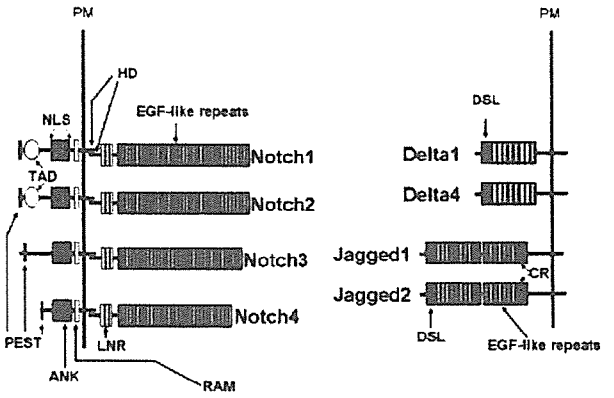

Figure 1. Protein structure of Notch receptors and their ligands. There are 36 EGF-like repeats in Notch1 and Notch2, 34 repeats in Notch3, and 29 repeats in Notch4. Some of the EGF-like repeats serve as a ligand-binding site. LNR has inhibitory function against the cleavage. HDs in the extracellular subunit and transmembrane subunit consist of 103 and 65 amino acids, respectively. RAM associates with CSL protein. ANK associates with proteins to form a complex. PEST negatively regulates the half-life of Notch proteins. DSL is a binding site for Notch. Abbreviations: ANK, ankyrin repeat; CR, cysteine-rich repeat: DSL, Delta-Serrate-Lag2 domain; EGF, epidermal growth factor; HD, heterodimerization domain: LNR, Lin-Notch repeat: NLS, nuclear localization signal; PEST, PEST domain; PM, plasma membrane; RAM, ram domain; TAD, transactivation domain.

that are in direct contact. Binding triggers the cleavage of the extracellular region of the Notch transmembrane subunit (S2 cleavage) [26]. This cleavage facilitates the next cleavage, which occurs within the transmembrane domain (S3 cleavage) [26, 27]. In a simplified scheme, the consequence of S3 cleavage is nuclear translocation of the cleaved intracellular domain of Notch (NICD) and its association with the constitutive DNA-binding protein CSL (after CBF1, Suppressor of hairless, Lag-1; CBF1 is also called RBP-Jκ). This turns the CSL complex from a transcriptional repressor to a transcriptional activator [28], for which the mastermind adaptor protein is an essential component [29, 30]. The most well-defined targets of the NICD-CSL complex are the HES family [31] and their homologs, the Hey (also called HERP) family [9] of basic helix-loop-helix transcription factors. The regulated intramembrane proteolysis (RIP) of transmembrane proteins was first recognized to have biologic significance in the amyloid precursor protein (APP). In APP, RIP occurs at more than one site, which is believed to be linked to the pathogenesis of Alzheimer disease. Interestingly, a second RIP (designated S4 cleavage) has also been detected in Notch1 [32, 33]; this RIP might be related to the Notch signaling pathway. A number of Notch signal-modifying proteins have been identified, and the presence of noncanonical, CSL-independent pathways has been advocated, which are not included in this review.

Notch Signaling in Stem Cells During Embryonic Development

During embryogenesis, it is not always easy to distinguish between primordial cells and tissue-specific stem cells and between stem cells and progenitors. Therefore, stem cells are most broadly considered in this section. Studies using gene-modified animals, which have demonstrated roles of Notch signaling in stem and early progenitor cells, are summarized in Table 1.

Phenotypes of Mice with Mutated *Notch* and Ligand Genes and Their Roles in Embryogenesis

Mutations have been introduced in mice for each of the four *Notch* genes (*Notch1–Notch4*) and four Notch ligand genes (*Delta-like1*, *Delta-like4*, *Jagged1*, and *Jagged2*). Mice, homozygously disrupted with either *Notch1* [34, 35] or *Notch2* [36] are fatal at approximately embryonic day (E) 11. *Notch3*-null [37] and *Notch4*-null mice [38] survive without any apparent phenotypic abnormalities, but the contribution of *Notch3* and *Notch4* to embryonic vascular development has been clarified [38, 39]. Homozygous inactivation of *Delta-like1*, *Delta-like4*, or *Jagged1* causes embryonic lethality during E9.5–E12.5 [40–43], and *Jagged2*-null mice die perinatally [44]. These findings indicate that most of the individual *Notch* genes and ligand genes have nonredundant roles in mouse embryogenesis. Somitogenesis, abnormal vasculature formation, increased cellular apoptosis, excessive neuronal differentiation, etc., are observed in these mutant mice. There are, however, both similar and dissimilar phenotypes in these mice, and the causes of fatality in early to midgestation stages are not likely to be uniform in the knockout mice for each gene.

