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Minireview

Multiple functions of Notch signaling in self-renewing organs and cancer

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Abstract In recent years a substantial body of evidence has accumulated to support the notion that signaling pathways known to be important during embryonic development play important roles in regulating self-renewing tissues. Moreover, the same pathways are often deregulated during tumorigenesis due to mutations of key elements of these pathways. The Notch signaling cascade meets all of the above-mentioned criteria. We discuss here the pleiotropic roles of the Notch signaling pathway in three different self-renewing organs (intestine, hematopoietic system and skin) and how its deregulation is involved in tumorigenesis.

Keywords: Notch signaling; Stem cell; Differentiation; Oncogene; Tumor suppressor

1. The Notch pathway

The Notch pathway is evolutionarily conserved and found in organisms as diverse as worms and humans. The consequences of partial loss-of-function (haplo-insufficiency) of the *Notch* gene were first described in *Drosophila* in the early 20th century when fruit flies were observed with notches at the margins of their wing blades. The gene causing this particular phenotype was cloned in the mid 1980s (nearly 70 years later) and encodes a single pass transmembrane (TM) receptor, harboring a large extracellular domain involved in ligand binding and a cytoplasmic domain involved in signal transduction. *Drosophila* has one Notch receptor that is bound by two TM bound ligands while mammals possess 4 Notch receptors (Notch1–4) and five ligands (Jagged1, and 2 and Delta-like 1, 3 and 4) [1]. The receptors are synthesized as single precursor proteins that are cleaved during transport to the cell surface where they are expressed as heterodimers. Notch signaling is initiated by ligand–receptor interaction between two neighboring cells resulting in two successive proteolytic cleavages. The first is mediated by a metalloprotease of the ADAM family (TACE, tumor necrosis factor-α-converting enzyme), which cleaves the receptor in the extracellular domain, close to the TM domain. The released extracellular domain is then transendocytosed by the ligand-expressing cell. The second cleavage occurs within the TM domain and is mediated by the γ-secretase activity of a multi-protein complex consisting of presenilin, nicastrin, APH1 and PEN2 [2]. This final cleavage liberates the cytoplasmic domain of the Notch receptor (NICD), which subsequently translocates to the nucleus where it binds to its downstream transcription factor CSL (*C*BF1 in humans, *S*uppressor of Hairless in *Drosophila* and *L*AG in *Caenorhabditis. elegans*, also known as RBP-J in mice) and thereby activates transcription (Fig. 1). To date only a few Notch target genes have been identified, some of which are dependent on Notch signaling in multiple tissues, while others are tissue specific. Members of the basic helix-loop-helix transcription factor family, Hairy enhancer of split (*Hes*) are among the best known Notch target genes. They negatively regulate transcription of genes including the *achete scute* gene family, which is well known for mediating neuronal differentiation [3]. Other Notch target genes include the related *Herp* (Hes-related repressor protein) transcription factor family, the cell cycle regulator *Cdkn1a* (also known as cyclin dependent kinase inhibitor (CDKI)p21), the gene for Notch regulated ankyrin repeat protein (*Nrarp*), *Deltex1* and the *pre-T-cell receptor* α gene (reviewed in [1]).

Notch signaling has been shown to regulate a broad range of events during embryonic and post-natal development, including proliferation, apoptosis, border formation, and cell fate decisions [4]. In self-renewing organs in vertebrates and during tumorigenesis, inhibition of differentiation, lineage specification at developmental branch points and induction of differentiation are relevant functions of Notch signaling (Fig. 2). The ability of the Notch pathway to inhibit differentiation was first proposed for the nervous and the hematopoietic systems. The classical example of Notch signaling regulating binary cell fate decisions at developmental branch points is the development of the peripheral nervous system in flies. Equipotent precursors give rise to two alternative cell fates (epidermal versus neuronal) depending on whether an uncommitted progenitor cell receives a strong Notch signal or not. In a different context (keratinocytes, for example), Notch induces terminal differentiation (Fig. 2C). Thus, the question arises; how can the Notch pathway that is not only evolutionarily but also mechanistically conserved, lead to so many different and sometimes opposing outcomes? One obvious, but also superficial explanation is that

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Abbreviations: NICD, Notch intracellular domain; BM, bone marrow; HSC, hematopoietic stem cell; AGM, aorta-gonad-mesonephreos; TA cells, transient amplifying cells; Hes, hairy enhancer of split; FoBs, follicular B cells; MzBs, marginal zone B cells; T-ALL, T cell acute lymphoblastic leukemia; TAN1, translocation associated Notch homologue; CDKI, cyclin dependent kinase inhibitor; NFAT, nuclear factors of activated T cells; Shh, sonic hedgehog

