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The Notch pathway in the developing hematopoietic system

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ABSTRACT The main function of the Notch signaling pathway is to generate cell diversity during both embryonic development and adult tissue homeostasis. The extended use of this pathway, together with its conservation during evolution, is indicative of its importance. During embryonic development, the vascular and hematopoietic systems are intimately associated and Notch signals are responsible for the correct specification of both systems. More explicitly, Notch is required for the induction of the arterial program; however, it is simultaneously or consecutively also involved in the generation of hematopoietic stem cells. Although both genetic programs are different, they are both implemented in endothelial cells of the dorsal aorta in the midgestation embryo. This close association during the development of arteries and blood has hindered our understanding of Notch function in the generation of hematopoietic stem cells. Here, we will review the work from recent years showing how Notch participates in the embryonic development of hematopoiesis in the mouse, but also in other organisms such as chick, zebrafish and flies.

KEY WORDS: *Notch, AGM, yolk sac, hematopoiesis, embryo*

The Notch pathway and cell fate specification

During evolution, multicellular organisms have developed regulatory mechanisms to ensure the fine-tuned and reproducible development of the different organs and tissues. Direct cell-cell interactions between neighbouring cells are crucial to govern most of these processes and one of the important pathways regulating these interactions is the Notch pathway [reviewed in (Lai 2004)].

Notch was first identified in 1914 by genetic experiments in *Drosophila* and received its name from indentations (notches) that form in the wing of mutant flies (Dexter 1914) (Morgan TH,CB 1916). Since then, Notch function has been the focus of extensive research and nowadays it is known that it regulates a wide variety of developmental processes such as neurogenesis, miogenesis, hematopoiesis, intestinal and pancreatic differentiation, wing formation and somite segregation among others (reviewed in Lewis, 1998). In this review, we will present an extensive overview of the role of Notch signaling pathway in vascular and hematopoietic development.

Key players and mechanisms

The Notch signaling pathway includes Notch receptors, the Delta and Serrate/Jagged ligands and the nuclear transcription factor CSL (that accounts for CBF1; recombinant binding protein-J kappa (RBPjκ); Suppressor of Hairless (Su[H]); Lag-1). Indicative of the importance of this pathway, orthologues of most Notch family members are found during evolution from nematodes to mammals as summarized in Table 1.

The Notch receptor

The Notch receptor is a transmembrane protein involved in transducing specific extracellular signals to the nucleus in response to ligand binding. The extracellular part of the receptor contains multiple epidermal growth factor (EGF) repeats. In flies, repeats 11 and 12 of the Notch receptor are responsible for ligand-binding (Rebay *et al.,* 1991). The extracellular part of the receptor also contains the Lin/Notch repeats (LNR), involved in maintaining the heterodimeric structure by disulphide bridges, thus preventing ligand-independent activation (Weng *et al.,* 2004). The intracellular Notch (NotchIC) contains several functional domains including the RAM domain and the ankyrin (ANK) repeats, required for the interaction with its intracellular partners; the nuclear localization signals; the transactivation domain and

Abbreviations used in this paper: AGM, aorta-gonad-mesonephros; HSC, hematopoietic stem cell; KSL, c-kit$^+$Sca1$^+$Lin$^-$; P-Sp, para-aortic splanchnopleura; YS, yolk sac.

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the C-terminal PEST domain that regulates protein stability (Fryer *et al.,* 2002; Thompson *et al.,* 2007). Notch molecule is translated from a single mRNA transcript, that in the Golgi complex is first cleaved (S1 site) by a furin convertase and subsequently reassembled by disulphite bridges into the functional heterodimeric receptor present at the cell surface [reviewed in (Maillard *et al.,* 2003)] (see Fig. 1).

Jagged and Delta ligands

Signaling through the Notch receptor is triggered by the interaction with one of the Notch ligands expressed in the adjacent cell (presenting cell) (see Fig. 1). The Notch ligands are transmembrane proteins that contain multiple EGF-like repeats and a characteristic DSL domain (DSL accounts for Delta, Serrate and Lag2). Both EGF and DSL repeats are involved in Notch receptor interaction, however peptides solely containing the DSL domain are sufficient to activate Notch in some systems (Shimizu *et al.,* 2000). Two different ligands have been identified in *Drosophila* (Delta and Serrate), whereas five different ligands are present in vertebrates (Jagged1,2, Delta1,3,4), and an additional ligand, Delta2, specific for *Xenopus*. Serrate and its orthologues Jagged1 and 2 differ from Delta ligands in the number of EGF-like repeats and in the presence of a cysteine-rich domain [reviewed in (Ohishi *et al.,* 2003)]. The intracellular domain of Jagged and Delta is composed by a few aminoacids and it is not known whether it displays any function in the presenting cell.

Glycosilation of the Notch receptor by Fringe glycosyltransferases (Radical, Lunatic and Manic Fringe) is responsible for modulating ligand specificity. Fringe proteins can regulate Notch activity and contribute to the generation of cell diversity [reviewed in (Irvine 1999)].

The importance of ubiquitination is evidenced by the Notch-loss of function phenotypes produced by mutations in the Delta-specific E3 ubiquitin ligases *Neuralized* and *Mind bomb* (Lai *et al.,* 2001; Itoh *et al.,* 2003). Recent studies have shown that ubiquitination regulates endocytosis of the Delta ligand that is required for Notch activation [reviewed in (Ben-Yaacov *et al.,* 2001)].

TABLE 1

BASIC COMPONENTS OF THE NOTCH SIGNALING PATHWAY

Notch pathway element	*C. elegans*	*D. melanogaster*	*Mammals*
Receptor (Notch)	LIN-12 GLP-1	Notch	Notch1 Notch2 Notch3 Notch4
Ligand	LAG-2 APX-1 ARG-2 F26B12.2	Delta Serrate	Delta1/Delta-like1 (Dll1) Delta3/Delta-like3 (Dll3) Delta4/Delta-like4 (Dll4) Jagged1 (JAG1) Jagged2 (JAG2)
DNA-binding protein (CSL)	LAG-1	Suppressor of Hairless (Su(H))	CBF1/RBPjk RBPL

Adapted from Lai (2004). Notch receptor, ligand and transcription factor in different species (*Caenhorabditis elegans*, *Drosophila melanogaster* and mammals).

Activation of the Notch pathway

The unbalanced levels of Notch receptors and ligands on the surface of adjacent cells trigger the activation of the Notch pathway (see Fig. 1). Binding of the Notch receptor to one of its ligands leads to two successive proteolytic cleavages in the Notch molecule (Kopan *et al.,* 1996) which in turn, is translocated to the nucleus to exert its function. The first cleavage (S2) occurs in the extracellular domain at the LIN/Notch repeats (LNR) and it is mediated by an ADAM metalloprotease called TACE (TNF-α converting enzyme) in vertebrates or *Kuzbanian* in *Drosophila*. It has recently been demonstrated that endocytosis of the Delta ligand bound to extracellular domain of Notch facilitates the S2 cleavage (Nichols *et al.,* 2007). Following this first cleavage, Notch receptor suffers a conformational change that converts the receptor into a substrate for a multiprotein complex formed by presenilin, nicastrin, Aph1 and Pen-2 that displays γ-secretase activity. This complex cleaves Notch within the transmembrane domain thus leading to the release of its intracellular fragment (Notch^{intra} or NotchIC) that is the active form of the receptor [reviewed in (Lai 2004)]. Blocking γ-secretase activity with pharmacological inhibitors or by genetic inactivation of members of the γ-secretase complex prevents Notch signaling (Zhang *et al.,* 2000).