On the other hand, successful progression to midgestation stages implies that Notch signaling is unnecessary for the very early stage of embryogenesis, including the fertilized egg stage [45]. This would be consistent with the fact that activation of Notch signaling in ESCs, which are derived from the inner cell mass, does not block differentiation of ESCs [46].

Roles in the Central Nervous System During Embryogenesis

Among undifferentiated neuroectodermal cells with the same potential during *Drosophila* embryogenesis, some cells will eventually express Delta at higher levels, which sends a signal to surrounding cells that uniformly express the Notch receptor. Cells receiving the signal are blocked from differentiating to neuroglioblasts (NGBs) and eventually assume another differentiation fate. On the other hand, cells that express Delta differentiate to NGBs and subsequently to neurons and glial cells [47]. Accordingly, insufficient levels of Notch signals result in the "neurogenic phenotype," in which all cells with neuronal potential differentiate into neurons.

Precocious neuronal differentiation observed in Notch pathway-deficient neurocompetent cells is also detected in mammals, such as in mice with inactivated *Notch1* [48]. A conditional *Notch1* knockout study [49] provides further support that Notch signaling inhibits the premature onset of neurogenesis. These studies, as well as those of *rbpsuh* (gene for CSL) knockout mice [50], suggest that, in addition to the differentiation blockade, this pathway is required for the maintenance and expansion of the neural stem/progenitor cell pool. Inactivation of *HES1* [51]; *HES1* and *HES5* [52]; and *HES1*, *HES5*, and *HES3* [53] in mice variably induces precocious neuronal differentiation accompanied by a decrease in neural progenitors. Together, these studies and HES protein overexpression studies [54] indicate that *HES* genes are the conserved targets of Notch-CSL signaling for regulating the expansion and differentiation of neural progenitors [6, 31, 54].

Table 1. Gene-modified animal studies demonstrating roles of Notch signaling in stem and early progenitor cells

Stem/early progenitor cell system	Observations	Genes and modifications	Specific references	Review article
Embryogenesis				
CNS development	Precocious neuronal differentiation	Notch1-null, Notch1-cond null, rbpsuh-null, Hes1-null	48, 49, 50, 51	6, 31, 47
	Decrease in neural stem/progenitor cell pool	Hes1-null/Hes5-null, Hes1-null/Hes5-null/Hes3-null	52, 53	6, 31, 47
	Expansion of neural stem cells	Hes1:Tg, Hes5:Tg	54	6, 31, 47
HSC generation	Deficient HSC generation at P-Sp	Notch1-null, rbpsuh-null	70, 72	5
	Failure in contribution to hematopoiesis	Notch1-null ES cell chimera	71	5
Melanocyte generation	Severe coat color dilution in the initial hair	rbpsuh-null	73	
Vasculature system development	Abnormalities in blood vessel formation and remodeling	Notch1-null, Notch1-null/Notch4-null, rbpsuh-null	38, 74	2
		Jagged1-null, Hey1-null/Hey2-null	43, 76	2
	Failure in arterial patterning of endothelial cells	Dll4-hetero, rbpsuh-null	41, 42, 74	2
	Failure in arterial specification of smooth muscle cells	Notch3-null	39	2
Organogenesis	Hypoplastic gromeruli and heart	Notch2-ΔEGFrep	82	
	Eye dysmorphology	Jagged1-null	43	
	Abnormalities of bile duct, heart, eyes, and vertebrae[a]	Jagged1-hetero (Aligille syn)[a]	83, 84	
	Excess endocrine cells in pancreas	Dll1-null, rbpsuh-null, HES1-null	86, 87	
	Insulin-deficient diabetes with both endocrine and exocrine pancreatic hypoplasia	rbpsuh-cond null	88	
	Gall bladder agenesis, hypoplastic bile duct	HES1-null	89	
	Differentiation to pancreatic cells from bile duct epithelium	HES1-null		
	Excess differentiation to secretary cells in small intestine	HES1-null	87	
	Amplification of intestinal progenitor pool			
	Inhibition of intestinal cell differentiation	aNotch1-cond Tg	90	
Stem cell systems in adults				
Skin and hair systems	Hair loss; epidermal hyperkeratinization and epidermal cyst formation	Notch1-cond null, Notch1-cond null/Notch2-cond null	92, 93, 95	
		Notch1-cond null/Notch2-cond null/Notch3-null	92, 93, 95	
		PS1-cond null/PS2-null, rubsuh-cond null; Notch1-Tg	92, 93, 95	
	Generation of squamous carcinoma of skin	Notch1-cond null	97	
Hematopoietic and immune systems				
HSC maintenance in bone marrow	Increase in bone marrow HSCs	CaPTHER-Tg (Jagged 1 overexpression in trabecular bone)	100	
Early T cell development	Impaired thymocyte development, increased B lineage cell development in the thymus	Notch1-cond null, 1-Fringe-Tag	123, 125, 126	
Intestinal mucosal system	Conversion of proliferative crypt cells to postmitotic Goblet cells	rbpsuh-null	146	
Skeletal muscle system	Restoration of regenerative potential in old mouse skeletal muscle by Notch activation using Notch-stimulatory antibody[b]		140, 149	
	Impairment of regenerative potential in young mouse skeletal muscle by Notch inhibition using Jagged1-Fc			