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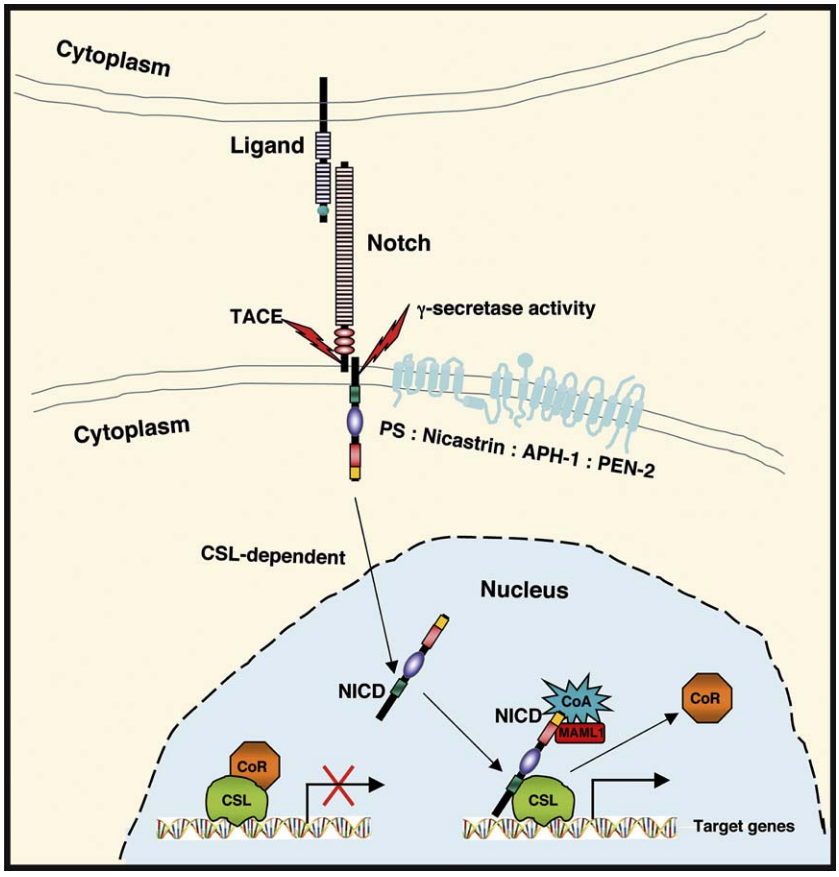

Fig. 1. Notch signaling. Notch signaling is initiated between neighboring cells upon ligand receptor interactions resulting in two successive proteolytic cleavages. The first cleavage within the extracellular domain is mediated by TACE, while the second cleavage occurs within the TM domain is mediated by the γ-secretase activity of a multi-protein complex including Presenilins, Nicastrin, APH-1 and PEN-2. The liberated NICD translocates into the nucleus and heterodimerizes with the transcription factor CSL (CBF1 in *humans*, Suppressor of hairless in *Drosophila* and LAG in *C. elegans*). This interaction leads to transcriptional activation by displacing co-repressors and simultaneously recruiting co-activators (CoA) including mastermind-like proteins (such as MAML1).

Notch function is context dependent. We need a better understanding of the intersecting pathways that interact with and/or influence Notch signaling in a given tissue or cell population to better define "context". Alternatively, different Notch receptors induce different gene expression programs or Notch function might be controlled at the level of Notch ligands. In the next paragraphs we will discuss examples of various Notch functions within different self-renewing organs and how deregulation of this pathway contributes to cancer.

2. Notch: a gate-keeper of intestinal progenitor cells

The mammalian intestine is a prototype self-renewing organ as the intestinal epithelium has one of the highest turnover rates in the body and comprises stem cells, transit amplifying (TA) cells and terminally differentiated cells (Fig. 3A). The gut resembles a tube containing two major parts; the small and the large intestine, each of which can be further divided into anatomically different structures with different functions (absorption of nutrients and compaction of stools). The small intestine is much longer in length than the large intestine, and contains finger like protrusions called villi that dramatically increase the cell surface area to more efficiently absorb nutrients, as well as invaginations called Crypts of Lieberkühn (Fig. 3A). By contrast, the large intestine lacks villi and comprises only crypts. The epithelia of both comprises four different cell lineages: absorptive enterocytes, mucus secreting goblet cells, hormone secreting enteroendocrine cells and lysozyme and cryptidin producing Paneth cells (Fig. 3A). For reasons of simplicity we will concentrate on the small intestine. Paneth cells are the only terminally differentiated cells found at the bottom of the crypts. Intestinal stem cells are thought to localize just above the Paneth cells within the crypts and give rise to proliferating TA cells, which constitute the majority of cells within the crypt compartment. TA cells migrate upward and stop proliferating upon reaching the top of the crypts where they differentiate into the different cell lineages. The enterocytes, enteroendocrine and goblet cells continue migrating upwards towards the tips of the villi, and then undergo apoptosis and are shed into the lumen of the intestine, a process called exfoliation [5].