After cleavage, NotchIC translocates to the nucleus where it associates with its downstream effector, the transcription factor CSL. In the absence of Notch activation, CSL is bound to specific binding sites in the DNA (C/T)GTGGGAA) and represses specific gene transcription by recruiting corepressors (NcoRs) and histone deacetylases (HDACs) (Kao *et al.,* 1998). Binding of NotchIC to CSL displaces transcriptional repressors and recruits coactivators, such as

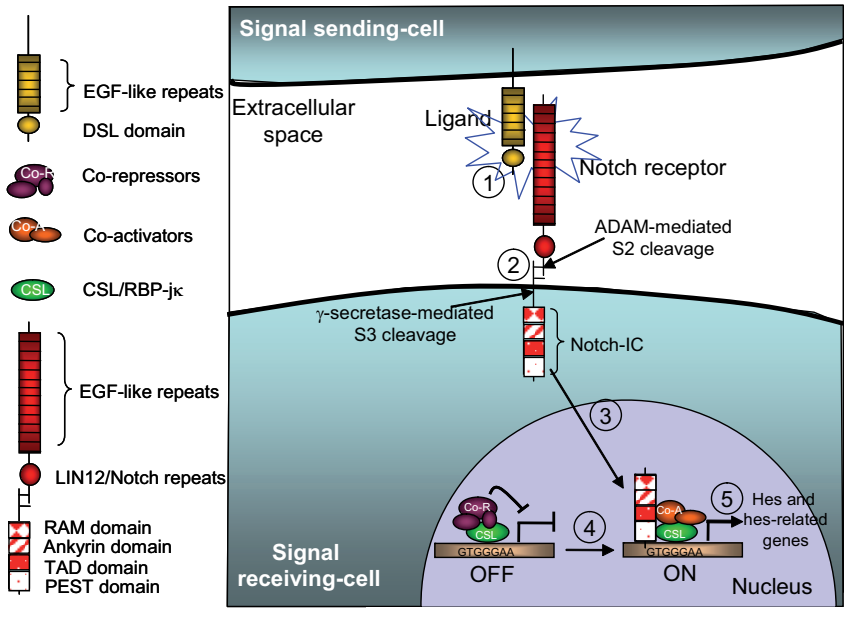

Fig. 1. Sequence of events in the canonical Notch signal. *Interaction between ligand and receptor* **(1)** *leads to a cascade of proteolytic events: one extracellular (ADAM-metalloproteases) and one intracellular (γ-secretase)* **(2)**, *resulting in NotchIC translocation to the nucleus* **(3)**, *exchange of a repressor complex by an activator complex* **(4)** *and NotchIC/RBPjκ dependent target gene expression such as hes and hes-related genes* **(5)**.

p300, or the 'specific' Notch coactivator *Mastermind*, leading to gene activation (see Fig. 1). The best-characterized Notch target genes are the Hairy and Enhancer of Split (hes) and hes-related (hrt) family of transcription factors. There are seven hes genes (hes1-7) in mammals, based on sequence homology, but only hes1 (Jarriault *et al.,* 1995), hes5 (Ohtsuka *et al.,* 1999) and hes7 (Hirata *et al.,* 2000) as well as hrt1/herp2 and hrt2/herp1 are activated by Notch [reviewed in (Iso *et al.,* 2003)]. Hes and Hrt proteins are basic helix-loop-helix (bHLH) factors that generally function as transcriptional repressors in association with *Groucho/TLE* by binding to N-box sequences (CACNAG). In general, Hes and Hrt proteins mediate most of the Notch effects on cell differentiation in several systems including inhibition of neurogenesis, myogenesis, hematopoiesis or intestinal differentiation [reviewed in (Ohishi *et al.,* 2003)].

Control of cell-fate decisions

During development but also during adult life, complex systems formed by cells with different functional qualities are generated from pluripotent cells with equivalent developmental potential. In most of them, the interactions between Notch and Jagged/Delta are responsible for inducing cellular diversity from common progenitors through the activation of specific genetic programs [reviewed in (Lewis 1998)]. Two different mechanisms have been proposed to explain how Notch regulates cell diversity: lateral inhibition and lateral induction.

Lateral inhibition model

In the lateral inhibition model, a hypothetical initial population of equivalent cells that express similar levels of both Notch receptor and ligand. By stochastic events, one cell upregulates the expression of the ligand, which will be responsible to activate Notch receptor in the neighbouring cells. This leads to the subsequent downregulation of the ligand by negative feedback loops, allowing the maintenance and intensification of the differences in expression of receptors and ligands generating a "salt-and-pepper" mosaic of cells. As a result Notch is specifically activated in the receptor-expressing cells to inhibit differentiation whereas ligand-expressing cells differentiate (see model in Fig. 2A) [reviewed in (Lewis 1998)]. The neural-epidermal choice in *Drosophila* is the paradigm of the lateral inhibition model. Specification of the neural lineage requires the expression of the achaete-scute bHLH transcription factors that are repressed by Notch activation through the Enhancer of split Complex [E(spl)-C]. Thus, Notch activation results in the inhibition of the neural fate permitting the epidermal differentiation (Parks *et al.,* 1997). In mammals, this mechanism is conserved and controls neurogenesis, intestinal differentiation and myogenesis through the repression of Math1, Mash1 and MyoD by the ortholog of Hairy/E(spl)-related bHLH repressors, Hes [reviewed in (Artavanis-Tsakonas *et al.,* 1999)].

Lateral induction model

Some Notch effects also fit with a lateral induction model. In this case, a given cell type that expresses Notch ligands induces Notch activity in a different compartment of adjacent cells that will differentiate and diverge from the initial population. In this case, expression of active Notch induces rather than inhibits differentiation (see Fig. 2B) [reviewed in (Lewis 1998)]. In *Drosophila*, one example of lateral induction is the delimitation of the wing margin through Notch-mediated activation of the transcriptional coactivator *vestigial* in the wing imaginal disc (Couso *et al.,* 1995). In vertebrates a classical example is somite formation. Somites are regularly spaced blocks of mesoderm that split off from the presomitic mesoderm in a periodic oscillatory manner. Somite boundaries are generated by a Notch-dependent transcriptional activation of hes and lunatic fringe followed by a negative feedback of Lunatic fringe on Notch activity (Dale *et al.,* 2003). Although these two models are useful to explain some of the Notch effects, other developmental events are likely to be more complex.

The ontogeny of the hematopoietic system

The hematopoietic system has evolved to ensure nutrient supply and protection from external challenges in multicellular organisms. The blood is composed of a large variety of mature cell types with a limited life-span (i.e two days for neutrophils, thirty days for erythrocytes), that need to be constantly replenished from a pool of hematopoietic stem cells (HSCs). This process is known as hematopoiesis [reviewed in (Godin and Cumano 2002)].

