MINIREVIEW

Notch Signaling: Linking Embryonic Lung Development and Asthmatic Airway Remodeling

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ABSTRACT

Lung development is mediated by assorted signaling proteins and orchestrated by complex mesenchymal-epithelial interactions. Notch signaling is an evolutionarily conserved cell-cell communication mechanism that exhibits a pivotal role in lung development. Notably, both aberrant expression and loss of regulation of Notch signaling are critically linked to the pathogenesis of various lung diseases, in particular, pulmonary fibrosis, lung cancer, pulmonary arterial hypertension, and asthmatic airway remodeling; implying that precise regulation of intensity and duration of Notch signaling is

imperative for appropriate lung development. Moreover, evidence suggests that Notch signaling links embryonic lung development and asthmatic airway remodeling. Herein, we summarized all-recent advances associated with the mechanistic role of Notch signaling in lung development, consequences of aberrant expression or deletion of Notch signaling in linking early-impaired lung development and asthmatic airway remodeling, and all recently investigated potential therapeutic strategies to treat asthmatic airway remodeling.

Introduction

Lung development originates from the anterior foregut and is orchestrated by complex mesenchymal-epithelial interactions that coordinate the temporal and spatial expression of multiple regulatory factors for normal development (Morrissey and Hogan, 2010). Lung development occurs through sequential stages: embryonic stage (week 3–7 in humans; embryonic day (ED) ~8.0–9.5 in mice), pseudoglandular stage (week 7–17; ED 9.5–16.5), canalicular stage (week 17–27; ED 16.5–17.5),

saccular stage (week 27–36; ED 17.5 to postnatal day 5 (P5) in mice), and alveolar stage (week 36 to ~7–10 years in humans; P5 to ~P30 in mice) (Warburton et al., 2010; Schittny, 2017). Stages of lung development and expression of Notch signaling are depicted in Fig. 1. Later in gestation, epithelial progenitors are differentiated into basal cells, ciliated cells, goblet cells, and neuroendocrine cells (in proximodistal axis), submucosal glands consisting of mucous and serous cells (in the proximal region), and ciliated and Clara cells (in bronchioles that are distal to the trachea and bronchi). Alveoli, most distal part of the lungs, are layered with flattened type-I pneumocytes and cuboidal surfactant-producing type-II pneumocytes (Rawlins and Hogan, 2006). Airway epithelial cells are similar in mouse and human lung, but there are some differences. For instance, a pseudostratified epithelial layer including basal cells merely

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ABBREVIATIONS: α -SMA, smooth muscle α -actin; ADAM, A disintegrin and metalloprotease; ANK, ankyrin repeats; bHLH, basic-helix-loop-helix; CSL, C promoter-binding factor-1, also known as RBP-j κ in mammals, suppressor of hairless, Su(H) in *D. melanogaster*, longevity-assurance gene-1, and LAG-1 in *C. elegans*; DAPT, tert-butyl (2S)-2-[[[(2S)-2-[[2-(3,5-difluorophenyl)acetyl]amino]propanoyl]amino]-2-phenylacetate; DC, dendritic cells; DBZ, dibenzazepine; DLL, Delta-like ligand; ECM, extracellular matrix; ED, embryonic days; EGF, epidermal growth factor-like repeats; OK EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; ERK, extracellular signal-regulated kinase; HD, heterodimerization domain; HES, Hairy and enhancer-of-split family of genes; HEY, Hairy and enhancer-of-split-related with a YRPW motif family of genes; IL, interleukin; Jag, Jagged (Serrate-family ligands); LNR, Lin-12-Notch repeats; MAML, Mastermind-like; NECD, Notch extracellular domain; NEXT, Notch extracellular truncated form; NICD, Notch intracellular domain; p, postnatal days; PDGF, platelet-derived growth factor; PNEC, pulmonary neuroendocrine cells; PEST, proline-glutamic acid-serine-threonine-rich motifs; RAM, RBP-j κ -associated molecule; RBP-j κ , recombination binding protein for immunoglobulin kappa J region; RSV, respiratory syncytial virus; SMRT, thyroid hormone receptor; TAD, transactivation domain; TMD, transmembrane domain; TN-C, tenascin-C; Treg, regulatory T-cell.

Fig. 1. Notch signaling and lung developmental stages in humans and mice. During embryonic stage (ED ~8.0–9.5 in mouse, ~3–7 weeks in human), lung development is initiated by the emergence of lung buds from foregut endoderm for the onset of lung specification. In the pseudoglandular stage (ED 9.5–16.5 in mouse, 7–17 weeks in human), numerous Notch ligands, receptors, and extracellular modulators are expressed within proximal-distal airways and surrounding mesenchyme that coordinate the proximal-distal patterning of branching morphogenesis. Most mesenchymal and epithelial cells start to form during this stage. In the canalicular stage (ED 16.5–17.5 in mouse, 17–27 weeks in human), Notch signaling regulates the balance of proliferation and differentiation of the distal airway epithelium and results in the appearance of AECI/II and formation of blood capillaries. In the saccular stage to alveolar stage (ED 17.5 to postnatal day 5 in mouse, 27–36 weeks in human), Notch signaling may organize the vascularization and alveolarization (sac-like structures), which is accompanied by the enlargement of lymphatic and capillary networks, and production of surfactant. In mouse, the alveolar stage starts postnatally (P5 to ~P30) whereas, in humans, it starts in utero (36 weeks to ~7–10 years). Arrows represent the expression of Notch and its components at different stages of lung development.

originates in the main stem bronchus and trachea in mouse lung, whereas in human lung basal cells are distributed in the terminal bronchioles. Further, large numbers of Clara cells are thoroughly distributed in mouse lung, but in the human lung Clara cells are only found in the bronchiolar epithelium. Human lung contains extensive branches in the bronchial tree compared with mouse lung. Branching pattern is also different in both mouse and human lung (Metzger et al., 2008). Alveolar development starts from the saccular stage (ED 17.5) in mouse, whereas in human lung, alveolar expansion starts from the late canalicular stage (~24 weeks). Nevertheless, precisely coordinated signaling pathways regulate lung developmental processes, and disruptions of these signaling pathways could result in neonatal respiratory disorders, such

as lung cancer, pulmonary fibrosis, chronic obstructive pulmonary disease, asthma, and airway remodeling.

Asthmatic airway remodeling, first described in 1922 (Huber and Koessler, 1922), is characterized by structural changes in the airway walls, such as thickened epithelium with mucous gland hypertrophy (mucus hypersecretion), thickened subepithelial basement membrane, deposition of extracellular matrix, fibrosis, angiogenesis, neovascularization, hyperplasia, and hypertrophy of airway smooth muscle cells and increased airway vascularity (Elias et al., 1999; James et al., 2012; Prakash, 2013; Fehrenbach et al., 2017). Asthmatic airway remodeling mediates the airway hyper-responsiveness, airway obstruction, pulmonary dysfunction, and infiltration of inflammatory cells, followed by

chemokines, cytokines, and growth factors, under the influence of various signaling proteins, also known as morphogens, such as Wnt/ β -catenin, TGF- β /BMP, fibroblast growth factor, epidermal growth factor, Sonic hedgehog, and Notch.