Due to the very high turnover rate of the intestinal epithelium, processes such as proliferation, differentiation, migration and cell death must be tightly regulated in order to ensure homeostasis. Despite the diversity of cellular responses, these processes are apparently controlled by a relatively small number of signaling pathways, including Wnt, TGFβ/BMP, Hedgehog and Notch. We will focus here on the functions of the Notch pathway within the intestine. Those readers

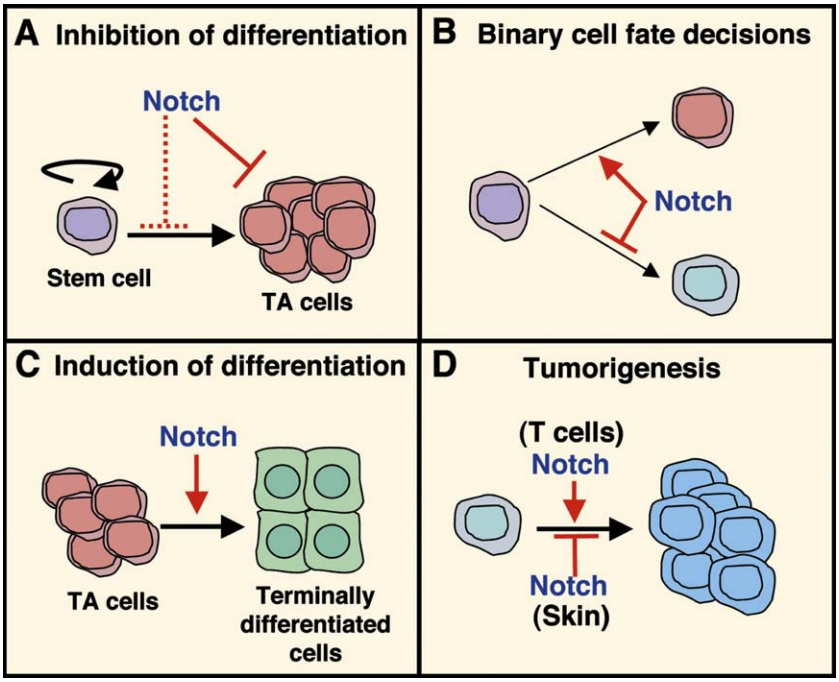

Fig. 2. Pleiotropic effects of Notch signaling. The four major roles of the Notch cascade that are relevant within self-renewing tissues or during tumorigenesis are schematically illustrated. (A) Gate-keeper function: Notch maintains stem and/or TA cells in an undifferentiated state. In the intestine for example, Notch prevents crypt progenitor cells (TA) from differentiating. (B) Binary cell fate decisions: In the lymphoid system Notch specifies the T cell lineage at the expense of the B cell lineage from a (at least) bi-potent early thymocyte progenitor. (C) Induction of differentiation. In the skin, Notch induces terminal differentiation events of TA cells, and during thymocyte differentiation Notch1 promotes differentiation of pro-T-cells into pre-T cells. (D) Tumorigenesis: overexpression of Notch within hematopoietic BM cells or in T cell progenitors results in T cell leukemias and as such Notch functions as an oncogene. However, in the skin Notch functions as a tumor suppressor since loss of Notch signaling results in the development of basal cell carcinoma-like tumors.

interested in the function of the other signaling pathways are referred to other recently published reviews [5,6].

The first, direct genetic evidence implicating Notch signaling in homeostasis of the mammalian intestine derives from inducible gut specific inactivation of the *CSL/RBP-J* gene that mediates Notch signaling of all Notch receptors in the mouse. Postnatal inactivation of CSL/RBP-J within the crypt compartment results in the complete loss of proliferating TA cells followed by their conversion into mucus secreting goblet cells [7]. In reciprocal experiments expression of a dominant active form of the Notch1 receptor (NICD) in the gut inhibits differentiation of crypt progenitor cells [8]. The intestines of these mice consist primarily of undifferentiated TA cells. These reciprocal loss- and gain-of-function data demonstrate that Notch functions as a gate-keeper for intestinal crypt progenitor cells in mice (Fig. 3A). Indirect evidence supporting such an important role for Notch is derived from toxicology studies of γ-secretase inhibitors, which are currently being developed by pharmaceutical companies to inhibit the protease activity (γ-secretase) of the presenilin multi-protein complex for the treatment of Alzheimer's disease. The primary target of these drugs is the disease-causing amyloid precursor protein. However Notch receptors are also cleaved by this protease upon ligand-mediated activation resulting in liberation of the NICD (Fig. 1). However, rodents treated with γ-secretase inhibitors exhibit unwanted side effects such as a large increase in goblet cells (goblet cell metaplasia) within the crypt compartment due to the inhibition of Notch signaling [7,9,10]. The fact that loss of Notch signaling results in goblet cell differentiation at the expense of enterocytes suggests an additional function for Notch signaling in lineage specification of enterocytes. Support for this comes from gene-targeted mice for *Hes1* (a well known Notch target gene). Fetal intestines of Hes1 mutant mice exhibit increased mucus secreting and enteroendocrine cells at the expense of absorptive enterocytes [11]. The reciprocal phenotype is observed in intestines of gene-targeted mice for the *Math1* gene, which is transcriptionally repressed by Hes1 as their intestines are only populated by enterocytes, suggesting that Math1 is required for the secretory cell lineages (goblet and enteroendocrine cells) [6] (Fig. 3A). Taken together these results indicate that Notch has at least two functions during intestinal homeostasis; one to maintain undifferentiated crypt progenitor cells, and the other is to control binary cell fate decisions of progenitor cells that have to choose between the secretory and adsorptive cell fates, most likely by Notch induced expression of Hes1.