During embryonic development, the major site of hematopoiesis shifts from one organ to another in a dynamic temporal and

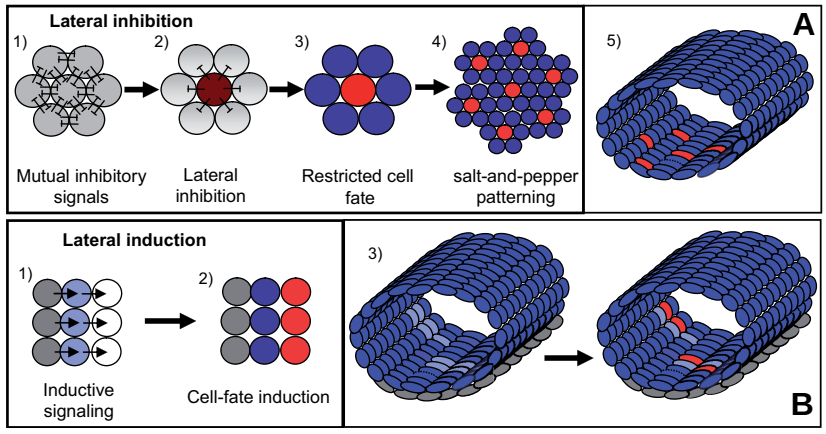

Fig. 2. Lateral inhibition (A) and induction (B) models. (A) *The lateral inhibition model: (1) Equivalent cells expressing receptor and ligand exert mutual inhibitory signals. (2) One of these cells expresses high levels of ligand, inhibiting the expression in the surrounding cells. (3) The ligand expressing cell switches on a different genetic program than the neighbouring cells and adopts a different cellular fate (hematopoietic in red). (4) As a result, a salt-and-pepper pattern is generated that (5) in the aorta could induce the formation of hematopoietic cells while preserving endothelial integrity.* **(B)** *Lateral induction model. (1) A group of cells (mesenchyme in grey) signal to the adjacent blue cells (hemogenic endothelium/hemangioblast in light blue). (2) Subsequently, these cells induce a new fate in the neighbouring cells expressing Notch (hematopoietic cells in red) whereas the rest of cells remain endothelium with hemogenic capacity. (3) In the aorta, few hemangioblastic cells may have the capacity to become hematopoietic by restricting the endothelial fate through lateral inhibition signals and a combination of both models may be operating. (Notch models are inspired in Artavanis-Tsakonas* et al., *(Artavanis Tsakonas* et al., *1995)).*

spatial manner (reviewed in this number). In the extra-embryonic yolk sac, at E7 in the mouse, the mesoderm layer develops into structures referred as blood islands, responsible for the first wave of hematopoiesis also known as primitive hematopoiesis (Silver and Palis 1997). This process mainly generates nucleated erythrocytes that are characterized by the expression of embryonic hemoglobins (ε and βH1). During the 6-8 somite pair stages (E8-8.5), the mouse embryo suffers the process of "turning" or axial rotation, in which achieves the characteristic "fetal" position ((Kaufman 1994)), and shortly after, circulation between the embryo and the yolk sac through the vitelline vessels starts. Beginning E9, the intra-embryonic para-aortic splanchnopleura (P-Sp) mesoderm gives rise to the fused aorta surrounded by gonads and mesonephros, a region referred as AGM. The definitive/adult HSCs (defined as those cells able to self-renew and repopulate the whole hematopoietic system when transplanted into adult irradiated mice) are first detected in the AGM region as early as E9 (Godin *et al.,* 1995; Medvinsky and Dzierzak 1996). However, at E8.5 and before circulation between the yolk sac and the embryo is stablished, intraembryonic mesodermal-derived regions are able to generate hematopoietic cells under specific experimental conditions (Cumano *et al.,* 1996). The HSCs and other progenitors mainly develop from the ventral part of the dorsal aorta in the AGM (Garcia-Porrero *et al.,* 1995) but also from other major vessels such as the umbilical and vitelline arteries (de Bruijn *et al.,* 2000). Original studies by Dièterlen-Lièvre using chick and quail chimeras demonstrated that, at least in this system, hematopoiesis comes entirely from the embryo proper (Lassila *et al.,* 1978), however, evidence has also been collected that supports the origin of hematopoietic cells in the yolk sac (Yoder and Hiatt 1997; Yoder *et al.,* 1997; Yoder *et al.,* 1997), the placenta (Muller *et al.,* 1994; Medvinsky and Dzierzak 1996; Kumaravelu *et al.,* 2002; Gekas *et al.,* 2005; Ottersbach,Dzierzak 2005), the allantois (Zeigler *et al.,* 2006; Corbel *et al.,* 2007) and the subaortic patches (mesenchyme under the floor of the dorsal aorta) (Manaia *et al.,* 2000; Bertrand *et al.,* 2005). Although some controversy still exists, cell tracing experiments have recently demonstrated that HSC are originated from a VE-cadherin expressing cell population present in midgestation embryos (Zovein *et al.,* 2008; Chen *et al.,* 2009). Recently, life imaging technology has provided new evidence that further supports the emergence of hematopoietic stem cells from the endothelial cells in zebrafish and mouse embryos (Boisset *et al.*, 2010, Kissa and Herbomel, 2010, Lam *et al.*, 2010).

Around E11, fetal liver becomes active as hematopoietic site with HSC activity. However, since HSCs appear in the embryo before fetal liver is formed, this activity may necessarily be originated in other hematopoietic niches and next colonize the liver for further differentiation and expansion. Consistent with this, stem cell activity is detected in embryonic circulating blood just before it is found in fetal liver. Colonization of both the fetal thymus (where T-cell differentiation occurs) and the spleen (responsible for B-cell generation) by HSCs starts around E12 (Godin *et al.,* 1999). Near the end of gestation (E15-16), the presence of HSC in fetal liver and spleen regresses concomitant with the migration of these cells to the bone marrow that will the principal hematopoietic organ through adult life (Metcalf *et al.,* 1971). However, spleen and thymus will remain adult hematopoietic organs, responsible for the final maturation of B and T cells respectively, together with the lymph nodes.

The ontogeny of the human and mouse hematopoietic systems are comparable. The first blood islands appear in the human embryo around day 18 of gestation, and YS primitive erythropoiesis takes place from weeks 3-6. HSCs generation in the AGM occurs at weeks 5-7. From weeks 6-22, fetal liver acts as the major hematopoietic site and finally the bone marrow becomes the lifelong site of blood-cell production [reviewed in (Palis,Yoder 2001)].

Direct relationship between hematopoietic and endothelial lineages

In 1917, Florence R. Sabin showed that hematopoietic cells in the blood islands of the yolk sac developed closely in time and space to endothelial cells [re-published in (Sabin 2002). This observation raised the possibility that a common mesodermal progenitor for both lineages, the hemangioblast, exists. This could also be the case in the AGM region where hematopoietic clusters emerge from the ventral part of the dorsal aorta within, or in close association with, the endothelial cell layer (Garcia-Porrero *et al.,* 1995; de Bruijn *et al.,* 2000; de Bruijn *et al.,* 2002). In the mouse, hematopoietic clusters contain HSCs that express CD34, CD45 and c-Kit (Tavian *et al.,* 1996; North *et al.,* 1999; North *et al.,* 2002) markers. Comparable structures have been described in bird, zebrafish, amphibian and human embryos (Tavian *et al.,* 1996; Thompson *et al.,* 1998; North *et al.,* 1999). Cells within aortic/AGM clusters also express transcription factors required for definitive hematopoiesis such as Runx1, c-Myb, Gata2 and Scl (North *et al.,* 1999; de Bruijn *et al.,* 2002; Bertrand *et al.,* 2005; Robert-Moreno *et al.,* 2005).

Recent work using transgenic mice that express the green fluorescent protein (GFP) under the control of Ly-6A/Sca-1 promoter, a well characterized HSC marker, has revealed the presence of a Sca-1-expressing cell population that resides in the endothelial layer of the dorsal aorta in the AGM region and express the endothelial marker CD31. This population displays long-term repopulating activity, strongly suggesting a common endothelial-hematopoietic origin of HSCs in the AGM (de Bruijn *et al.,* 2002). Multiple experimental evidences support the existence of this common precursor. For example, endothelial cells, characterized by the presence of exclusive endothelial markers, are able to generate hematopoietic cells *in vitro* (Eichmann *et al.,* 1997). Moreover, murine embryoid bodies contain blast-colony forming cells that can differentiate into endothelial or hematopoietic cells upon secondary replating (Cho,Choi 1998). Finally, Wang's group identified an endothelial-like subpopulation (PECAM-1, VE-cadherin, Flk-1 positive) within human ES cells with hemangioblastic properties (Wang *et al.,* 2004).