[a] Alagille syndrome (human disease).

[b] Gene-modified animals are not used.

Abbreviations: aNotch1, constitutive active form of Notch1; caPTH, constitutively active form of parathyroid hormone receptor; CNS, central nervous system; cond, conditional; ΔEGFrep, deletion of EGF-like repeat(s); hetero, heterozygous null mutation; HSC, hematopoietic stem cell; l-Fringe, lunatic Fringe; null, homozygous null mutation; P-Sp, paraaorta-splanchnopleura; Tg, transgenic.

Importantly, Notch signaling either promotes or, by default, facilitates glial cell fate, perhaps as a consequence of inhibiting neuronal cell fate [55–59]. It further induces astrocyte differentiation [60, 61]. Therefore, Notch signaling might act on neural stem cells in two steps: (a) initially inhibiting neuronal fate while allowing for glial cell fate; and (b) then promoting differentiation to astrocytes while inhibiting differentiation to both neurons and oligodendrocytes [61]. These concepts fit the conventional view of the fundamental function of Notch signaling to prevent cells from taking the first pathway while guiding them into a secondary pathway.

To add to the complexity, neural stem cells have been identified in glial cell populations, particularly in astrocytes [47]. It remains to be clarified how Notch signaling is involved specifically in the maintenance of stem cell characteristics and the promotion of glial differentiation.

Notch signaling is also involved in the regulation of apoptosis in mammals. It remains controversial, however, whether it is pro- or antiapoptotic in developing neural stem/progenitor cells. Conditional deletion of *Notch1* [49] and both *Notch1* and *Notch3* [62] genes causes apoptosis and massive loss of developing neural progenitors as well as newly differentiating neurons. These studies suggest an antiapoptotic activity of Notch signaling. The results of other studies that analyzed conditional transgenic and knockout mice, however, led to the opposite conclusion. Conditional expression of a constitutively active form of Notch1 leads to p53-dependent apoptosis of early neural progenitors. Conversely, disruption of *presenilin-1*, the gene for a component of γ-secretase that is essential for Notch signaling, and conditional deletion of *Notch1* result in reduced apoptosis of early neural progenitor cells [63]. Proapoptotic activity of Notch signaling occurs in keratinocytic [3, 64] and endothelial [65, 66] cells, partly through inactivation of the phosphatidylinositol 3-kinase (PI3K) pathway, whereas activation of the PI3K pathway and antiapoptotic effects have also been demonstrated in other cells, including tumor cells [67, 68]. Thus, the regulation of apoptosis and survival by Notch signaling must be highly context-dependent, and reciprocal directions should be considered on this axis for understanding the development of neural stem/progenitor cells.

Roles in Generating Hematopoietic Stem Cells

In mice, phenotypically defined endothelial cells that possess hematopoietic potential, called "hematogenic endothelial cells," are generated by E9.5 at the paraaorta-splanchnopleura (P-Sp) region. On E10.5, when the P-Sp develops to become the aorta-gonad-mesonephros (AGM), hematogenic endothelial cells develop into hematopoietic stem cells that have the potential to engraft in adult mouse bone marrow [69]. In the *Notch1*-null mutant mouse embryo, apparent hematogenic endothelial cells are recognizable, but they do not develop into hematopoietic stem cells [70]. Using different approaches, either *Notch1*-null ESC-chimeric mice [71] or *rbpsuh*-null mutant mice [72] produced similar results, indicating that Notch1 has an indispensable role at the point right before hematopoietic stem cells are generated.

On the other hand, Notch1 is dispensable for primitive hematopoiesis and secondary hematopoiesis that is derived from progenitors, not from HSCs, in the yolk sac. Thus the requirement of Notch signaling during hematogenesis is most prominent in adult-type HSC generation [70, 71].