Another well known signaling cascade that has been implicated in the maintenance of crypt progenitors is the Wnt pathway. Loss of Wnt signaling in the intestine results in loss of the proliferative crypt compartment [5]. Thus the Notch and the Wnt pathways synergize as gate-keepers of self-renewal in the intestinal epithelium (Fig. 3A).

Recent gene profiling experiments have revealed a highly conserved expression pattern between crypt cell progenitors and colorectal cancer cells [12]. This symmetry also applies to the Notch and Wnt pathways as multiple Notch and Wnt signaling components are expressed both in adenomas of APC min (multiple intestinal neoplasia) mice as well as in wild

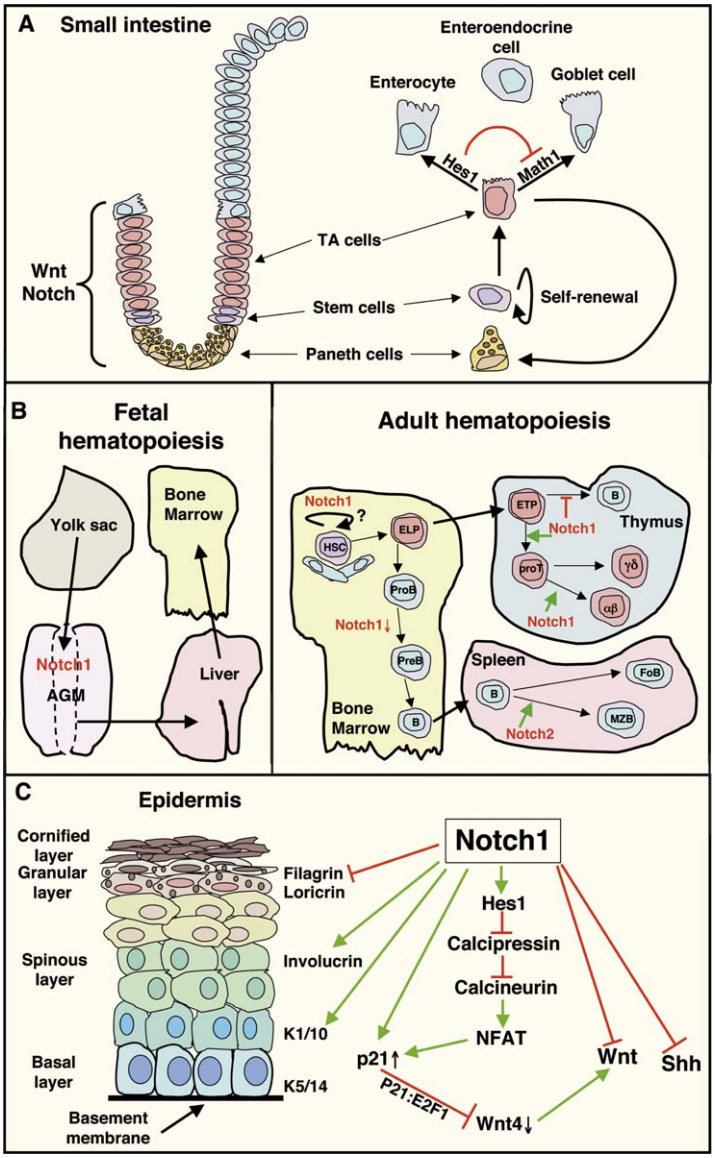

Fig. 3. Notch functions within self-renewing tissues. (A) Notch signaling in the small intestine. Schematic representation of the crypt/villus of the small intestine. Stem cells (in grey) and transient amplifying (TA) cells (in purple) localize to the crypt compartment, which is maintained by both Notch and Wnt signaling. All differentiating TA cells, with exception of Paneth cells which localize to the bottom of the crypt, migrate upwards and stop cycling at the crypt/villus boundary. Migration of non-proliferating differentiated cells continues towards the tip of the villus where they are shed into the lumen of the intestine. One function of Notch signaling within the small intestine is to maintain proliferative crypt progenitors in the undifferentiated state, while a second function is to influence a binary cell fate decision of TA cells that have to choose between the adsorptive and the secretory lineages such as goblet cells and enteroendocrine cells. This process seems to be regulated by the Notch target gene Hes1, which transcriptionally represses Math1. Math1 is required for the development of secretory lineages while Hes1 expression favors the development of adsorptive cells. (B) Notch signaling in hematopoiesis. In fetal hematopoiesis, Notch1 signaling is necessary for developing stem cells within the AGM region. In adult BM progenitors Notch signaling has been proposed (based mainly on gain-of-function studies) to inhibit differentiation of stem cells (HSCs). Downregulation of Notch1 signaling is required in BM B cell progenitors to allow normal B cell development. In the thymus Notch1 signaling is essential for T lineage specification in an early thymocyte progenitor, while at subsequent developmental stages it promotes differentiation of pro-T cells into pre-T cells of the $\alpha\beta T$ lineage. In the spleen Notch2 signaling specifies MzB. FoBs. (C) Notch signaling in the skin. Schematic representation of the murine skin showing some proteins that are expressed in specific cellular layers. The epidermis is a stratified squamous epithelium that is composed of multiple cell layers. The basal cell layer localizes to the basement membrane and consists mostly of TA cells intermingled with a few stem cells. The basal cell layer gives rise first to the spinous layer followed by the granular layer and then the cornified layer. Notch1 signaling induces expression of early differentiation markers such as Keratin1 and Involucrin, and partially represses the expression of Loricrin and Filagrin, two late differentiation markers. Moreover Notch1 induces expression of the cell cycle regulator $p21^{CIP1/WAF}$ by at least two mechanisms. First, Notch1 targets the $p21^{CIP1/WAF}$ promoter directly, and second Notch1 upregulates $p21^{CIP1/WAF}$ through the activation of calcineurin/NFAT activity mediated by the downregulation of calcipressin via the Notch target gene Hes1. Both Wnt- and Shh-mediated signaling are normally repressed in the murine epidermis by Notch1. Repression of the Wnt pathway is at least partially mediated by the downregulation of Wnt4 through a $p21^{CIP1/WAF}$:E2F-1-dependent mechanism.