Notch, discovered in 1913 (Morgan, 1917), is an exceedingly conserved signaling system. Molecular mechanism of Notch-signaling activation and transduction is multifarious; however, fundamentally, after ligand binding, γ -secretase cleaves the released Notch intracellular domain (NICD), then this NICD translocates to the nucleus. The NICD interacts with the DNA-binding transcriptional repressor (CBF-1/RBP-j κ) and converts it into a transcriptional activator complex (NICD-CBF-1/RBPj κ -MAML) that induces the transcription

of target genes (Fig. 2). Notch regulates differentiation, development, proliferation, and apoptosis (McCright, 2003; Amsen et al., 2009; de la Pompa, 2009). Mutation in Notch components results in genetic disorders, such as spondylocostal dysostosis, T-cell acute lymphoblastic leukemia, alagille syndrome, cerebral dominant autosomal arteriopathy, and schizophrenia, as well as the commencement of multiple tumors (Mašek and Andersson, 2017). Moreover, aberrant expression of Notch has been implicated in the pathogenesis of lung diseases. In this review, we briefly discuss the molecular basis of Notch signaling, its role in lung development, and the consequences of aberrant expression of Notch signaling in the initiation and progression of asthmatic airway remodeling. Moreover, we emphasize relevant

Fig. 2. Notch signaling (canonical and noncanonical), Notch receptor, and Notch ligand. Notch receptors and ligands are single-pass transmembrane receptors. Notch receptors contain 29–36 EGF-like repeats, followed by three LNRs while all Notch ligands, except for Dll-3, contain variable numbers of EGF-like repeats, followed by DOS domain and DSL domains. The newly translated Notch receptor protein is glycosylated to mature receptor after proteolytic cleavage by furin at site 1 (S1) and then targeted to the cell surface as a heterodimer. Membrane-bound Notch receptor is activated by binding with ligand on a neighboring cell, which results in S2 cleavage at site 2 to generate the membrane-anchored NEXT fragment, by ADAM; followed by S3/S4 cleavage at site 3 (S3) to site 4 (S4) to release the stable form of NICD and N β peptide with γ -secretase. The NICD contains the RAM domain, two nuclear localization signals, six ANK repeats, and a PEST sequence that regulates NICD degradation. Released NICD then translocates to the nucleus where it associates with the DNA-binding protein CSL. In the absence of NICD, CSL may associate with ubiquitous corepressor (Co-R) proteins and histone deacetylases to repress transcription. In the presence of NICD, allosteric changes may occur in CSL which displaces the transcriptional repressors, then MAML recognizes the NICD/CSL interface to recruit additional coactivators (Co-A) for the transcription. Noncanonical Notch signaling may occur via the membrane-bound, uncleaved Notch receptor or via the NICD. Noncanonical Notch signaling is independent of CSL and allows for interaction with PI3K/AKT/mTORC2, Wnt/ β -catenin, IKK α / β , NF κ B, YY1, and HIF1 α pathways at the cytoplasmic and/or nuclear level. DOS, Delta and OSM-11-like proteins; DSL, Delta-Serrate-LAG-2; EDF, epidermal growth factor; HIF-1 α , hypoxia-inducible factor-1 α ; LNR, Lin-12-Notch repeats; MNLL, N-terminal domain of Notch ligands; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K, phosphoinositide-3-kinase; vWF, von Willebrand factor; YY1, Yin and Yang 1.

potential therapeutic targets to regulate/inhibit the Notch signaling to facilitate the appropriate treatment of asthmatic airway remodeling.

Regulation of Notch Signaling

The taxonomy and structural features of the molecules involved in and the commencement mechanism of Notch receptors are different among *Drosophila melanogaster*, *Caenorhabditis elegans*, and mammals. For this reason we discuss the Notch signaling pathway in mammalian cells. Four Notch receptors (Notch-1, Notch-2, Notch-3, and Notch-4) and five major ligands [three Delta-family ligands (Delta-like ligands Dll-1, Dll-3, and Dll-4) and two Serrate-family ligands (Jagged-1 and Jag-2)] have been recognized in mammals (Kopan and Ilagan, 2009; Guruharsha et al., 2012) (Table 1). Although all Notch receptors exhibit the same structures, they show considerable differences in the protein domains. Notch receptors are synthesized as large precursors in the endoplasmic reticulum, travel to the plasma membrane through the exocytic pathway, and then undergo various post-translational alterations. During the maturation process, in the endoplasmic reticulum, the sequence of signals located at the N-terminus is cleaved off, and the extracellular domain of Notch is subjected to modification mediated by glycosyltransferases (Rana and Haltiwanger, 2011). For instance, O-fucosylation by protein O-fucosyltransferase 1 (*Pofut-1*) and consequent elongation of chain with O-linked β -D-N-acetylglucosamine (O-GlcNAc) by *fringe* genes (*radical*, *manic*, and *lunatic fringe*) regulate the

ligand specificity in Golgi complex (Bruckner et al., 2000; Okajima et al., 2003; Kovall et al., 2017). Moreover, O-glucosylation by protein-O-glucosyltransferase (Poglut) in the endoplasmic reticulum is essential for activation of the receptor (Acar et al., 2008), and further elongation of chain with O-xylose negatively regulates Notch receptor (Lee et al., 2013). In Golgi complex, Notch extracellular domain undergoes its first proteolytic cleavage by a Furin-like proprotein convertase at site S1 (Logeat et al., 1998), although S1 cleavage is not necessary for signal activation but facilitates exocytosis (Gordon et al., 2009) (Fig. 2). After cleavage, Notch receptors are presented as heterodimers of a large, modular, N-terminal portion of the plasma membrane, which is exposed to extracellular spaces known as Notch extracellular domain (NECD). NECD is noncovalently attached to the C-terminal part of the multidomain intracellular portion that contains transcriptional activity, referred to as NICD; hence, Notch receptors contain large extracellular and small intracellular domains. The extracellular domains of Notch-1 and Notch-2 include 36 epidermal growth factor-like repeats (EGF) each, whereas those of Notch-3 and Notch-4 contain 34 and 29, respectively (Radtke and Raj, 2003). These domains bind to calcium ions and provide a necessary function in receptor-ligand binding (Cordle et al., 2008) as well as in signaling efficacy (Raya et al., 2004). Generally, NECD is acknowledged as inhibitory, because EGF-like repeats are followed by a negative regulatory region, which comprises three cysteine-rich Lin-12-Notch repeats (LNR) and a Notch heterodimerization region that perform a crucial role in inhibiting inappropriate Notch activation