Role in Melanoblast Survival

In embryonic skin, melanoblasts (Mbs) develop in the suprabasal and basal layers of the epidermis. The cleaved form of Notch1 is detected and the HES1 promoter is activated in the embryonic Mbs. A γ-secretase inhibitor induces apoptosis of embryonic Mbs, and *tyrosinase* promoter-dependent deletion of the *rbpsuh* gene results in severe coat color dilution in the initial hair [73]. These results indicate that the embryonic Mbs use the Notch1-CSL-Hes1 pathway to protect themselves from apoptosis.

Roles in Vasculature Formation

Mice with disrupted *Notch*-related molecules display various abnormalities in blood vessel formation, such as proliferation and migration of endothelial cells, smooth muscle differentiation, vascular remodeling processes [2], and arterial-venous identification [41, 42, 74, 75]. These studies describe the involvement of Notch1 [38], Notch3 [39], and Notch4 [38] receptors and Delta-like4 [41, 42, 74] and Jagged1 [43] ligands in vasculature formation.

In the vascular system, Hey1, Hey2, and HeyL are considered candidate Notch-CSL targets [2]. In fact, mice lacking both *Hey1* and *Hey2* are embryonic lethal due to abnormalities in the heart and vasculature [76]. The phenotypes of these mice, however, do not precisely recapitulate those of *Notch1/Notch4* double mutant mice or *Delta-like4* heterozygous mice. Thus, further studies are required before definitive conclusions can be drawn as to whether the Hey family alone comprises the transcription factors functioning downstream of the Notch signals in vasculature development. Endothelial cells of the artery and vein have distinct gene expression patterns at the early developmental stage. Many Notch-related molecules are preferentially expressed in the arterial endothelial cells [77, 78]. Strikingly, signals from Delta-like4 to the Notch receptors confers the identity of the arterial endothelium to the precursor cells, which is implicated by the analysis of *Delta-like4* heterozygous mice [41, 42, 74]. *rbpsuh*-null mice have a similar arterio-venous misidentification [74]. In addition to the endothelium, Notch signaling is also involved in the differentiation and maturation of vascular smooth muscle cells, particularly their specification to arterial smooth muscle cells [39].

It has been proposed that the default pathway of the endothelial cells is "venous fate" and that Notch signaling instructively modulates endothelial cell fate to the "arterial fate" [79, 80]. It was recently reported, however, that the venous fate of endothelial cells is induced by a transcription factor, COUP-TFII, that inhibits Notch signaling [75]. In any case, it is clear that the Notch pathway is a critical determinant for arterial-venous endothelial cell fate.

Gain-of-function and loss-of-function experiments in vascular development sometimes yield similar results [81]. These findings often appear to contradict those observed in neural progenitors in which gain- and loss-of-function studies exhibit mirroring phenomena, that is, progenitor expansion with blocked differentiation by the gain-of-function studies and precocious differentiation with decreased progenitor pool by the loss-of-function studies. This might indicate that appropriate

Notch signaling levels are most important for survival of the endothelial cells, given that Notch signals often affect endothelial cells in a proapoptotic manner [65, 66].

Roles in Organogenesis

Analyses of *Notch* gene knockout mice and identification of *Jagged1* as a responsible gene for a hereditary disease that accompanies defects in organ morphogenesis provide evidence that Notch signaling has important roles in the development of kidney, liver, pancreas, heart, etc.

Notch2-null mouse embryos die before mesonephros generation [36] (described above). *Notch2*-hypomorphic mice, in which Notch2 lacks one or two epidermal growth factor-like domains in the extracellular region, survive until birth. In these mice, the glomeruli are severely hypoplastic, and the heart is also variably hypoplastic [82].

Alagille syndrome is an autosomal-dominant human disorder characterized by intrahepatic cholestasis; abnormalities of heart, eyes, and vertebrae; and a peculiar facial appearance. Haploinsufficiency of *Jagged1* is responsible for this disorder [83, 84], suggesting that reduced Jagged1-Notch signaling results in organogenesis failure. Both hepatocytes and biliary epithelial cells are derived from common liver progenitor cells or liver stem cells [85]. Whether adequate Jagged1 function is required for the biliary epithelial cell induction from liver stem cells is not known. The phenotypes of *Jagged1*-disrupted mice [43] and *Notch2*-hypomorphic mice [82] are similar to that of Alagille syndrome.

In the pancreas of *Delta-like1* or *rbpsuh* gene knockout mice, there is an excess of endocrine cells [86], suggesting that Notch signaling inhibits endocrine-lineage differentiation from the common exocrine and endocrine progenitors during pancreatic development. Analysis of *HES1*-null mice leads to a similar conclusion about the role of Notch signaling in pancreatic development [87]. Recently, somewhat different results were obtained from conditional deletion of the *rbpsuh* gene; the mice exhibited insulin-deficient diabetes with both endocrine and exocrine pancreatic hypoplasia [88]. *HES1*-null mice show agenesis of the gall bladder and hypoplastic bile ducts. Furthermore, bile epithelial cells differentiate to pancreatic cells in these mice [89], suggesting that Notch signaling physiologically inhibits pancreatic differentiation from bile epithelial cells, which have the potential to take the pancreatic cell fate in the absence of Notch signaling.