type crypt cells [6]. These data support the hypothesis that activation of the Notch and Wnt pathways occurs simultaneously in proliferating adenomas and intestinal crypts. This leads to the question whether proliferating adenoma cells can be differentiated and withdrawn from the cell cycle by inhibiting Notch signaling, similarly to what is observed with crypt progenitors.

Indeed, treatment of APC *min* mice with γ-secretase inhibitors induces goblet cell differentiation and reduces proliferation in such adenomas [7], suggesting that specific inhibition of the Notch pathway can drive cells out of cycle despite the fact that Wnt signaling remains active. This 'proof of principle' experiment highlights the Notch pathway as potential drug target for the treatment of intestinal neoplasia.

3. Notch and hematopoiesis

The hematopoietic system is certainly the best studied and characterized self-renewing system. Although hematopoietic stem cells (HSCs) were first identified 25 years ago [13], the molecular mechanisms and specific microenvironments that regulate self-renewal versus differentiation are far from being fully understood. Initially the wide expression pattern of Notch receptors and their ligands (reviewed in [1]) within the adult hematopoietic system, suggested that Notch might play an important role during hematopoiesis. Such a role has been confirmed during embryonic hematopoiesis. In the developing embryo hematopoiesis starts in the yolk sac, shifts first to a region within the embryo called the aorta-gonad-mesonephros (AGM), then to the fetal liver and finally localizes to the bone marrow (BM) (Fig. 3B). The importance of the Notch pathway in embryonic hematopoiesis was shown using gene-targeted mice for *Notch1* and *Notch2*, both of which die around E10.5 due to multiple defects [14]. Hirai and colleagues showed that while Notch1 is dispensable for primitive hematopoiesis within the extraembryonic yolk sac, it is essential for the reconstitution ability of fetal HSCs derived from the AGM region (Fig. 3B). By contrast, Notch2 appears to be dispensable for both primitive and definitive embryonic hematopoiesis [14]. During the onset of definitive hematopoiesis in the embryo Notch1-RBP-J-dependent signaling leads to the activation of GATA2 [15], which has been shown to be an essential transcription factor for hematopoiesis [16]. The importance of Notch1 for embryonic hematopoiesis was further confirmed by generating chimeric mice using Notch1 deficient and wild type embryonic stem cells (ES). Although, hematopoietic cells derived from Notch1 deficient ES cells were initially found in these chimeric embryos, the level of chimerism declined rapidly. While Notch1 deficient ES cells contributed efficiently to other organ systems, at E15.5 they no longer contributed to the hematopoietic system [17]. Taken together these results underscore the important role of Notch1 during early embryonic hematopoiesis.