TABLE 1
Notch pathway components in vertebrates and mammals

Component	Types	Vertebrates and mammals	References
Receptor		Notch-1, Notch -2, Notch -3, Notch -4	Kopan and Ilagan, 2009; Guruharsha et al., 2012; Kovall et al., 2017
Ligand	DSL/DOS DSL only DOS Coligands Noncanonical	Dll-1, Jagged-1 and Jagged-2 Dll-3 and Dll-4 DLK-1, DLK-2/EGFL9 DNER, NB 3/Contactin6, F3/Contactin1, MAGP-1 and MAGP-2,	Andersen et al., 2012
Receptor proteolysis	Furin convertase (site 1 cleavage) Metalloprotease (site 2 cleavage) γ -Secretase (site 3/site 4 cleavage)	Furin, PC5/6 ADAM-17/TACE, ADAM-10/Kuzbanian PEN-2, Nicastrin, APH-1a-c, PSEN-1 and -2	Logeat et al., 1998 Kopan and Ilagan, 2009; Weinmaster and Fischer, 2011 Bray, 2006; Jorissen and De Strooper, 2010
Glycosyltransferase modifiers	O-Fucosyl-transferase β 1,3-GlcNAc-transferase	Pofut-1 Lunatic, manic, and radical fringe	Brückner et al., 2000; Okajima et al., 2003; Kovall et al., 2017
Endosomal sorting/membrane trafficking regulators	Ring finger E3 Ubiquitin ligase (ligand endocytosis) Ring finger E3 Ubiquitin ligase (receptor endocytosis) HECT domain E3 Ubiquitin ligase (receptor endocytosis) Negative regulator	Skeletrophin, Mindbomb, Neuralized 1-2 Deltex-1, Deltex-2, Deltex-3, Deltex-4 Itch/AIP4, Nedd4	Bray, 2006; Guo et al., 2016
NICD degradation	F-Box ubiquitin ligase	Numb, Numb-like, ACBD3 Fbw-7/SEL-10	Chapman et al., 2006 Kopan and Ilagan, 2009
Nuclear effectors	CSL DNA-binding transcription factor Transcriptional coactivator Transcriptional corepressors	RBP-jk/CBF-1 MAML-1, MAML-2, MAML-3 KyoT2, NCoR/SMRT, Mint/SHARP/SPEN HES/ESR/HEY	Bigas et al., 2013; Kovall et al., 2017 Mukherjee et al., 2014 Chen et al., 1997; Ostroukhova et al., 2006 Chen and Evans, 1995; Rana and Haltiwanger, 2011
Canonical target bHLH repressor genes			

AP2, adaptor protein-2; APH-1, anterior pharynx-defective 1; CSL, CBF1/RBP-jk/Su(H)/Lag-1; DOS, Delta and OSM-11-like proteins motif; DSL, Delta/Serrate/LAG-2 motif; E3, ubiquitin ligase; NICD, Notch intracellular domain; PEN, Presenilin enhancer; PSEN, Presenilin; SMRT, a silencing mediator for retinoid or thyroid hormone receptors.

(Malecki et al., 2006; Kopan and Ilagan, 2009) as well as modulating the communications between NECD and NICD (Greenwald, 1994). Notch intracellular domain consists of the recombination binding protein for immunoglobulin kappa J region (RBP-j κ)-associated molecule (RAM) domain, a nuclear localization signal, seven ankyrins (ANK) repeats, a transactivation domain (TAD), and a degradation domain [glutamine-rich repeat/proline-glutamic acid-serine-threonine-rich motifs (PEST) to regulate the stability (Bigas et al., 2013)] (Fig. 2). Classic TAD is present in Notch-1 (strong TAD) and Notch-2 (weak TAD) and is not present in Notch-4 (Ong et al., 2006). Notch-3 contains potent but specific TAD for activation of

the Hairy and enhancer-of-split (HES)-5 promoter. It has been revealed that RAM domains exhibit a crucial role in NICD and RBP-j κ interaction, whereas ankyrin repeats are also critical for transcriptional activation complex formation and recruitment of Mastermind-like (MAML)-1 (Nam et al., 2006). In addition, NICD contains nuclear localization sequences and target sites for ubiquitination (Fryer et al., 2004), phosphorylation, and hydroxylation (Romain et al., 2001). A list of proteins that interact with NICD by phosphorylation, ubiquitination, and hydroxylation are described in Table 2.

In mammals, Notch ligands are type-1 transmembrane proteins with a relatively small intracellular domain and a

TABLE 2
Various proteins interacting with NICD

Symbol	Protein	Interface with NICD
Regulation of NICD by phosphorylation		
GSK3 β	Glycogen synthase kinase 3	Phosphorylates Notch, which can lead to degradation or stabilization
CycC	Cyclin C	Target NICD along with CDK8 for phosphorylation that subsequently acts as substrate for ubiquitylation and degradation
CDK8	Cyclin-dependent kinase 8	Phosphorylates NICD with CycC that subsequently acts as substrate for ubiquitylation and degradation
G-CSF	Granulocyte colony-stimulating factor	Phosphorylation of NICD that subsequently leads to its inactivation
Regulation of NICD by ubiquitylation		
Fbxw7/Cdc4	F-box/WD-repeat protein 7	Ubiquitylates the NICD leading to its degradation
Numb	Numb homolog	Suppresses Notch signaling by recruiting E3 ubiquitin ligases to ubiquitylate Notch
Dtx1–4	Deltex 1–4	Controls Notch ubiquitylation, processing, and internalization
Itch	Itchy, E3 ubiquitin protein ligase	Promotes ubiquitylation of NICD
Regulation of NICD by hydroxylation		
FIH	Factor inhibiting HIF1 α	Hydroxylates the Notch and represses Notch
HIF1 α	Hypoxia-inducible factor 1, alpha subunit	Stabilizes the NICD and synergizes with it in transcription of Notch target genes
Miscellaneous proteins interacting with NICD		
Axin	Axin	Synergies with NICD to regulate the stability of β -catenin and controls the trafficking of NICD in association with adenomatous polyposis coli
Apc	Adenomatous polyposis coli	Control the trafficking of Notch
CSL/RBP-j κ	CBF1, Su(H) and LAG-1/RBP-j κ	Key canonical transcriptional cofactor for NICD
Ctnnb1	β -catenin	Synergies with NICD/CSL on Notch target gene
Dab	Disabled	Acts as link to Abelson tyrosine kinase (Abl) protein in noncanonical Notch axon guidance
Dvl/Dsh	Dishevelled	Dishevelled regulates ligand-dependent Notch trafficking and inhibits canonical Notch signaling
MAML-1/2	Master mind-like-1/2	Coactivator of NICD/CSL
NF- κ B	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	NICD blocks NF- κ B transcription of NF- κ B target genes through binding to p50/cRel
Nrarp	Notch-regulated ankyrin repeat protein	Nrarp binds and inhibits the NICD/CSL
p73 α (TA)	Tumor protein p73 alpha (transactivating form)	Binds NICD and inhibits the NICD/CSL-mediated transcription
RITA/C12ORF52	RBP-j κ interacting and tubulin associated	Shuttles NICD between the nucleus and cytoplasm on tubulin networks
SMAD	Smad family members	Smads augment Notch signaling, Notch fine-tunes signaling via Smads
SNW1/SKIP/NCOA-62	SNW domain-containing protein 1/Ski-interacting protein/Nuclear receptor coactivator NCoA-62	Can bind both NICD and corepressor SMRT, but these are mutually exclusive; forms multimers with NICD and MAML, which then associates with CSL to activate transcription
Tacc3	Transforming, acidic coiled-coil containing protein 3	Binds NICD and blocks transcription from Notch target promoters; can be reversed by overexpression of CSL
Trio	Triple functional domain (PTPRF interacting)	A guanine nucleotide exchange factor (GEF) for Rho GTPases that acts as link to Abelson tyrosine kinase (Abl) proteins in noncanonical Notch axon guidance

