Hb) concentration as a surrogate marker for the red cell mass (RCM), irrespective of the fact that the Hb concentration is influenced by iron deficiency, which is prevalent in PV patients. Indeed, these concerns have been addressed and confirmed in several studies showing that a high proportion of ET patients (approx. 45-65%) did not meet the World Health Organization (WHO) diagnostic criterion