Whether Notch signaling is similarly important for adult HSC self-renewal and/or maintenance is controversial. The first experiments indirectly supporting an important role for Notch in HSC maintenance were derived from Notch gain-of-function studies using hematopoietic cell lines that could no longer be differentiated due to the expression of a dominant active form of the Notch1 receptor (NICD) [18–20]. These results suggested that Notch inhibits differentiation of hematopoietic progenitor cells. This notion was further confirmed by similar gain-of-function studies using primary BM progenitors which showed increased HCS self-renewal in vivo [21] (Fig. 3B), and in one case led to immortalization of hematopoietic progenitor cells with myeloid and lymphoid differentiation potential [22]. This enhanced HSC self-renewal is possibly mediated by Notch1-induced Hes1 expression since transplanted Hes1 expressing HSCs resulted in increased numbers of cells with side population activity [23], characterized by the active efflux of the DNA dye Hoechst 33342; a hallmark of long-term HSCs [24]. Moreover, co-culture assays in which murine or human HSCs were incubated with immobilized or soluble Notch ligands, or together with ligand-expressing feeder cells maintained or even enhanced HSC self-renewal in vitro (reviewed in [1]). Recently Duncan et al., retrovirally expressed a dominant negative form of CSL/RBPJ in HSCs. These cells showed accelerated differentiation in vitro and reduced levels of chimerism in recipient mice after transplantation [25]. Although this large body of evidence supports the notion of an important function for Notch signaling in HSC self-renewal and/or maintenance, none of the genetic conditional loss-of-function models support this hypothesis. Specifically, neither inducible inactivation of CSL/RBP-J [26], which mediates Notch signaling of all four Notch receptors, nor conditional loss-of-function of Notch1 [27] or Notch2 [28] in adult BM cells lead to a HSC phenotype.

Components of the Notch signaling pathway have also been suggested to participate in the HSC niche, because osteoblast specific expression of the activated parathyroid hormone related protein receptor results in increased Jagged1-expressing osteoblasts, and correlates with increased HSC numbers. These data led to the hypothesis that Jagged1-mediated Notch signaling may regulate HSC homeostasis [29]. However, once again, the genetic data do not support this hypothesis since conditional inactivation of Jagged1 in BM progenitors and/or stroma does not perturb hematopoiesis [30]. Despite the fact that there is no consensus between gain- and genetic loss-of-function experiments regarding the role of Notch signaling in HSC self-renewal and/or maintenance, both experimental settings have demonstrated an essential role for Notch1 in T cell commitment in the adult lymphoid compartment [1]. Inducible inactivation of Notch1 in BM progenitors results in a block in T cell development and ectopic B cell development in the thymus suggesting that Notch1 instructs an early lymphoid progenitor to adopt a T cell fate. In the absence of a Notch1 signal an early lymphoid progenitor chooses the B cell fate by default (Fig. 3B). An identical phenotype is observed in mice in which the *CSL/RBP-J* gene was inactivated in BM progenitors [1], strongly indicating that T cell specification is mediated by Notch1/RBP-J dependent signaling. Interfering with Notch signaling by transgenic expression of negative modulators (such as Fringe, Deltex or Nrarp), or dominant negative forms of transcriptional co-activators (MAML1) also blocks T cell development concomitant with B-lymphopoiesis in the thymus (reviewed in [31]). Reciprocal gain-of-function studies overexpressing NICD in BM progenitors results in ectopic T cell development at the expense of B cell development [31]. Thus, both gain and loss-of-function studies demonstrate that Notch1 is essential for T lineage commitment. In addition, Notch1-RBP-J signaling promotes differentiation of pro-T cells into pre-T cells within the thymus by controlling rearrangement of the T cell receptor (TCR) β locus [31] through regulating chromatin accessibility [32], thereby assuring the successful generation of a pre-TCR complex, which is essential for thymocyte development (Fig. 3B).

An additional role for Notch signaling has been shown for splenic B cell differentiation. Immature BM derived B cells enter the spleen where they differentiate into either follicular B cells (FoBs) or marginal zone B cells (MzBs). Notch signaling is important for MzB differentiation, a process that is regulated

by Delta1:Notch2-mediated CSL/RBP-J dependent signaling [31] (Fig. 3B). Other functions of Notch signaling during adaptive immunity are (reviewed in [31]). Taken together the clear cut physiological roles of Notch signaling in hematopoiesis is influencing lineage decision of progenitors at developmental branch points as well as the induction of differentiation.