CDK, cyclin-dependent kinase; E3, ubiquitin ligase; HIF, hypoxia-inducible factor; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; RITA, RBP-j κ interacting and tubulin-associated; SKIP, Ski-interacting protein; SMRT, a silencing mediator for retinoid or thyroid hormone receptors.

large extracellular domain. Synthesis, trafficking, and exocytosis of the Notch ligands are the same as for Notch Receptors. In Notch ligands, three interconnected structural patterns are described: EGF-like repeats (variable numbers), Delta and OSM-11-like proteins (DOS) domain (a specialized tandem EGF-repeat) and Delta-Serrate-LAG-2 (DSL) domain (a cryptic EGF-like repeat); DOS and DSL domains, both, are implicated in receptor binding (Kopan and Ilagan, 2009) (Fig. 2). Nevertheless, because of the presence or absence of a cysteine-rich domain, Notch ligands can be classified into the Delta-like group or the Jagged/Serrate group (D'Souza et al., 2008). Notch signaling pathway components are depicted in Table 1.

Canonical Notch Signaling. Importantly, Notch signaling is initiated upon interaction of signal-dispatching (Notch ligand-containing) and signal-receiving (Notch receptor-containing) cells, the result of which is initiation of a trans-endocytosis process and binding of ligands to the extracellular domain of Notch receptor.

For ligand endocytosis, an E3 ubiquitin ligase, Mindbomb-1, performs the critical role in the mono-ubiquitination of the intracellular domains of ligands in mammals (Koo et al., 2007), and Notch signal activation fails in the absence of ligand ubiquitination. After ligand binding and endocytosis, the ligand induces a conformational change on the extracellular domain of Notch, exposing the S2 proteolytic cleavage site operated by A disintegrin and metalloproteinase (ADAM) at site S2 (Weinmaster and Fischer, 2011) (Fig. 2). S2 cleavage is a key step for Notch activation, but some uncertainties still exist regarding enzymes responsible for cleavage. Indeed, ADAM10 is generally considered to be the protease that is responsible for ligand-inducible cleavage, whereas ADAM17 is responsible for ligand-independent cleavage (Kopan and Ilagan, 2009). However, cleaved S2 (second cleavage) remains entrenched in the membrane and is known as Notch extracellular truncated form (NEXT). NEXT acts as a substrate of the γ -secretase complex, an intramembrane protease (Jorissen and De Strooper, 2010). In turn, Notch exposes the S3 cleavage site to γ -secretase complex, which is composed of Presenilin 1, anterior pharynx-defective 1, Presenilin enhancer 2, and Nicastrin, undergoes S3 cleavage (third cleavage), and eventually releases the NICD from the membrane (Bray, 2006) (Table 1). The exact subcellular location of γ -secretase cleavage is still controversial, i.e., γ -secretase cleavage occurs at multiple sites within the transmembrane domain; however, most probably occurs at the plasma membrane and in the endocytic vesicles (Yamamoto et al., 2010). The pharmacological effects of Notch signaling can be minimized by targeting the S3 cleavage via γ -secretase inhibitors. NICD then enters the nucleus and interacts with DNA-binding protein, to accumulate a transcriptional complex comprising NICD, CSL [C promoter-binding factor-1 (CBF-1) also known as RBP-j κ in mammals; suppressor of hairless, Su(H) in *D. melanogaster*; longevity-assurance gene-1, LAG-1, in *C. elegans*], and coactivator of Mastermind (MAML-1, MAML-2, MAML-3 in mammals and MAM in *Drosophila*) through ANK and RAM domains of NICD and converts it into transcriptional activator to induce transcription of target genes. In the absence of Notch, CSL, mainly CBF-1/RBP-j κ in mammals, recruits transcriptional corepressors such as Ski-interacting protein, Hairless (H), C-terminal-binding protein-1, SPEN [also known as SHARP/MINT (SMRT/HDAC-1-associated

repressor protein, SHARP, in human/MINT in mouse homologs) and Msx2-interacting nuclear target], RBP-j κ interacting and tubulin-associated protein, Groucho to recruit histone deacetylases, a silencing mediator for retinoid or thyroid hormone receptors (SMRT), and further repressive cofactors to depress the expression of Notch target genes (Chen and Evans, 1995; Oswald et al., 2005). In the presence of NICD, SKIP collaborates with ankyrin repeat domain of NICD to assist NICD function by dissociating the repressor complex (Zhou et al., 2000) and recruits the CBF-1 transcriptional coactivators, like chromatin-remodeling complexes and histone acetyltransferases (Kurooka and Honjo, 2000), to create a short-lived transcriptional activation complex, NICD-CBF-1/RBP-j κ -MAML, which then activates a downstream target gene via the addition of an extra coactivator, such as histone acetyltransferase p300 (Fryer et al., 2002; Wallberg et al., 2002) (Fig. 2). The best-known Notch targets are the components of basic-helix-loop-helix (bHLH) family genes such as Hairy and enhancer-of-split family of genes (HES-1, -3, -5 and -7) and Hairy and enhancer-of-split-related with a YRPW motif (HEY) family of genes (HEY-1, HEY-2 and HEY-L) (Ranganathan et al., 2011; Chen et al., 2014). These components act as transcription repressors either by direct binding to the E and N regions, or attaching corepressor (Groucho), or by a unique mechanism still mysterious but independent of direct protein binding. In addition to HES and HEY family genes, Cyclin-D1 (cell-cycle promoter), c-Myc (proliferation-related gene), Bcl-2 (anti-apoptotic gene), the gene for HER-2, Deltex-1, p21Cip1/Waf1, Notch-regulated ankyrin repeat protein (Nrarp), and the pre-T-cell receptor gene have also been described as Notch target genes (Takebe et al., 2014; Wakabayashi et al., 2015). Mostly Notch-mediated processes need a transient activation, but a few procedures require prolonged activation. Hence, continuous signal activation is controlled at a regulation point that shuts off the Notch signaling via phosphorylation of NICD within the PEST domain by kinases such as cyclin-dependent kinase-8 (CDK8) (Fryer et al., 2004) and is targeted for polyubiquitination via E3 ubiquitin ligases such as SEL10/FBXW7, which results in proteasome-mediated degradation and termination of Notch signaling and resets the cells for the next round of signaling (Guo et al., 2016; Kovall et al., 2017). It yet needs to be recognized whether CDK8 and FBXW7 are common mediators of NICD degradation, because some other kinases and E3 ubiquitin ligases are considered to be contributors to NICD regulation in context-dependent circumstances. Additionally, Notch activity can also be determined by the ubiquitylation status of the receptor. Thereby phosphatases, ubiquitin ligases, and kinases remain to be identified and characterized. The proteins that interact with NICD and influence the output of signaling pathway are described in Table 2.