In agreement with their possible common origin, hematopoietic and endothelial cells share a great number of cell surface markers: PECAM-1/CD31 (platelet endothelial cell adhesion molecule-1), angiopoietin receptor Tie-2, CD34, VE-cadherin and the VEGF receptor-2, Flk-1 (Young *et al.,* 1995; Hamaguchi *et al.,* 1999; North *et al.,* 1999; Hsu *et al.,* 2000; Huber *et al.,* 2004). In addition, several targeted mutations in endothelial genes strongly compromise the hematopoietic development in mice. For example, mice deficient for Flk-1 (Shalaby *et al.,* 1995) or the transcription factor Scl (Robb *et al.,* 1995) die at

early stages of development due to severe hematopoietic and endothelial disorders. However, since endothelial development is a prerequisite for hematopoiesis to occur it is difficult to establish whether these mutations affect hematopoiesis *per se* or this is a side effect of endothelial failure. In fact, Flk-1-deficient ES cells failed to contribute not only to the vascular endothelium but also to primitive and definitive hematopoiesis in mouse chimeras (Shalaby *et al.,* 1997), but these results cannot distinguish between both possibilities. More recently, a cell population that expresses the *brachyury* mesodermal marker and Flk-1, first detected in the primitive streak of the mouse embryo, was shown to be the precursor of both endothelial and hematopoietic cells of the yolk sac (Huber *et al.,* 2004).

Further supporting the idea that endothelial and hematopoietic cells come from a common progenitor, chimeras of quail splanchnopleural mesoderm into chicken embryos revealed that HSCs in the AGM come from mesodermal cells that migrate and integrate into the floor of the aorta to generate hematopoietic clusters (Dieterlen-Lievre *et al.,* 1997).

The arterial program precedes hematopoiesis

Blood vessels are formed during embryonic development by two different processes known as vasculogenesis and angiogenesis. The former comprises the differentiation and assembly of mesoderm-derived endothelial cells into a network of primitive blood vessels homogeneous in size, while the latter involves the morphological changes of this vascular plexus required to generate the mature vascular system. A great number of signaling pathways have been implicated in the control of both processes, including the vascular endothelial growth factor (VEGF) or the Angiopoietin/Tie receptor and, more recently, the Notch signaling pathway.

Lineage tracing experiments in the zebrafish embryo provided evidence that endothelial precursor cells are specified in the lateral posterior mesoderm to form either the artery or the vein but never both (Zhong *et al.,* 2001). Notch-regulated gene gridlock/hrt2 is expressed in the artery-forming angioblasts and it is responsible for the correct segregation of these cells. Thus, induction of Notch signaling is a crucial step in artery formation. How is Notch activity induced in the arterial precursors? It has been shown that hedgehog (Hh) pathway mutants as well as embryos treated with the Hh signaling inhibitor cyclopamine fail to establish arterial identity in the dorsal aorta concomitant with the loss of the arterial marker ephrinB2 (Lawson *et al.,* 2002; Gering and Patient 2005). These embryos lack VEGF expression that it is required for arterial specification, however vegf cannot restore the arterial fate in Notch mutants (Lawson *et al.,* 2002). Taken together this data indicates that, at least in zebrafish, Hh activates the expression of VEGF, which is upstream of Notch activation during arterial specification. In the mouse, gene-targeting studies confirm most of the findings in zebrafish and support the conservation of this signaling cascade. Definitive hematopoiesis in the embryo is closely associated to arteries (de Bruijn *et al.,* 2000) and it is generally assumed that hematopoietic development is not possible without previous artery specification. This assumption is supported by the absence of hematopoiesis in most arterial-defective mutants and the presence of ectopic hematopoiesis in the vessels of mutants with vein-to-artery conversion, such is the case for COUP-TFII-deficient embryos (You *et al.,* 2005). However, it has not been demonstrated that hematopoiesis cannot occur in the absence of arterial specification. In zebrafish, hematopoietic cells (runx1$^+$ and/or c-myb$^+$) can originate from vessels that do not express EphrinB2 (EfnB2) after Notch1IC induction (Burns *et al.,* 2005) and some TGFβ-family mutant mouse embryos with arteriovenous malformations (activin receptor like kinase1, ACVRL1, ALK1 (Urness *et al.,* 2000) or endoglin (Urness *et al.,* 2000; Lawson *et al.,* 2001; Lawson *et al.,* 2002) mutants) present ectopic hematopoiesis (CD34$^+$ cells) in veins characterized by the absence of EfnB2 expression. These observations suggest that cells expressing hematopoietic markers are generated in the absence of EfnB2 expression, however whether lack of EfnB2 implies abrogation of arterial specification and if so what is the functional HSC potential of these hematopoietic cells arising in EfnB2-negative vessels, remains unknown.

Notch in the vascular development

The analysis of different mouse embryos with targeted mutations or carrying Notch-activated transgenes has revealed the importance of the Notch pathway in the regulation of angiogenic development. Most of Notch family mutants develop an homogenous capillary plexus in the yolk sac but lack major angiogenic remodelling of the main vessels (Xue *et al.,* 1999; Krebs *et al.,* 2000). As described above, analysis of Notch mutants in zebrafish first demonstrated the important role of Notch in artery specification (Zhong *et al.,* 2001; Lawson *et al.,* 2002; Gering,Patient 2005). The Notch function in vascular remodelling, including artery specification, is conserved in the mouse being both Notch1 and Notch4 receptors essential albeit some functional redundancy (Conlon *et al.,* 1995; Krebs *et al.,* 2000). Delta4 is the most important Notch ligand in vascular development, although Jagged1 mutants have some vascular defects. Conversely, the transcription factor COUP-TFII inhibits Notch to allow the formation of veins (You *et al.,* 2005). Some of the downstream effectors of Notch signaling in angiogenesis include Gridlock/hrt2 but also EphrinB2 that has recently been identified as a direct Notch-target (Zhong *et al.,* 2000; Timmerman *et al.,* 2004).

In addition to its function in artery versus venous specification, Notch is important in the decision between endothelial and smooth muscle cells, pulmonary versus systemic vessels and large vessels versus capillaries (for an extensive review see (Iso *et al.,* 2003)). One important aspect of vascular remodelling resides in the control of the growing tip when new vessels are formed from the existing ones. Tip cells are specialized endothelial cells that extend filopodia that guide the growth of the sprout blood vessel along VEGFα gradients (Gerhardt *et al.,* 2003; Gerhardt *et al.,* 2004). Notch and Delta4 signaling have been demonstrated to regulate tip cell number, filopodia extension and branching of angiogenic sprouts in HUVEC cells, in the mouse retina and in the hindbrain (Ridgway *et al.,* 2006; Hellstrom *et al.,* 2007; Lobov *et al.,* 2007; Suchting *et al.,* 2007), in the zebrafish embryo (Leslie *et al.,* 2007; Siekmann,Lawson 2007) and in tumors (Noguera-Troise *et al.,* 2006; Scehnet *et al.,* 2007).

Further indicating the relevance of Notch in vascular development, mutations in members of the pathway including the

Notch ligand Jagged1 and Notch3 are responsible for human diseases that involve vascular defects such as the Alagille and CADASIL syndromes, respectively (reviewed in (Iso *et al.,* 2003)).