4. Notch and T cell neoplasisa

There is increasing evidence that aberrant Notch signaling plays an important role in a number of cancers (Fig. 2D). The first link between Notch and human tumors was made in the late 1980s and early 1990s by Jeff Sklar's group which cloned and sequenced a t(7;9) chromosomal translocation breakpoint in a small number of patients suffering from T cell acute lymphoblastic leukemia (T-ALL). The chromosomal translocation juxtaposes the C-terminal region of EGF-like repeat 34 of human *NOTCH1* to the *TCR* β-enhancer. This leads to the expression of a truncated and constitutively active form of the NOTCH1 receptor which was named TAN1 for translocation-associated Notch homologue [33]. The causative role of aberrant Notch signaling for T-cell leukemia was shown in multiple mouse models by expressing NICD in murine BM progenitors [34]. Similarly, constitutive expression of Notch1-ICD or Notch3-ICD in thymocyte progenitors also leads to T cell leukemia suggesting that the oncogenic potential is not restricted to Notch1 signaling [31]. Moreover these experiments indicate that not only can NICD transform BM progenitors (most probably HSCs), it can transform also more committed thymocyte progenitors. However it is interesting to note that the oncogenic potential of Notch seems to be restricted to T cell malignancies as no myeloid malignancies have been reported to date. The mechanistic reason for this restriction is currently unknown. It is possible that Notch needs to cooperate with a T cell specific signal to cause T cell malignancies. Experiments supporting this hypothesis are derived from NICD-expressing BM progenitors from RAG deficient mice (which cannot rearrange B and TCRs) which do not seem to develop T-ALL, suggesting that Notch cooperates with a TCR-mediated signal [35]. However the molecular details of these TCR-mediated signals are still not well understood and require further investigation.

The t(7;9) chromosomal translocation in humans occurs rarely, and is found in less than 1% of all T-ALL patients thus questioning the clinical importance of this finding. However in a recent study, Aster and colleagues identified activating mutations within the NOTCH1 receptor in more then 50% of the 96 primary T-ALL tumors analyzed. These mutations were found to localize within the heterodimerization domain and/or PEST domain, which regulate protein stability of the receptor. In approximately 20% of cases mutations were found in both domains [36]. These data show that activating mutations within the NOTCH1 receptor are one of the major causes for the development of T cell leukemias thereby pushing Notch into the center of T-ALL pathology.

5. Notch and skin

The skin and its appendages including hair follicles, represents a physical barrier that is constantly renewed. Two stem cell pools have been described, one in the skin epidermis and a second in the bulge region of hair follicles. The epidermis consists of multiple layers of keratinocytes that are separated from the dermis by the basement membrane (Fig. 3C). Slowly cycling multipotent stem cells, as well as rapidly cycling TA cells are found within the epidermal basal cell layer that is characterized by expression of keratins 5 and 14. After a limited number of cell divisions, TA cells are withdrawn from the cell cycle, and differentiate by detaching from the basement membrane to form the suprabasal spinous layer that expresses keratins 1 and 10. Keratinocytes from the spinous layer continue migrating towards the outer surface to form the granular layer, characterized by cells that acquire lipid-containing granules that release their contents in the intercellular space. At this stage the cells synthesize Filagrin and Loricrin, which participate in the formation of the cornified envelope in the outermost layer before eliminating their nuclei and cytoplasmic organelles, a process called cornification (Fig. 3C) (reviewed in [37]).

Hair follicles also undergo self-renewal throughout life. Hair follicle stem cells reside within the bulge region which is located in the upper part of the hair follicle at the level of the insertion of the arrector pili muscle [38]. Hair follicle stem cells were first defined by their label retaining ability [38] and their capacity to generate hair follicles, sebaceous glands and epidermis [39]. Recently very sophisticated studies showed that a single cell isolated from the bulge region of either a hair follicle [40] or a whisker follicle [41], can produce long-term proliferating clones in vitro, indicating that these cells do indeed have self-renewal capacity. More importantly, such clonally expanded cells were able to form intact hair follicles and sebaceous glands, and to participate in formation of the epidermis in transplantation experiments, demonstrating that these cells have multi-lineage potential [40,41]. Moreover, Barrandon and colleagues re-isolated hair follicle stem cells from the first transplant and performed serial transplantation experiments thus demonstrating the self-renewal ability of clonally expanded single hair follicle stem cells in vivo [41].

The multi-lineage potential of bulge stem cells as well the ability of progeny derived from label retaining cells to contribute to the epidermis in response to wounding [42] has fueled the notion that bulge stem cells are also responsible for long-term self-renewal of the skin epidermis. However a recent study in which bulge cells were ablated, combined with fate mapping experiments demonstrated that hair follicle stem cells are not required for normal homeostasis of the skin epidermis. However they can contribute transiently to the epidermis after wounding [43].

In the human epidermis NOTCH1, NOTCH2 and NOTCH3 show high mRNA expression in the basal cell layer and weaker expression in the suprabasal layers. Delta1 and Jagged1 expression is confined to the basal layer [44], with Delta1 expression being highest in regions where potential stem cells seem to reside. These observations led to the suggestion that Delta1-mediated Notch signaling induces a TA cell phenotype [45]. In the epidermis of newborn mice Notch1 and Notch2, as well as Jagged1 and Jagged2 are expressed in the suprabasal layers [46]. Despite these differences in expression patterns of Notch receptors and ligands between human and mouse skin, in vitro data indicate that Notch signaling induces differentiation of keratinocytes [45,46]. Activation of Notch1 signaling causes cell cycle arrest in mouse keratinocytes by increasing expression of the cell cycle regulator $p21^{WAF1/Cip1}$