Non-canonical Notch Signaling. Several recent studies have revealed the existence of numerous modes of Notch signaling, in addition to canonical Notch signaling, commonly referred as noncanonical Notch signaling. Noncanonical Notch signaling is related to other, different transcription factors, such as hypoxia-inducible factor-1 α , β -catenin, estrogen receptor (ER α), Yin and Yang 1, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), instead of CSL (CBF1, suppressor of hairless, and Lag-1) transcription

factor (Fig. 2). Interestingly, canonical Notch signaling is associated with various normal cellular processes such as early development of embryo, lungs, and other physiologic processes, whereas noncanonical Notch signaling is mainly concerned with potentially pathologic conditions like cancer and activation of the immune system. Hence, blockade of noncanonical Notch signaling may possibly establish new opportunities to inhibit pathologic conditions without harming normal physiologic processes; A more detailed description of noncanonical Notch signaling pathway can be found elsewhere (Andersen et al., 2012) and is beyond our topic of discussion.

Regulatory Role of Notch Signaling in Fetal Lung Development

Early Proximodistal Cell Fates. It is challenging to evaluate the participation of Notch signaling in normal lung development owing to the complexity of Notch receptor/ligand interactions, but experimental data has revealed that Notch signaling performs the crucial role in regulating the patterning and proximodistal cell fate in the early lung development (Tsao et al., 2008). Genetic ablation of *RBP-jk* or *Pofut-1* from developing epithelium or mesenchyme demonstrated the substantial differentiation defect in proximal airways, including complete ablation of the Club-cell secretory lineage and overpopulation of ciliated cells and neuroendocrine cells; the same defects were also found in the DAPT (γ -secretase inhibitor)-treated proximal airways explants (Tsao et al., 2009; Morimoto et al., 2010).

In addition, expression patterns of Notch receptors, ligands, and other constituents (ED 11.5 to maturity) also suggest the participation of Notch signaling in regulating proximodistal cell fates. For instance, Notch-1 is persistently expressed in the distal lung endoderm, Notch-2 and Notch-3 both are articulated in fetal lung mesenchyme, and Notch-4 is endothelial-specific, although Notch-3 can be articulated in mesenchyme and endothelial cells of the airway (Post et al., 2000). During budding, Dll-1 is restricted to the proximal area, whereas Jag-1 and Jag-2 are found in the distal region (Kong et al., 2004). Jag-1 is highly expressed in proximal airways (ED 16.5) (Zhang et al., 2013a) and Jag-2 expression (ED 13) is observed in peripheral lung mesenchyme (Post et al., 2000). Dll-1 is first expressed in the secondary bronchi (ED 13.5) and gradually rises within bronchioles and branch points until birth (Post et al., 2000). Postnatally, Dll-1 expression has been identified in endothelial cell lining the lung vasculature throughout the lung tissue (Beckers et al., 1999; Post et al., 2000). Hence, Dll-1 is well located to relate a subset of Notch proteins expressed in SPNC cells/Club-like cell that surrounds neuroepithelial bodies (Morimoto et al., 2010; Guha et al., 2014). Dll-4 expression is restricted to endothelial cells in the lung (Yoneya et al., 2001). Further, HES-1 expression starts from the early pseudoglandular stage (ED 12) and gradually rises until birth, and it can also be promptly detected in nonendocrine airway epithelial cells in the fetal lung (Ito et al., 2000). Predominantly, HES-1-reactive cells are differentiated into Club cells in distal airway epithelium (ED 17 at low levels), and remnants continue up to fetal lung development (Hackett et al., 1992). HEY-1 is notably expressed in developing lung, but HEY-2 expression is low (Steidl et al., 2000). HEY-L is expressed in lung vasculature (Leimeister et al., 2000).

Differentiation of Pseudostratified Epithelium. Notch signaling regulates the differentiation of pseudostratified epithelial layer, including basal cells, mainly toward secretory lineages in the airway (Rock et al., 2011), whereas genetic and pharmacological inhibition of Notch-3 leads to the aberrant expansion of basal cells and altered pseudostratification (Mori et al., 2015). Further, Notch is required to maintain the Club cells (Pardo-Saganta et al., 2015), although conditional deletion of *HES-1* or *RBP-jk* or *Pofut-1* produced a remarkable elevation in ciliated cell count and attenuated Club-cell count; in fact, in the dearth of such signaling, cells are generally intended to turn into ciliated cells through a default program (Tsao et al., 2009; Morimoto et al., 2010). Transgenic misexpression of the Notch-1 intracellular domain in distal epithelial cells demonstrated ectopic expression of Club cells (Guseh et al., 2009). Furthermore, Jag-1 actively participates in controlling Notch-dependent differentiation of Club cells during lung development, because spatial-temporal deletion of Jag-1 in the airway epithelium caused aberrant cell-fate specification, with ciliated cells in excess at the expense of Club cells, a phenotype correlated with downregulation of HES-1 (Zhang et al., 2013a). Moreover, mouse trachea and human airway basal cells both undergo self-renewal and generate ciliated and Club-cell lineages (Engelhardt et al., 1995; Rock et al., 2009). In the *Scgb1a1*-CreER knockin mouse, the majority of Club cells in bronchioles undergo both self-renewal and generate ciliated cells while in the trachea; Club cells generate ciliated cells but do not undergo self-renewal (Rawlins et al., 2009). Taken together, differentiation of airway epithelium into the secretory, ciliated, Club, and neuroendocrine cell types in the developing and adult murine lung is regulated by Notch signaling, but little human data are available beyond reports that Notch-1 and Notch-3 pathways regulate the differentiation of basal cells into ciliated and secretory cells (Gomi et al., 2015) via Jag-1 mediation (Gomi et al., 2016).

Pulmonary Neuroendocrine Cell Differentiation. Pulmonary neuroendocrine cells (PNEC) differentiation is regulated by Notch signaling (Ito et al., 2000). HES-1 and Mash-1 are expressed in nonneuroendocrine cells and pulmonary neuroendocrine cells (and neuroepithelial bodies in lungs), respectively. However, analyses of both Mash-1 and HES-1 knockout mice showed a significant correlation between Notch/Notch-ligand pathways and the regulation of bHLH proteins in the cell-fate decision in developing lung. An elevated level of Mash-1 mRNA and pulmonary neuroendocrine cells has been observed in HES-1-deficient mice, whereas lack of pulmonary neuroendocrine cells has been noticed in Mash-1-deficient mice (Borges et al., 1997; Ito et al., 2000). Nevertheless, the exclusive interaction between HES-1 and Mash-1 is the consequence of direct suppression of Mash-1 promoter by HES-1, and the ability of Notch-1, HES-1-independent, to promote degradation of Mash-1 (Chen et al., 1997; Sriuranpong et al., 2002). Hence, Notch-1 might control the expression of HES-1 and inhibit the fate of pulmonary neuroendocrine cells, in view of the fact that antisense oligonucleotides of Notch-1 promoted the differentiation of PNEC, and transgenic expression of Notch-1 intracellular domain blocks the differentiation of PNEC (Kong et al., 2004; Shan et al., 2007). Expression of the Dll-1 in neuroendocrine cells, and Notch-1, -2, and -3 in nonneuroendocrine cells further authenticated this model (Xu et al., 2010). Consequently, Dll-1-mediated activation of Notch could significantly encourage the expression of HES-1 and suppression