The same mice exhibit excess differentiation to secretory cells, such as Goblet cells, in the intestinal mucosa at the expense of enterocytes [87]. A mirroring phenotype has been reported in *Villin* promoter-controlled Cre and Rosa-Notch transgenic mice. These mice, in which constitutively active Notch1 is expressed in the intestinal epithelium, survive until birth at Mendelian ratios but die of malnutrition within 3 days after birth. The architecture of the intestinal epithelium in neonatal mice is markedly altered by amplification of the intestinal progenitor pool and inhibition of cell differentiation [90]. These findings together suggest a role of Notch signaling in the developing intestinal epithelium to regulate specification of the intestinal progenitor cells.

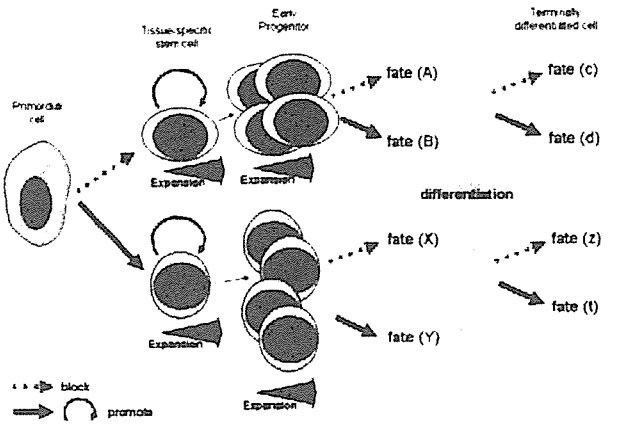

Figure 2. Influence of Notch signaling on the fate of stem and progenitor cells. The biologic effects of Notch signaling can be generalized as depicted in this figure. Notch signaling guides cells to differentiate or not and to differentiate to A instead of B or to B instead of A. Thus, Notch signaling has a role in increasing the number of stem or early progenitor cells.

Cell Fate Determination by Notch Signaling During Embryogenesis

The above observations indicate that from the viewpoint of cell differentiation, Notch signaling has three major roles during embryonic development. First, it affects differentiation from primordial cells to tissue-specific stem cells in the early- to midstage embryo. In the traditional view of *Drosophila* nervous system development, differentiation from undifferentiated neuroectodermal cells to NGBs is inhibited. In mammals, however, induction rather than inhibition is postulated for HSC generation from upstream progenitors. The effect of Notch signaling on differentiation fate from primordial cells to tissue-specific stem cells might be context-dependent. Second, it inhibits tissue- or organ-specific stem cells or immature progenitors from further differentiation and presumably helps them expand while maintaining the immature state. Third, it blocks the default pathway and promotes the alternative pathway, which is typically observed during mid- to late-stage embryo development, such as during organ formation (Fig. 2).

Roles of Notch Signaling in Stem Cells Postdevelopment

Adult stem cells are considered to maintain homeostasis of cells and tissues throughout life. The adult stem cells maintain the number of stem cells, as well as terminally differentiated cells, during normal turnover and repair damage after injury. Involvement of Notch signaling occurs during both normal status and injury in various stem cell systems. Studies along this line using gene-modified animals are summarized in Table 1.

Roles in the Skin

Involvement of Notch signaling in postdevelopmental stem cell systems is best understood in the skin, particularly in the hair follicles. Notch1, Notch2, and Notch3 are expressed and differentially localized to various layers of the hair follicle [91, 92]. Notch signals do not affect the pattern of skin formation during embryogenesis. Thin, short, and wavy hair grows when *Notch1*

is inactivated specifically in the hair follicles during embryogenesis, where Notch1 activity in the cortical progenitors affects the neighboring inner root sheath and medulla cells from their progenitors in a nonautonomous manner [92].

After birth, the first hair cycle of *Notch1*-null mice shows a shortened anagen phase and premature entry into the catagen phase, and inactivation of *Notch1* in adult mice results in almost complete hair loss followed by cyst formation. These observations indicate that Notch1 is essential for postnatal hair follicle development and homeostasis [92, 93]. Compared with the phenotypes of *Notch1*-null mice, *Notch1* and *Notch2* double-null mice display stronger hair follicle phenotypes. The phenotypes of triple-null (i.e., *Notch1*-, *Notch2*-, and *Notch3*-null) mice appear to be even stronger than those of double-null mice. Presenilins are necessary for hair follicle maintenance in mice, and the hair and skin phenotypes of the *Notch1*-, *Notch2*-, and *Notch3*-null mice are very similar to those of *presenilin-1* and *presenilin-2* double-null mice [92].