(also known as CDKIp21) in two different ways (Fig. 3C). On one hand Notch1 induces $p21^{WAF1/Cip1}$ expression by directly targeting NICD-RBP-J to the p21 promoter [46], while on the other hand Notch1 indirectly activates the calcineurin/nuclear factors of activated T cells (NFAT) pathway, which acts positively on the $p21^{WAF1/Cip1}$ TATA-box proximal region. This indirect activation is mediated by the Notch target gene Hes1, by downregulating calcipressin, a negative regulator of the serine/threonine phosphatase calcineurin [47]. Activated calcineurin dephosphorylates NFAT proteins, thereby inducing their subsequent translocation to the nucleus where they participate in regulating gene expression programs. Another property of Notch1 activation in keratinocytes is induction of early differentiation markers (such as Keratin1/10 and Involucrin), down modulation of integrin expression and partial repression of late differentiation markers such as Loricrin and Filagrin [46] (Fig. 3C).

Tissue specific inactivation of the Notch1, RBP-J and Presenilin1 and 2 genes in the murine epidermis results in hyperproliferation of the skin, hair loss and epidermal cyst formation within less than one month [48–51]. Moreover, mice in which Notch1 has been ablated in the skin are more susceptible to chemical induced carcinogenesis, in part explained by reduced $p21^{WAF1/Cip1}$ protein levels [48], since $p21^{WAF1/Cip1-/-}$ mice are also more sensitive to chemical induced carcinogenesis [52]. Since the carcinogen-induced mutation event is predominantly found within the *HA-ras* oncogene it is possible that loss of Notch1 function may have cooperative effects with activated ras during the transformation process of keratinocytes. Indeed, if Notch1 deficient keratinocytes forced to express an oncogenic form of the *ras* gene are injected subcutaneously into nude mice they form aggressive squamous cell carcinomas while wild type control cells do not [48].

Over time, mice with skin specific inactivation of Notch1 develop spontaneous, highly vascularized, basal cell carcinoma-like tumors. In mice and humans this tumor type is frequently associated with aberrant sonic hedgehog (Shh) signaling, and the absence of Notch1 in the mouse epidermis leads to aberrant Gli2 expression, a downstream component of the Shh pathway. Consistent with this, human basal cell carcinomas show reduced expression of NOTCH1, NOTCH2 and JAGGED1 [44], indicating that loss of NOTCH signaling in the human epidermis could also lead to aberrant Shh signaling and thus contribute to the development of basal-cell carcinomas.

The Wnt/β-catenin pathway is another signaling cascade that is deregulated as a consequence of loss of Notch1 signaling in the mouse skin. Notch1 deficiency results in increased β-catenin mediated signaling in keratinocytes and tumors, while Wnt signaling can be repressed by activated Notch1 expression [48]. Suppression of Wnt signaling by Notch1 activation seems (at least in part) to be mediated indirectly by increasing levels of $p21^{WAF1/Cip1}$ protein that subsequently associates with E2F-1 transcription factors at the Wnt4 promoter causing down modulation of Wnt4 gene expression [53].

Taken together, the function of Notch signaling in the epidermis and keratinocytes is to induce terminal differentiation processes as well as to withdraw proliferating cells from the cell cycle. A long-term consequence of loss of Notch1 activation in murine skin is the development of basal-cell carcinoma like tumors, suggesting that the Notch pathway exerts tumor suppressive functions in the skin (Fig. 2D).

6. Concluding remarks

The Notch pathway is a key regulator of many developmental processes during fetal and adult differentiation. Many of the general Notch functions such as stem cell gate keeper, influencing binary cell fate decisions or induction of terminal differentiation processes were first described in invertebrates and subsequently confirmed in self-renewing organ systems of mammals. Although the Notch pathway is mechanistically relatively simple and highly conserved its physiological function within different self-renewing tissues is unpredictable despite their common structure. In the intestine Notch and Wnt play a gate-keeper function for crypt progenitor cells. In addition Notch seems to influence binary cell fate decisions of cells that have to choose between the secretory and adsorptive lineages in the gut. Although deregulation of the Wnt pathway plays a central oncogenic role in the development of colorectal cancers in humans it remains to be shown whether deregulation of Notch signaling also follows the Wnt cascade in this respect. Although a gate-keeper function of Notch has also been postulated for HSC in the BM, the best established role of Notch within the hematopoietic systems is the ability to influence and/or specify cell fates of lymphoid progenitors. Moreover it has become clear that aberrant Notch signaling in humans due to activating mutations in the NOTCH1 receptor plays a key role in the development of T-ALL. Thus Notch1 is an established oncogene in the hematopoietic system. However, this dramatically contrasts with the function of Notch1 in the skin where Notch1 seems to induce terminal differentiation processes and moreover functions as a tumor suppressor. This obviously leads to the question of how Notch can have such opposing functions in different self-renewing organs. Questions concerning specific Notch target genes, mechanistic insights into activating Notch mutations and cross talk between Notch and other pathways need to be answered in order to expand our limited understanding of this 'simple' signaling pathway.

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