Notch in the development of the hematopoietic system in mammals

Role of Notch in HSC self-renewal

There is controversial data on the role of Notch in HSC self-renewal. On one side, conditional deletion of Notch1, Notch2 or Jagged1 genes induced by Mx-cre in adult bone marrow (Radtke *et al.,* 1999; Mancini *et al.,* 2005; Maillard *et al.,* 2008) or specific expression of a dominant negative *mastermind* transgen in fetal liver (Radtke *et al.,* 1999; Mancini *et al.,* 2005; Maillard *et al.,* 2008) have no effect on adult hematopoietic stem cells suggesting that, Notch is dispensable for the maintenance of this cell compartment. However, there are multiple evidences that e*x vivo* expansion of HSC can be achieved by manipulating the Notch pathway. For example, retroviral transduction of the Notch1IC in the KSL subpopulation (c-Kit$^+$Sca1$^+$Lin$^-$ enriched for murine hematopoietic progenitors and HSCs) leads to immortalization of these cells preserving their ability to repopulate myeloid and lymphoid lineages when transplanted into lethally irradiated mice (Varnum-Finney *et al.,* 2000). Ectopic expression of Notch1IC also expands the number of bone marrow repopulating cells in secondary transplants and promotes their lymphoid differentiation (Stier *et al.,* 2002) similar to Notch4IC overexpression in human Lin$^-$ cord blood cells (Vercauteren and Sutherland 2004). Moreover, addition of soluble Jagged1 to *ex vivo* cultures of human CD34$^+$CD38$^-$Lin$^-$ cord blood cells expand the number of cells capable to repopulate the hematopoiesis of NOD/SCID mice (Karanu *et al.,* 2000) similar to that found by incubating bone marrow KSL progenitors with a Delta1 fusion protein (Varnum-Finney *et al.,* 2003). Moreover, many of these effects are reproduced in CD34$^-$KSL cells retrovirally transduced with hes1, suggesting that this Notch-target gene is responsible for the effects of Notch1 on HSCs self-renewal (Kunisato *et al.,* 2003). Recent work performed by Duncan *et al.,* demonstrated that Notch signaling is active in the KSL subpopulation located in the bone marrow niche and decreases as these cells differentiate. Moreover, Notch inhibition by a dominant negative RBPjκ/CSL leads to accelerated differentiation of HSCs *in vitro* and depletion of HSC activity *in vivo* indicating that Notch is required for the maintenance of the undifferentiated state of HSCs (Duncan *et al.,* 2005)

Independently of the physiological role of Notch in stem cell maintenance, these results are optimistic about the possibility of targeting Notch in clinical procedures that involve stem cell transplantation or hematopoietic cell therapy.

Notch regulation of lymphoid cell-fate decisions

Notch signaling regulates several cell-fate decisions in the lymphoid lineage and different studies demonstrate that Notch activation promotes T- while inhibits B-cell fate from a common progenitor. For example, deletion of RBPjκ/CSL results in increased B-cell differentiation and blockage of T-cell development (Han *et al.,* 2002), conversely, expression of Notch1IC blocks B-cell differentiation and leads to generation of immature CD4$^+$CD8$^+$ T cells (Pui *et al.,* 1999). However, it is still unclear whether this B- versus T-cell decision takes place in the common lymphoid progenitor (CLP) or in the recently described early T-cell progenitor (ETP) [reviewed in (Maillard *et al.,* 2003)]. Similarly, conditional inactivation of RBPjκ/CSL arrests T-cell development at the DN3 stage, prior to β-selection (Tanigaki *et al.,* 2004). Following β-selection, Notch signalling has to be downregulated for differentiation to proceed. In the last step of CD4$^+$CD8$^+$ double positive cells, it is unclear whether Notch regulates the decision for generating CD4$^+$ and CD8$^+$ single positive (Robey *et al.,* 1996; Izon *et al.,* 2001). Finally, Notch1 also favours the αβ choice at expenses of the γδ cell differentiation (Washburn *et al.,* 1997). In addition, Notch2 is required to generate the marginal zone B cells in TCR (T-cell receptor) lineage.

Notch in myeloid differentiation

Early studies using myeloid cell lines showed that forced Notch1 or Notch2 activation could inhibit myeloid differentiation in a cytokine-dependent manner (Milner *et al.,* 1996; Bigas *et al.,* 1998), likely through expression of Gata2 (Kumano *et al.,* 2001). A similar result was recently reported using isolated KSL population co-cultured on an OP9 stromal cell line expressing Delta1 (de Pooter *et al.,* 2006). In apparent contradiction with *in vitro* data, the analysis of different conditionally-targeted Notch alleles showed that Notch activity is only required for T and B lymphoid differentiation *in vivo* (Radtke *et al.,* 1999). Nevertheless, it has recently been reported that mice defective in the FX protein, that converts the GDP-mannose to GDP-fucose, which is required for Fringe-dependent glycosylation of Notch signalling, develop a myeloproliferative disease. This phenotype has been attributed to a defect in the Notch pathway since fucosylation-deficient myeloid progenitors were not able to respond to Notch-ligands and failed to differentiate compared to the wild type cells (Zhou *et al.,* 2008). Together, these results suggest that Notch is dispensable for myeloid cell generation but its activity is required for the maintenance of myeloid homeostasis *in vivo*.

Expression of Notch family members in hematopoietic cells

In agreement with the pleiotropic effects of Notch in hematopoietic cell differentiation, different Notch molecules are expressed in hematopoietic tissues at various stages of development [reviewed in (Ohishi *et al.,* 2003)]. Activation of Notch in hematopoietic precursors is achieved by interactions with stromal supporting cells but also by interactions among hematopoietic cells.

In the adult, Notch1 and Notch2 expression was first detected in the CD34$^+$Lin$^-$ bone marrow fraction of hematopoietic precursors (Milner *et al.,* 1994; Ohishi *et al.,* 2000), suggesting a role for Notch in hematopoietic progenitor and/or stem cells. Conversely, Notch-ligands Jagged1 (Walker *et al.,* 2001) as well as Delta1 and Delta4 (Karanu *et al.,* 2001) are expressed in the bone marrow stromal cells. Similarly, all three ligands are expressed in thymic epithelial cells, consistent with the important role of Notch in T-cell development (Felli *et al.,* 1999; Mohtashami,Zuniga-Pflucker 2006). In the myeloid lineage, monocytes but not granulocytes express high levels of Notch1 and 2 (Ohishi *et al.,* 2000). Finally, bone marrow erythroid progenitors express Notch1 and its expression decreases in more mature erythroid cells such as acidophilic normoblasts (Ohishi *et al.,* 2000; Walker *et al.,* 2001).

In the embryo, we have previously reported that Notch1 and

Notch4 receptors together with Jagged1, Jagged2 and Delta4 ligands are expressed in the E9.5-E10.5 aortic endothelium (Robert-Moreno *et al.,* 2005). However, expression of Notch receptors or ligands is not a synonymous of Notch activity since it is the imbalance between them what triggers Notch activation. This was demonstrated by experiments in *Drosophila* or mouse chimeras in which cells with two wildtype alleles preferentially activated Notch compared to cells with only one Notch allele (Heitzler and Simpson 1991; Washburn *et al.,* 1997). A better indication of Notch activity in the aorta endothelium and the hematopoietic clusters is the expression of its downstream targets hes1, hrt1 and/or hrt2 genes (Fig. 3E) (Robert-Moreno *et al.,* 2005; Robert-Moreno *et al.,* 2008), however since hes1 can be activated by other transcription factors (Curry *et al.,* 2006; Fernandez-Majada *et al.,* 2007), its expression does not necessarily implies Notch activity. Recently a very powerful tool has been developed that is now widely used to assess Notch1 activation. This is the antibody that recognizes Val1744 of the Notch1 protein, which is the cleavage site of γ-secretase (Cheng *et al.,* 2003). We have recently shown that cells in both the AGM endothelium and the hematopoietic clusters stain for this antibody indicating that Notch is activated in these cells (Fig. 3D) (Robert-Moreno *et al.,* 2008).