Notch overactivity in hair follicle cells also leads to abnormal hair formation [94, 95]. Notch1 overactivity in the cortex affects neighboring cells, indicating a nonautonomous role for Notch-regulated transcripts [94]. Transgenic mice expressing constitutively active Notch1 in the suprabasal epidermal keratinocytes and inner root sheath of the hair follicle have epidermal hyperplasia and delayed inner root sheath differentiation, which leads to hair shaft abnormalities and alopecia associated with defects in the anagen phase of the hair cycle [95]. Thus, it is noteworthy that both loss and gain of Notch signaling similarly result in hyperkeratosis and hair loss due to hair cycle disturbances.

Analysis of mice with a specific deletion of *rbpsuh* in the skin using a *nestin* promoter-controlled Cre-lox system reveals that Notch signaling inhibits stem cells in the bulge, the stem cell niche in the hair follicle, from differentiating into epidermal cells and promotes hair formation [96]. This conclusion is consistent with observations that defects in Notch signaling result in markedly impaired hair formation and excess differentiation to the epidermal cells during the catagen phase, which eventually causes epidermal hyperplasia and epidermal cyst formation [92, 93]. Moreover, a lack of *Notch1* caused by a tamoxifen-inducible *K5* promoter-controlled Cre-lox system leads to skin tumor generation [97], indicating that Notch signaling is sometimes tumor-suppressive [98].

In summary, Notch signals are likely to promote the selection of hair formation in bulge stem cells. Thus, ex vivo expansion of hair stem cells with the use of an artificial stem cell niche could revolutionize the dermatology/cosmetology field. Notch signaling should be an important component of such an artificial niche for hair stem cells. Moreover, the regulation of Notch signaling might be considered as a means for skin surface management.

Roles in Hematopoietic and Immune Systems

In the hematopoietic and immune systems, Notch1, Notch2, and Notch3 are expressed in immature, as well as mature, blood cells and lymphocytes. Notch ligands are mainly expressed in the stromal cells and antigen-presenting cells, yet subsets of hematolymphopoietic cells also express some Notch ligands [4]. A number of studies demonstrate that Notch signaling inhibits myeloid differentiation from progenitor cells [5]. Whether this signaling pathway is involved in HSC maintenance in the bone marrow niche and whether ex vivo HSC expansion is feasible using Notch signaling activators are issues that remain to be examined.

HSC Maintenance in Adult Bone Marrow. Osteoblasts on the surfaces of trabecular bone have been identified as one of the bone marrow HSC niches [99–101]. Sinusoidal endothelial cells in the bone marrow and spleen also provide a niche for HSCs [102]. Two groups have proposed that Notch signaling is actively involved in HSC maintenance/growth in the osteoblastic niche using different experimental animals. One group, using transgenic mice that express constitutive active parathyroid hormone receptor under the control of the collagen type-IV promoter, reported that the trabecular bone mass is increased and the Notch ligand, Jagged1, is overexpressed in the osteoblasts. The authors of this report argue that the increase in bone marrow HSCs is a direct consequence of the increased osteoblastic niche area and overexpression of Jagged1 in the niche cells [100]. This finding does not necessarily imply that physiologic levels of Notch signaling contribute to HSC maintenance, particularly as the long-term culture-initiating cells decreased only to basal levels, but not below basal levels, by a γ-secretase inhibitor in the coculture with bone marrow stromal cells derived from the transgenic mice. The other group used mice harboring green fluorescence protein as a reporter under the CSL binding sequence and explored Notch activation at the cellular level in situ. In these mice, green fluorescence protein is expressed in the c-Kit-positive cells near the trabecular bone area, indicating Notch activation in the HSCs, which contributes to inhibiting HSC differentiation [103].

Taken together, interactions between osteoblast-expressed Notch ligands such as Jagged1 and signal transmission to the Notch receptor-expressing HSCs might be one of the molecular mechanisms underlying the regulation of HSC in the osteoblastic niche in the bone marrow. The conditional deletion of both *Notch1* and *Jagged1*, however, fails to show a clear-cut role for the Jagged1-Notch1 pathway in HSC maintenance [104]. Therefore, truly convincing evidence must be demonstrated before we can draw conclusions about the physiologic role of Notch signaling for HSC maintenance in the bone marrow niche, including the osteoblastic and sinusoidal endothelial niches.