of neuroendocrine differentiation via lateral inhibition (Noguchi et al., 2015). Importantly, *Jag-1* deletion resulted in an increased number of PNEC (Zhang et al., 2013a). On the other hand, conditional removal of *RBP-jk* in lung endoderm has no robust influence on the number of PNECs or expression of HES-1. Moreover, an involvement of noncanonical Notch signaling in neuroendocrine cell-fate selection is determined by Notch-1, -2, -3 triple knockouts, which results in a massive expansion of neuroepithelial bodies (Morimoto et al., 2010; Morimoto et al., 2012). Hence, under normal physiologic conditions, most probably a noncanonical pathway might compel inhibition of pulmonary neuroendocrine cells fate and expression of HES-1. However, such mechanisms could use signaling different from JAK, Alk5, FGFR, or extracellular signal-regulated kinase (ERK) kinases (Niwa et al., 2007; Yoshiura et al., 2007; Xing et al., 2010).

Pulmonary Goblet Cell Fate. Notch signaling also regulates the fate of pulmonary goblet cells, but it is complicated to evaluate the precise physiologic participation of Notch signaling in this context owing to the paucity of goblet cells in the murine airway epithelium. It has been reported that *Dll-4* augmented the number of Muc5AC goblet cells in both human airway cell cultures and murine tracheal explants studies (Guseh et al., 2009). Interestingly, mice with conditional inactivation of *RBP-jk* or *Pofut-1* via *Tgfb3-Cre* could survive to adulthood with aberrant airway phenotype characterized by obvious goblet cell metaplasia, increased ciliated cell numbers, and attenuated Club-cell count (Tsao et al., 2011). Moreover, goblet-cell metaplasia is observed in adult *Jag-1*-deleted mice lungs, indicating that *Jag-1* contributes to repression of mucin production and goblet cell fate, accompanied by downregulation of *Dll-1*, HES-1, and HES-5 (Zhang et al., 2013a). Recently, it has been demonstrating that Notch signaling, particularly Notch-2, directly promoted the pulmonary goblet cells, whereas anti-Notch-2 antibodies prevented the interleukin (IL)-13, as well as allergen-driven goblet cell metaplasia (Danahay et al., 2015; Lafkas et al., 2015).

Alveolar and Microvasculature Development. Alveolar development takes place because of coordinated proceedings of endothelium, epithelium, and mesenchymal stroma in the distal lung. Notch signaling exhibits a pivotal role in the cell differentiation and cell-fate specification in the vascular and parenchymal compartments to coordinate the alveolar and microvasculature development. Constitutive expression of Notch in the peripheral (Dang et al., 2003) and distal (Guseh et al., 2009) lung epithelium in mice caused perinatal lethality owing to aberration and/or inhibition of differentiation of alveolar cells into type-I and type-II cells. Additionally, dilated cysts, which appear on proximal airway epithelium, were lacking some markers, particularly SP-C, keratin-5, β -tubulin, transformation-related protein 63 (p63), Club-cell 10-kDa protein (CC10), but not all markers (Guseh et al., 2009). In contrast, conditional deletion of *RBP-jk* or *Pofut-1* in lung epithelium exhibited no negative impact on the differentiation of alveolar epithelial cells and formation of alveolar saccules (Tsao et al., 2009; Morimoto et al., 2010). Interestingly, failure of alveolar septation and defective alveolar development have been reported in Notch-2^{+/-} Notch-3^{-/-} compound mutant (*lfn*) knockout mice owing to flawed differentiation and recruitment of myofibroblast cells rather than alveolar epithelial cells, whereas conditional deletion of *RBP-jk* (ED 14.5–18.5) impaired the myofibroblast

differentiation without affecting the alveolar epithelial cells (Xu et al., 2010).

Notch-deficient mice, such as *Jag-1* or *lfn* mutants, that survived postnatally helped the investigation of Notch-mediated events in alveolar development. Conditional deletion of *Jag-1* in lung epithelium that disrupted alveolar septation showed no effect on differentiation and maturation of alveolar epithelial cells (Zhang et al., 2013a). Moreover, overexpression of *lfn* in distal lung epithelium did not affect the spatial or temporal expression of HES-1 and Mash-1, nor the expression of proximal ciliated, nonciliated, distal epithelial cell, and mesenchymal cell markers (von Willebrand factor, α -SMA, PECAM-1) (van Tuyl et al., 2005). However, Tsao et al. (2016) used the epithelial Notch-2-null mice to determine that epithelial Notch signaling regulates alveologenesis by triggering the paracrine activation of platelet-derived growth factor (PDGF)-receptor- α signaling in alveolar myofibroblast progenitors, whereas overexpression of stimulated Notch-2 reversed the negative effect of Notch inhibition.

Of note, infiltration of growing microvasculature into emerging alveolar walls is necessary for alveolar development. This infiltration event is strongly coordinated with alveolar epithelium development to facilitate lung physiology at birth. In lung vasculature, Notch gene expression gradually rises from early to late lung developmental stages, suggesting that Notch pathway plays a significant role during alveolar microvasculature development (Post et al., 2000; Taichman et al., 2002; Xu et al., 2010). This suggestion is consistent with the identified role of Notch signaling in whole-body vascular development (Holderfield and Hughes, 2008; Siekmann et al., 2008). Most mutants with complete deletion of Notch ligand and such receptor genes as *Notch-1*, *Notch-2*, *Dll-1*, and *Jag-1* die during the early embryonic developmental process; therefore, the appropriate role of the Notch gene in microvasculature development of the distal lung is still unclear. Nevertheless, some facts supporting the role of Notch signaling in peripheral lung microvasculature development arise from *Foxf-1* heterozygous mutants, where *Foxfi-1* haploinsufficiency mutant showed abnormal morphogenesis of lung microvasculature and lethality of neonates, owing to disruption of pulmonary expression of Notch-2 signaling and HES-1, even though disrupted Notch-2 expression and the directly affected cell type, by *Foxf-1* haploinsufficiency, remains unclear (Kalinichenko et al., 2004). Notch-3 and Notch-4 null mice demonstrated almost normal lung physiology and were viable (Krebs et al., 2000; Domenga et al., 2004), whereas aberrant activation of Notch-4 in vascular endothelium exhibited pulmonary arteriovenous shunts resulting from vessel-sprouting inhibition and overgrowth of the vessel at the capillary bed interface (Miniati et al., 2010). The requirements of Notch and its transcription factors during lung development are substantially depicted in Table 3.