Notch implication in the ontogeny of the hematopoietic system

During the recent years, the function of Notch in embryonic hematopoiesis has partially been elucidated. The first *in vivo* proof that Notch signaling plays a key role in the generation of HSCs during murine embryonic development came from Kumano *et al.,* by studying the Notch1 null embryos. By repopulation experiments in new-born mice, they found that Notch1$^{-/-}$ yolk sac and embryonic P-Sp lack HSCs, whereas the number of progenitors in the yolk sac was similar in the mutant and the wild type. Despite these hematopoietic defects, E9.5 Notch1 null embryos display a similar number of CD34^{+}c-Kit^{+} and VE-cadh^{+}CD45^{-} in the endothelium in both YS and P-Sp, suggesting a deficiency in the commitment of HSCs from "hemogenic" endothelial cells (Kumano *et al.,* 2003). Further indicating that the absence of Notch signaling was responsible for this phenotype, we described similar abnormalities in the RBPjκ/CSL mutant embryos. These embryos completely lack intraembryonic hematopoiesis, but contain a similar number of progenitors in the yolk sac compared to the wild type. In addition, the fused dorsal aorta of the mutants displayed an expansion in the endothelial layer as detected by an increase in the number of cells expressing CD31 and VE-cadherin (Robert-Moreno *et al.,* 2005), suggesting that the endothelial lineage was favoured at expenses of the hematopoietic lineage from a putative common progenitor (hemangioblast) [reviewed in (Dieterlen-Lievre *et al.,* 2006)]. Consistent with the hematopoietic phenotype of Notch pathway mutants, in chimeric mice generated from wildtype and Notch1-deficient ES cells, mutant cells contribute to the yolk sac hematopoiesis but not to the long-term definitive hematopoiesis (Hadland *et al.,* 2004).

Given the fact that Notch is involved in arterial specification through the regulation of ephrin, and arterial differentiation is likely a prerequisite for HSC formation (as previously discussed), it is difficult to separate the contribution of Notch to each of these processes. The best evidence for a specific hematopoietic Notch function in the AGM came from the analysis of the Jagged1-deficient embryos. Although Jagged1 mutant embryos also have some angiogenic defects, the arterial program is mostly intact as indicates the expression of the arterial markers ephrinB2, CD44 and α-SMA. These embryos, however,

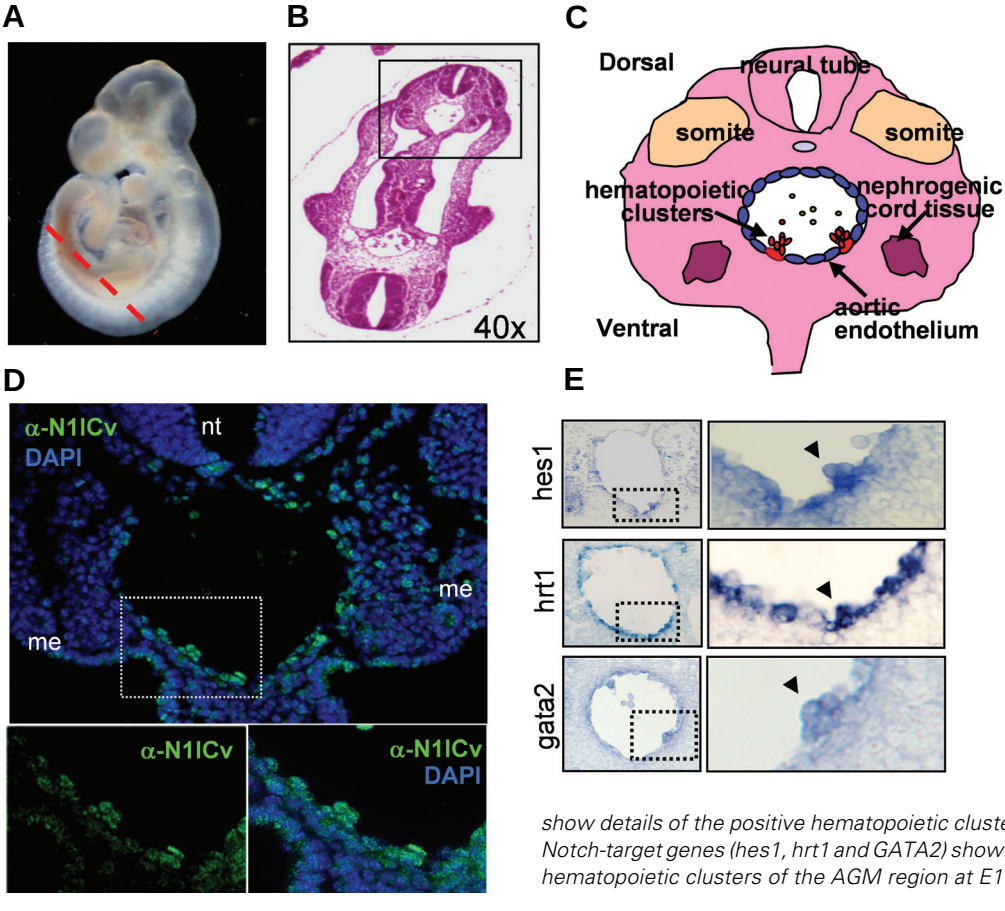

Fig. 3. Notch activity in the AGM region at E10.5. (A) *E10.5 mouse embryo. The discontinuous red line marks the section in B in the region of the trunk.* **(B)** *Hematoxiline-eosin staining of a transversal section through the trunk region of an E10.5'embryo. The black box shows the region represented in:* **(C)** *Representation of the aorta in the AGM region. From E10 to E12.5, clusters of hematopoietic cells appear as budding mainly from the ventral endothelium of the aorta in the AGM region.* **(D)** *Active Notch1 (Val1744) staining in the AGM aorta at E10.5. Lower panels show details of the positive hematopoietic clusters.* **(E)** *Detail of* in situ *hybridization of the Notch-target genes (hes1, hrt1 and GATA2) showing expression in the aorta endothelium and hematopoietic clusters of the AGM region at E10.5.*

share the intraembryonic hematopoietic defect of the previously analyzed Notch mutants and once again, this mutation does not affect the yolk sac progenitor population ((Robert-Moreno *et al.,* 2008) and unpublished results). Thus, two different Notch signals should be required in the mid-gestation aorta, the first one to establish the arterial fate and the second one to specify the HSC identity. These different signals could be initiated by different Notch receptors or different ligands. In this sense, Notch2, Notch3 and Notch4 mutants show altered vasculogenesis, but defects in embryonic hematopoesis have not been described in any of these mutants (Joutel *et al.,* 1996; Krebs *et al.,* 2000; McCright *et al.,* 2001; Kumano *et al.,* 2003). By expression analysis, others and we found that Notch4 is expressed at E9.5-E10.5 in the aortic endothelium but downregulated in the hematopoietic clusters (Shimizu *et al.,* 2000; Robert-Moreno *et al.,* 2005). However, Notch4-null embryos are viable and only when combined with Notch1-deficiency they display a severe vasculogenic defect (Conlon *et al.,* 1995; Krebs *et al.,* 2000) indicating an overlapping vascular function for Notch1 and Notch4 receptors in this tissue. We also found that not only Jagged1, but also Jagged2 and Delta4 were expressed in the E9.5-10-5 AGM, however we have analyzed the AGM hematopoiesis of the Jagged2 mutants, and it is comparable to the wildtype (Robert-Moreno *et al.,* 2008). Analysis of the hematopoiesis in the Delta4 null embryos has been unsuccessful since this mutation is lethal at E9.5 due to severe vasculogenic defects and loss of endothelial arterial cell fate (Duarte *et al.,* 2004) and in some backgrounds, lethality occurs even in heterozygosity (Krebs *et al.,* 2000). These data together with some unpublished observations suggests that activation of Notch1 by Delta4 is the right combination to specify vascular development. However, it is not known whether Delta4 plays any role in regulating hematopoietic stem cells *in vivo* as it has been suggested from *in vitro* experiments (Dando *et al.,* 2005).