Ex Vivo Expansion of HSCs Using Notch Signaling. Ex vivo expansion of HSCs maintained in the immature state has long attracted interest because of the potential utilization of expanded HSCs for transplantation, gene therapy, etc. [105]. Forced expression of a constitutively active form of Notch1 [106, 107] or wild-type HES1 [108] inhibits murine HSC differentiation and potentially expands the HSCs. There are no clear-cut data, however, showing ex vivo expansion of transplantable murine HSCs that retain full hematopoietic activity by stimulating with Notch ligands, although a number of studies have demonstrated the influence of Notch signaling on murine hematopoietic progenitor cells (Table 2) [109–121]. In contrast, investigations using human cord blood-derived cells demonstrated ex vivo expansion of severe combine immunodeficiency (SCID) mouse-repopulating cells (SRCs), which are established surrogates for HSCs (Table 2). For the latter, soluble forms of Delta-like1,

Table 2. Effects of Notch ligands on murine and human hematopoietic stem/progenitor cells in culture

Notch ligands[a]	Cytokines	Increased (maintained) progenitor cells and the source of culture	References
Murine hematopoietic cells			
Jagged1 (human) on 3T3 cells; Jagged1 (human) immobilized to beads	SCF, FL, IL6, IL11	Total CFC and CFU-Mix from BM KSL cells	109
Jagged1 (human) on S17 cells	Serum alone	Total CFC from $CD34^+$c-Kit^+ cells in AGM and fetal liver	110
Jagged2 (murine) on Rab2 fibroblasts	Serum alone	CFU-Mix, CFU-GM, CFU-G, and CFU-M from BM Lin^-c-Kit^+ cells	111
GST-Dll1 (human); Dll1 (human) immobilized to beads	SCF, FL, IL6, IL11, IL1, G-CSF, GM-CSF, EPO (no serum)[c]	Total CFC, CFU-Mix, CFU-GM, BFU-E, and CFU-S from BM Lin^- cells	112
Dll1-Fc (human) immobilized to flask[a]	FL, IL6, IL11	Short-term repopulating cell from BM KSL cells	113
Jagged1 (rat) immobilized to beads[a]	WEHI-3B-conditioned medium or SCF, TPO, FL	CFU-Mix, CFU-GM, BFU-E, and cobblestone area-forming cells from BM mononuclear cells and Lin^- cells	114
Human hematopoietic cells			
Jagged2 on 3T3 cells	FL, IL3/GM-CSF fusion protein	Total CFC, CFU-Mix, and CFU-GM from CB $CD3^+$ cells	115
Jagged1-Fc	SCF, FL, IL6, IL3, G-CSF (no serum)	SRC and total CFC from CB $CD34^+CD38^-Lin^-$ cells	116
Dll1-Fc (Dll4-Fc)[b]	SCF, FL, IL6, IL3, G-CSF (no serum)	SRC and total CFC from CB $CD34^+CD38^-Lin^-$ cells	117
Dll1-myc immobilized to flask	SCF, TPO, FL, IL6, IL3 (no serum)	SRC and total CFC from CB $CD34^+CD38^-$ cells	118
Dll4 on S17 cells (Dll4-Fc)	SCF, PEG-MGDF, FL, IL3	LTC-IC from CB $CD34^+CD38^-$ cells	119
Dll4 on S17 cells	SCF, TPO, FL, IL3	BFU-E and LTC-IC from FL $CD34^+CD38^-$ cells	120
Dll1-Fc immobilized to flask	SCF, TPO, FL, IL3, IL6	SRC from CB $CD34^+CD38^-$ cells[d]	121
	SCF, TPO, FL, IL3, FP6	SRC from CB $CD133^+$ cells	122

[a] Immobilization of proteins was required for the effects noted; free proteins were ineffective or antagonistic.
[b] SRC expansion was demonstrated only by Dll1-Fc.
[c] Fetal calf serum or horse serum is used unless indicated.
[d] Effective only in the low dose of Dll1-Fc.
Abbreviations: AGM, aorta-gonad-mesonephros; BFU-E, burst-forming units-erythroid; BM, bone marrow; CAFC, cobblestone area-forming cells; CB, cord blood; CFC, colony-forming cells; CFU-GM, colony-forming units-granulocytes/macrophages; CFU-Mix, colony-forming units-mixed; CFU-S, colony-forming units-spleen; Dll1, Delta-like1; Dll4, Delta-like4 (all the soluble proteins are consisted of extracellular domain of each ligand); EPO, erythropoietin; Fc, Fc portion of human immunoglobulin G; FL, flt-3 ligand; FP6, fusion protein 6; GST, glutathione *S*-transferase; KSL, c-Kit(+) Sca-1(+) Lin(-); Lin, lineage marker; PEG-MGDF, polyethylene glycol-megakaryocyte growth and development factor; SCF, stem cell factor; SRC, SCID-repopulating cells; TPO, thrombopoietin.

such as fusion with the Fc portion of human immunoglobulin G (Dll1-Fc), have been used to show the increase in the potential or the numbers of human SRCs when HSC-enriched cells are cultured in the presence of hematopoietic cytokines, such as stem cell factor and thrombopoietin [116, 118, 121, 122]. These findings raise the possibility of the clinical use of these proteins. To date, however, the degrees of SRC expansion have not been robust enough to establish clinically applicable ex vivo HSC expansion methods, although expansion of progenitors has been demonstrated to be more massive.