Pulmonary Vascular Development. The pulmonary vascular system at birth is subjected to a striking switch from a low-flow and low-pressure fetal status to a high-flow and higher-pressure postnatal status. The essential roles of Notch signaling in the vascular development have been supported by the fetal lethality of Notch signaling deficiency. For instance, either knockout of Notch-1, *Dll-4*, *Jag-1*, Notch-1 plus Notch-4, HERP-1 plus HERP-2, and presenillin-1 or continuous Notch-4 expression in mice or zebrafish led to embryonic death owing

TABLE 3
Notch pathway gene knockout and lung phenotypes

Genotype	Lung phenotype	Remarks	References
Notch-1 ^{-/-}	Developmental retardation and widespread cellular death	Embryonic lethality at ED 10	Swiatek et al., 1994; Conlon et al., 1995;
N1ICD ^{Δ/Δ} (Notch-1 ^{Δ/Δ})	Alveolar hyperplasia and apoptosis	Viable	Allen et al., 2011
Notch-2 ^{-/-}	Developmental retardation and widespread cellular death	Embryonic lethality at ED 11.5	Hamada et al., 1999
Notch-2 ^{-/-} (epithelial)	Abnormal enlargement of alveolar spaces, inhibition of Type II cell activation, decreased PDGF-A expression, and disturbance of integrity of bronchial and epithelial smooth muscle layer of distal airways	Viable (if stimulated by Notch-2 overexpression).	Tsao et al., 2016
Notch-3 ^{+/-}	No arterial defects	Viable	Krebs et al., 2000; Domenga et al., 2004
Notch-3 ^{-/-}	Apparently normal lung morphology with aberrant maturation of vascular smooth muscle cells (arterial defects) and abnormal aggregates of α -SMA	Viable and fertile	Krebs et al., 2000; Domenga et al., 2004; Li et al., 2009
Notch-3 ^{Δ/Δ}	Dramatic abnormalities of lung morphogenesis, delayed development of type II pneumocyte, and inhibition of type I pneumocyte differentiation, ductal formation, and dilated sacculi	Perinatal lethality	Dang et al., 2003
Notch-2 ^{+/-} + Notch-3 ^{-/-}	Alveolar septation failure, defective alveolar development, and flawed differentiation and recruitment of myofibroblast cells	Perinatal lethality	Xu et al., 2010
Notch-4 ^{-/-}	No obvious mutant phenotype.	Fertile and viable	Krebs et al., 2000
Notch-1 ^{-/-} + Notch-4 ^{-/-}	Vascular remodeling defects	Embryonic lethality	Roca and Adams, 2007
Notch-4 ^{Δ/Δ}	Vascular remodeling defects, arteriovenous shunting, lung hemorrhages, and respiratory insufficiency	Viable but lethal around 6–7 weeks	(Miniati et al., 2010)
<i>Pofut-1</i> ^{-/-}	Complete ablation of Club-cell secretory lineage, airways overpopulated with ciliated cells, goblet cell metaplasia, and attenuation of epithelium in airway and bronchioles,	Neonatal lethality	Tsao et al., 2009, 2011
<i>RBP-jk</i> ^{-/-}	Remarkably similar phenotype characterized by <i>Pofut-1</i> ^{-/-}	Neonatal lethality	Tsao et al., 2009, 2011
<i>Sox-2</i> ^{-/-}	Epithelium lacking Club, ciliated and goblet cells in conducting airway epithelium	Viable	Tompkins et al., 2009
<i>lfrng</i> ^{-/-}	Delayed-type I pneumocyte differentiation and defective alveolar septation and elastogenesis	Viable	Xu et al., 2010
<i>lfrng</i> ^{Δ/Δ} in distal lung epithelium	No obvious mutant phenotype.	Viable	van Tuyl et al., 2005
Low <i>Foxf-1</i> ^{+/-} (diminished Notch-2)	Severe fusions of right lung lobes with pulmonary hemorrhage, severe defects in peripheral lung saccules development, and abnormal microvasculature development	Postnatal lethality	Kalinichenko et al., 2004
High <i>Foxf-1</i> ^{+/-}	Normal microvascular development	Viable	Kalinichenko et al., 2004
<i>Foxf-1</i> ^{-/-}	Defects in extraembryonic mesoderm development	Embryonic lethality at ED 8	Kalinichenko et al., 2001
<i>HES-1</i> ^{-/-}	Hypoplastic lungs with attenuated Club-cell count and Notch-1 mRNA, and increased count of PNEC and Mash-1 mRNA	Peri- or postnatal lethality	Ishibashi et al., 1995; Ito et al., 2000; Tsao et al., 2009
<i>Mash-1</i> ^{-/-}	Hypoplastic lungs with disappeared Dll-1 mRNA and decreased count of PNEC	Perinatal lethality owing to hypoventilation	Guillemot et al., 1993; Ito et al., 2000
<i>Jag-1</i> ^{-/-}	Hemorrhage during early embryogenesis	Embryonic lethality at ED 10	Xue et al., 1999
<i>Jag-1</i> ^{-/-} in airway epithelium	Flawed alveologenesis, goblet cell metaplasia, and aberrant cell fate specification with excess ciliated cells at the expense of Club cells same as <i>HES-1</i> ^{-/-}	Viable	Zhang et al., 2013a
<i>Jag-2</i> ^{-/-}	Impaired differentiation	Perinatal lethality	Xue et al., 1999
<i>Dll-1</i> ^{-/-}	Patterning defects	Embryonic lethality at ED 12	Hrabě de Angelis et al., 1997
<i>Dll-4</i> ^{-/-}	Vascular remodeling defects	Embryonic lethality at ED 10.5	Gale et al., 2004
<i>PSEN-2</i> ^{-/-}	Vascular remodeling defects	Embryonic lethality	Herreman et al., 1999
<i>HERP-1</i> ^{-/-}	Vascular remodeling defects	Embryonic lethality	Doi et al., 2005

+/-, Deletion of single allele (heterozygous); -/-, Null allele (homozygous); Δ/Δ, overexpression of specific gene; PSEN-1, Presenilin 1.

to vascular remodeling defects (Gridley, 2007, 2010; Roca and Adams, 2007; Miniati et al., 2010), but its exact role in coordination of pulmonary vascular development remains ambiguous because of limited data.

Notch-3 receptor in embryonic pulmonary vascular smooth muscle cells is activated from an intracellular compartment (Ghosh et al., 2010). Defects in lung vascular smooth muscle have been reported in Notch-3-deficient mice, which were

viable throughout adulthood without other apparent lung abnormalities (Li et al., 2009). In this context, Ghosh and colleagues (2011) reported apparently similar morphology in Notch-3-deficient and wild-type (ED 18.5) mice at postnatal day 3. Vascular smooth muscle cells were noncohesive, dysmorphic, and vacuolated, with disordered smooth muscle α -actin (α -SMA) distribution, in the Notch-3-deficient mice. They reported that Notch-3 is expressed primarily in pulmonary artery vascular smooth muscle cells-derived Jag-1, which is activated from late fetal to early postnatal life, and played a role in maintaining the morphologic characteristics and gene expression profile of the pulmonary artery after birth. Moreover, Notch-3 activation promotes proliferation of the pulmonary vascular smooth muscles cell HES-1/p27Kip1–signaling pathway (Song et al., 2015).