Notch mutant phenotypes in embryonic hematopoiesis: the yolk sac and the AGM

There are two different waves of yolk sac hematopoiesis that occur before or after circulation is established between the embryo and the yolk sac. It is evident that hematopoietic cells found in the yolk sac before blood circulation (E8.5 in the mouse) are generated in this organ. These are primitive hematopoietic cells and mainly correspond to nucleated red cells and macrophages. As mentioned before, this pre-circulation hematopoiesis is not affected in the Notch-mutant mouse embryos that have been analyzed (Notch1, Notch2, RBPjκ/CSL, Jagged1, Jagged2). It is after E8.5, once the circulation is established, that the origin of the hematopoietic progenitors and HSCs residing in the yolk sac or the embryo becomes uncertain. Around E9, several types of myeloid and erythroid progenitors are detected in both the embryo and the yolk sac, and it is also around this time that the first stem cell activity is detected in the embryo and yolk sac by transplantation of busulphan-conditioned new-born mice (Yoder *et al.,* 1997). Interestingly, only this second wave of definitive hematopoiesis is abrogated in the Notch mutant embryos, including Notch1, RBPjκ/CSL or Jagged1 knockouts and the stem cell activity is completely lost in the Notch1 mutant embryos (Kumano *et al.,* 2003; Robert-Moreno *et al.,* 2007; Robert-Moreno *et al.,* 2008). Together this data supports the idea that there are two well-differentiated genetic programs responsible for embryonic hematopoiesis, being one Notch-dependent and the other Notch-independent. More accurate cell tracing experiments will be required to demonstrate the yolk sac or the intraembryonic origin of definitive HSCs, however, and independent of where HSCs are originated, they require Notch signaling to exist.

Notch signaling modulates red cell homeostasis in the yolk sac

As previously mentioned, pre- and post-circulation hematopoiesis in the yolk sac of RBPjκ/CSL null embryos is apparently normal (Robert-Moreno *et al.,* 2007). Nevertheless, when we analyzed the erythroid Ter119$^+$ cells in the blood islands of the RBPjκ null yolk sacs, we found an increase in the percentage and number of these cells that was attributed to reduced apoptosis. Consistently, we detected changes in the level of several pro-survival genes in the mutant cells, although part of the phenotype could be also explained by architectural defects in the yolk sac that leads to reduced oxygen availability. Nevertheless, incubation of wildtype yolk sac cells with the γ-secretase inhibitor DAPT resulted in a comparable decrease in the apoptosis of Ter119$^+$ cells *in vitro*, strongly supporting a function for Notch in regulating apoptosis in the erythroid lineage (Robert-Moreno *et al.,* 2007).

Notch target genes

Despite the multiple and diverse effects that Notch plays during embryogenesis and adult tissue homeostasis, to date only a few Notch target genes have been identified. This includes the well-characterized hes1 and hes-related genes [reviewed in (Jarriault *et al.,* 1995; Iso *et al.,* 2003) and the newly identified targets ephrinB2 in heart development (Timmerman *et al.,* 2004), c-myc in T-cell lymphoma (Weng *et al.,* 2006) or gata3 and il-4 in T cells (Amsen *et al.,* 2004; Dontje *et al.,* 2006). Interestingly, most of these recently identified targets only depend on Notch in specific tissues suggesting that its cooperation with other tissue specific factors is a general mechanism of Notch function. In the embryonic aorta, RBPjκ/CSL mutant embryos lack expression of at least three master hematopoietic transcription factors (Runx1, Gata2 and Scl) (Robert-Moreno *et al.,* 2005). Lack of gata2 expression was also found in the aortic endothelium of Jag1 null embryos (Robert-Moreno *et al.,* 2008). Although this may be a secondary effect of the hematopoietic defficiency, we found by chromatin immunoprecipitation experiments, from both myeloid progenitors (the 32D cell line) and E9.5 embryos, that gata2 was a direct target of Notch1/RBPjκ (Robert-Moreno *et al.,* 2005). Interestingly,

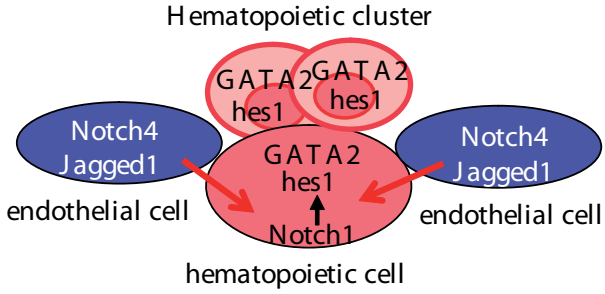

Fig. 4. Model for Notch signaling and downstream molecules involved in the determination of hematopoietic specification in the dorsal aorta.

gata2 had been previously identified as a Notch-dependent gene in 32D myeloid progenitor cells and F5-5 erythroleukemia cells (Kumano *et al.,* 2001; Robert-Moreno *et al.,* 2008). In contrast to our data, the authors proposed in this report that it is the Notch-target hes1, which maintains gata2 expression under differentiating conditions.

The hematopoietic transcription factor Runx1/Aml1 is absolutely required for the generation of HSCs from the aortic endothelium (Okuda *et al.,* 1996; North *et al.,* 1999; Chen *et al.,* 2009). Runx1 has been recently described to act downstream of Notch in the establishment of the hematopoietic stem cell fate in the zebrafish embryo (Burns *et al.,* 2005). In addition, ectopic expression of runx1 (but not gata2 or scl) partially rescues the hematopoietic defects of Notch1$^{-/-}$ AGM-derived cells *in vitro* (Nakagawa *et al.,* 2006). Although there is no evidence for a direct effect of Notch on the runx1 gene (Robert-Moreno *et al.,* 2005), it is possible that Notch regulates runx1 downstream of gata2 (Nottingham *et al.,* 2007). However we found that runx1 is expressed in 50% of the Jagged1 null embryos in which gata2 is absent. Moreover we have been able to partially rescue the hematopoietic defect of Jagged1 null cells by ectopic expression of gata2 (Kumano *et al.,* 2001; Robert-Moreno *et al.,* 2008), further supporting a role for Notch in specifically regulating embryonic hematopoiesis through gata2.

The classical Notch-targets hes1 and hes-related genes are also expressed in the endothelium lining the dorsal aorta and in hematopoietic clusters (Robert-Moreno *et al.,* 2005; Robert-Moreno *et al.,* 2008) suggesting that they play a role in this system (Fig. 3E). In agreement with this, Hes1 has been shown to inhibit differentiation and maintain HSCs (Kunisato *et al.,* 2003).

Together this data leads to speculate that different Notch downstream effectors, including general regulators such as hes1, but also tissue specific such as gata2, may cooperate in the acquisition of specific stem cell traits to generate, maintain or amplify HSCs in the embryo (Fig. 4).