A recent work by Delaney et al. indicates that low doses of immobilized Dll1-Fc are required for SRC expansion, whereas higher doses induce apoptosis of the cord blood-derived immature cells [121]. In addition, it appears that there are differences in the biologic effects of diverse Notch ligands if they are used ex vivo. These issues raise another possibility that the bone marrow microenvironment can be better mimicked if we learn more about the biologic outcomes of diverse levels of Notch signaling or Notch signaling by different ligands.

Effects of Notch Signaling on Early Lymphopoietic Cells. Several important conclusions have been drawn from hematolymphopoietic cells differentiated from HSCs, such as that Notch signaling guides further differentiation of HSC-derived hematolymphopoietic cells, for example: (a) T and B lineage determination (in the progenitor stage between the HSCs and the most early thymocytes, Notch signaling blocks B lineage differentiation and promotes T-lineage differentiation) [123–126], (b) further acceleration of thymocyte differentiation and proliferation [127–130], and (c) splenic marginal zone B cell forma-

tion [131–134]. Sambandam et al. identified "early T lineage progenitors" in the thymus, which express Flt3 and preserve B lineage potential, as the cell subset that first receives Notch signals rather than multipotent hematopoietic progenitors circulated in the blood [135]. Notch signals are also necessary for the differentiation, proliferation, and rescue from apoptosis at multiple checkpoints during thymocyte development. Interestingly, the glucose metabolism induced by activation of phosphatidylinositol-3-OH kinase is proposed to be the major contributor to apoptosis prevention by Notch signaling at the β-selection checkpoint [13]. It is now known that either Delta-like1 or Delta-like4 is sufficient to induce T-lineage cells down to the $CD4^+CD8^+$ stage from upstream progenitors in in vitro culture [134–136].

Information regarding the involvement of Notch signaling in the immune system is accumulating rapidly, and a comprehensive discussion of this topic is beyond the scope of this review. A number of review papers summarize the influence of Notch signals on lymphocyte development, particularly on T-cell development in the thymus [4, 7, 137, 138].

Roles in Intestinal Mucosal Cells

Intestinal epithelial stem/progenitor cells are localized in the basal area of the crypts and continuously supply multiple types of mature cells [139, 140]. These cells express Notch receptors and molecules necessary for Notch signaling [141, 142].

The administration of γ-secretase inhibitors induces gross histologic changes in the intestinal epithelial layer of mice, such as an increased number of Goblet cells, endocrine cells, and abnormal crypts [143–146]. Conditional inactivation of *rbpsuh* in the epithelium of the small intestine and colon by an inducible *Cyp1A* promoter-expressed Cre enzyme induces rapid and massive conversion of proliferative crypt cells to postmitotic Goblet cells [146]. These findings are consistent with observations of *HES1*-null mice [87] and *Villin*-Cre *Rosa*-Notch transgenic mice [90] and suggest that Notch signaling in adults functions to maintain intestinal epithelial stem/progenitor cells.

Roles in Skeletal Muscle Regeneration

Satellite cells are stem cells of skeletal muscle fibers [147]. In aged mice, satellite cells have a markedly impaired propensity to proliferate and produce the myoblasts necessary for muscle regeneration. This is attributed to insufficient upregulation of Delta1, in contrast to the injured muscle in young mice in which Delta1 is sufficiently upregulated. Ultimately, Notch signaling is insufficient for the regeneration of injured muscle in aged mice. Notch inhibition impairs regeneration in young mouse muscle, and forced Notch activation restores the regenerative potential to aged mouse muscle [148, 149]. Thus, Notch signaling is a key determinant of the muscle regenerative potential that declines with age.

CONCLUSION

This report reviews the current knowledge of the Notch signaling pathway in various types of stem and early progenitor cells, but not germ cells. Ex vivo stem cell expansion is fundamental to the success of stem cell-based regeneration medicine, and it is likely that Notch signaling has a role in stem cell expansion. The effects of Notch signaling on progenitor cell survival have been demonstrated, and tumorigenic aspects must be considered.

DISCLOSURES

The author indicates no potential conflicts of interest.

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