Notch functions in the hematopoietic development of zebrafish

The use of zebrafish has been a major advance in the research of embryonic hematopoiesis. The main advantage of this system is the transparency of the embryos and the similarity between mammals and fishes in the molecular programs that control hematopoiesis (Langenau *et al.,* 2003; Davidson,Zon 2004).

In zebrafish, primitive hematopoiesis does not occur in extra-embryonic tissue as in mammals, instead, primitive erythroblasts are generated in the intermediate cell mass of Oellacher, ventral to the notochord and placed in the trunk (Fouquet *et al.,* 1997; Herbomel,Levraud 2005). However, definitive hematopoiesis takes place on the floor of the dorsal aorta, as in vertebrates. The signalling pathways that controls hematopoiesis have been nicely characterized in this organism: secreted hedgehog from the floor plate, notochord and hypochord is at the top of this cascade, leading to the induction of vegf expression, which is upstream of Notch (Lawson *et al.,* 2002; Gering,Patient 2005). Then, Notch induces the arterial specification through regulation of gridlock (Zhong *et al.,* 2001) and the establishment of the definitive hematopoietic program, upstream of runx1 (Burns *et al.,* 2005). This essential role of Notch signalling in zebrafish hematopoiesis has been mainly characterized by the study of *Mind bomb* mutants, and has been confirmed by using γ-secretase inhibitors *in vivo* (Gering,Patient 2005; Lee *et al.,* 2009). *Mind bomb* is an E3-Ubiquitin ligase required for Delta maturation and Notch/Delta interaction. *Mind bomb* mutants display normal primitive hematopoiesis but impaired HSC development. Consistent with this, c-myb and runx1 express aorta of these mutants, while ectopic notch1 activation results in an expansion in the number of HSCs, dependent on Runx1 (Burns *et al.,* 2005). More recently, similar results for mind bomb gene have been reported in the mouse embryo (Yoon *et al.,* 2008).

From all this data we can conclude that zebrafish and murine hematopoiesis share the requirement of Notch signaling for definitive but not for primitive hematopoiesis (Burns *et al.,* 2005; Gering and Patient 2005).

Notch function during development of hematopoietic system in *Drosophila*

In the fly, Notch participates at least in two different phases during hematopoietic development (Lebestky *et al.,* 2003; Mandal *et al.,* 2004). The first one occurs in the *Drosophila* larvae, where cardiogenic mesoderm gives rise to nephrocytes (excretory), cardioblasts (vascular) and blood cells. Notch loss-of-function results in a decrease of nephrocytes and blood cells with an increase in the vascular fate indicating that Notch is required for blood cell specification (Mandal *et al.,* 2004). The second one takes place during adult fly hematopoiesis. This process occurs in a bilateral chain of tight clustered cells (20 cells approximately) flanking the lymph gland, a structure comparable to the dorsal aorta. *Drosophila* blood cells, called hemocytes, are divided into plasmatocytes, analogous to the vertebrate macrophages, and crystal cells, involved in the melanization of pathogens (Rizki,Rizki 1959; Hartenstein *et al.,* 1992). The *Drosophila* GATA homolog *serpent*, participates in the generation of both types of hemocytes, whereas the Runx1 homolog *lozenge* is only required for the specification of crystal cell precursors (Lebestky *et al.,* 2000). Similar to what occurs in mice, Notch induces *serpent* expression in *Drosophila* (Mandal *et al.,* 2004) that is essential for the expression of *lozenge* (Lebestky *et al.,* 2000; Nottingham *et al.,* 2007)

Altered Notch signaling in human disease

Three different congenital diseases due to mutations in Notch family members have been described. Mutations in the Jagged1 gene are responsible for Alagille syndrome, which results in impaired generation and disfunction of different organs such as heart, eye, liver and skeleton (Li *et al.,* 1997). Delta3 mutations are responsible for spondylocostal dysostosis, a developmental disease characterized by rib fusion and trunk dwarfism (Bulman *et al.,* 2000). Finally, mutations in the extracellular EGF-like repeats of Notch3 results in Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), characterized by migraines, strokes and dementia, further supporting that Notch is involved in vascular and neural development in humans (Joutel *et al.,* 1996). Notch also participates in tumorigenic processes in different tissues including mammary gland, skin, cervix and prostate [reviewed in (Lai

2004)], colon (van Es *et al.,* 2005) (Fernandez-Majada *et al.,* 2007; Rodilla *et al.,* 2009) or pancreas (Miyamoto *et al.,* 2003).

Hematopoietic disorders

The human Notch1 gene was first identified in a T-cell leukaemia as a translocation that involves the TCR. This resulted in a chimeric protein containing the extracellular domain of TCR-β fused to Notch1IC leading to constitutive Notch activation in T cells (Ellisen *et al.,* 1991). Although this rearrangement is a rare event, there is now evidence that point mutations occurring in the heterodimerization domain of Notch, that facilitates ligand-independent activation, or in the PEST domain, resulting in a more stable Notch1IC protein, are present in 50% of human T-ALL (Weng *et al.,* 2004). Moreover, different factors are known to cooperate with Notch in T-leukemogenesis including downstream targets such as c-myc (Palomero *et al.,* 2006) or NFκB (Vilimas *et al.,* 2007), however there are evidences that hes1 can reproduce the effects of Notch in promoting leukemias (Kawamata *et al.,* 2002). In B cells, Epstein-Barr virus or Kaposi's sarcoma-associated herpes viruses are known to activate Notch-target genes through viral proteins that bind to the RBPjκ/CSL consensus thus leading to cell immortalization and transformation [reviewed in (Milner and Bigas 1999)].

Pleiotropy and context specificity of Notch in adult versus embryonic hematopoietic stem cells

The mechanisms leading to the context specific functions of Notch may include 1) differences in the expression of Notch receptors (Cheng *et al.,* 2007), 2) accesibility of the Notch-presenting cell to different Notch ligands (Robert-Moreno *et al.,* 2005), 3) changes in the receptor-ligand affinity due to modification of the receptors by fringe glycosyltransferases (Hicks *et al.,* 2000; Moloney *et al.,* 2000), 4) ubiquitination of the ligands (Ben-Yaacov *et al.,* 2001; Bardin and Schweisguth 2006; De Renzis *et al.,* 2006) or 5) cooperation of Notch with tissue specific factors. All these variables are responsible for the wide range of Notch effects that are observed depending on the temporal phase, the tissue, and the cell type, among others. In general, Notch activation inhibits differentiation through hes and hrt transcriptional repressors [reviewed in (Artavanis-Tsakonas *et al.,* 1999)], however it may induce differentiation in some specific tissues. For example, in keratinocytes Notch promotes cell cycle arrest and terminal differentiation by inducing p21 expression (Rangarajan *et al.,* 2001).

In embryonic hematopoiesis, Notch may induce the hematopoietic fate through gata2 while maintaining the stemness through hes1. The high complexity of the hematopoietic process, including the expanded period of time when it occurs and the number of organs and cell types that are involved, may be responsible for the different and controversial effects that have been reported for Notch in this tissue.

Remarks

Generation, expansion and manipulation of HSC are the main challenges in clinical transplantation and regenerative medicine. As shown in this review, Notch signaling plays a crucial role in the ontogeny and maintenance of the hematopoietic system in both the embryo and the adult. Based on this, it is tempting to speculate that targeting the Notch pathway will be a valuable tool in HSC-based therapies. On the other side of the coin, we should be extremely cautious when manipulating this pathway since Notch activation precedes many tumorigenic processes including hematologic malignancies.

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