Notch Signaling as a Potential Biomarker in Asthmatic Airway Remodeling;

Immunologic Role of Notch Signaling in Allergic Asthmatic Airway Remodeling. Allergic asthmatic airway remodeling is characterized by infiltration of many inflammatory cells such as lymphocytes (T-lymphocytes), macrophages, neutrophils, mast cells, and eosinophils (Pascual and Peters, 2005). These inflammatory cells exhibit defensive roles in the pathogenesis of asthma. Activation of CD4⁺ T cells or Th-cells into Th-1 and Th-2 cell types, and Th-1/Th-2 imbalance (particularly an elevated level of Th-2), perform an important role in the pathogenesis of chronic asthma (Elias et al., 1999). Interleukins, such as IL-4, IL-5, IL-13 and IL-20, released as a result of Th-2 reactions, drive the asthmatic airway remodeling by interacting with airway smooth muscle cells and epithelial cells (Gong et al., 2014).

Notch/*RBP-jk* in CD4⁺ T cells exhibit the pivotal role in the induction of allergic asthma (KleinJan et al., 2013), because Notch is involved in the differentiation of CD4⁺ T cells into Th-1/Th-2 (Zhang et al., 2013b), and γ -secretase inhibitor reduced allergic asthma and goblet cell metaplasia by attenuating the Th-2 cytokine production (Kang et al., 2009). Elicitation of Th-2 cells requires GATA-3 (transcription factor) (Tanigaki et al., 2004). Notch directly governed the expression of GATA-3, and Notch and GATA-3 synergistically activated the IL-4 expression and Th-2 cell responses (Fang et al., 2007); this was further confirmed by inhibition of Notch/GATA-3 signaling pathway that resulted in the prevention of deterioration owing to allergic asthma (Chong et al., 2014). Further, intravenous immunoglobulin ameliorated allergic asthma by enhancing the Dll-4 levels, and by decreasing the GATA-3 levels and Jag-1 expression, suggesting the suppression of expected Th-2 response (Kaufman et al., 2011). Moreover, in asthma models, Notch and phosphoinositide-3-kinase (PI3K) coordinately regulated the activation and differentiation of T lymphocytes via downregulation of p27^{Kip1} of CD4⁺ T lymphocytes and upregulation of cyclin D-1 of lymphocytes (Zhang et al., 2013b), whereas CD4⁺ T lymphocytes exhibited the crucial role in regulation of asthmatic airway remodeling (Foster et al., 2002). Besides CD4⁺ T-cell, regulatory T-cell (Treg), and Th-17 cells also exhibit a role in asthma (Shi et al., 2011). For instance, γ -secretase inhibitor (L685,458) alleviated inflammation-induced allergic asthma in mice by down-regulating the differentiation of Th-17 cells (Zhang et al.,

2015a). In addition, in vivo study has recently shown that Notch signaling in T cells, but not Jag-1 or Jag-2 on dendritic cells (DCs), is important for the induction of house dust mite-mediated Th-2 responses, eosinophilia, and airway hyper-reactivity (Tindemans et al., 2017).

Eosinophils also exhibit the vital role in allergic asthma (Gaurav et al., 2014), and Notch regulates the differentiation and maturation of eosinophils via ERK pathway (Kang et al., 2005, 2007), and histone deacetylation of T lymphocytes and γ -secretase inhibitors inhibited the eosinophilic airway inflammation (Kang et al., 2009; Zhang et al., 2015b). In granulocyte-macrophage-colony-stimulating factor–stimulated eosinophils, activation of Notch receptors and subsequent transcription of HES-1 has been reported (Radke et al., 2009; Liu et al., 2015). Total ablation of eosinophil lineage in mice resulted in elevated mucus secretion and airway hyper-responsiveness similar to wild-type (acute and chronic phases), whereas eosinophil-deficient mice were considerably protected from deposition of airway smooth muscle cells and peribronchiolar collagen (Humbles et al., 2004). Various studies have demonstrated that eosinophils contribute to asthmatic airway remodeling through the secretion of cytokines (TGF- β) and eosinophil cationic protein, as well as through interaction with epithelial cells and mast cells (Kay et al., 2004; Venge, 2010). More detailed studies are required to evaluate the remarkable role of Notch in airway remodeling in response to allergic asthma.

Mucous/Goblet Cell Metaplasia. Mucous/goblet cell metaplasia is the hallmark of airway diseases in epithelial remodeling. Mucus, comprising Muc5AC and Muc5B, is secreted by the goblet cells and submucosal glands of the lung through epidermal growth factor receptor signaling and STAT6 activation, via IL-4, IL-5, and IL-13, during allergic disease, particularly asthma (Justice et al., 2002; Boucherat et al., 2013). The role of Notch signaling in the goblet cell metaplasia and mucus overproduction remains somewhat controversial. For instance, ablation of Notch signaling either by conditional inactivation of *Pofut-1* or by deletion of *RBP-jk* in mice resulted in striking goblet cell metaplasia and mucus metaplasia associated with significant downregulation of bHLH transcription repressor HES-5 (Tsao et al., 2009, 2011), whereas Jag-1 deletion–induced mucous metaplasia is mainly accompanied by downregulation of HES-1 and HES-5 (Zhang et al., 2013a). Another study has reported that Notch signaling directly downregulated the expression of Muc5AC via an HES-1-dependent mechanism (Ou-Yang et al., 2013). These findings suggest an inhibitory function of Notch in mucus overproduction and goblet cell metaplasia in asthmatics. However, in contrast, various studies have proposed that Notch signaling directly promotes the mucous metaplasia and goblet cell metaplasia (Rock et al., 2011; Danahay et al., 2015; Lafkas et al., 2015). Activation of Notch signaling in the entire lung epithelium (Guseh et al., 2009) or in airway basal cells (Rock et al., 2011) resulted in an elevated count of goblet cells and mucous cells. Most importantly, Notch ligand–treated STAT6-null cultured tracheal epithelial cells were able to cause mucous metaplasia, and unchanged expression of FOXA2 was noticed as well, demonstrating that Notch signaling acts via the STAT6-independent pathway; probably Notch and STAT6 may operate in parallel to promote goblet cell metaplasia (Guseh et al., 2009). FOXA2-independent goblet cell metaplasia was noticed in *Hoxa-5* deficient mice

accompanied by increased activity of Notch signaling, whereas γ -secretase inhibitor attenuated the goblet cell metaplasia (Boucherat et al., 2012). In addition, forced expression of the Notch-1 intracellular domain in lung epithelial cells induced both epidermal growth factor receptor (EGFR) and ERK phosphorylation with a Muc5AC expression even in the absence of epidermal growth factor. The phosphorylation of ERK induced by exogenous NICD was inhibited by a γ -secretase inhibitor (L-685,458) or introduction of small-interfering RNA directed against Notch-1 that antagonizes EGFR activity, indicating that Notch signaling induced Muc5AC expression by activating the EGFR pathway (Kang et al., 2011). Consistent with previous studies, antagonizing Notch prevented and reversed IL-13- and allergen-driven goblet cell metaplasia (Guseh et al., 2009; Kang et al., 2009; Danahay et al., 2015; Lafkas et al., 2015), as well as overturning the human airway epithelium disorder (Gomi et al., 2016), providing evidence to support the involvement of Notch signaling in goblet cell metaplasia and asthmatic airway remodeling. Taken together, controversies regarding the role of Notch in mucous/goblet cell metaplasia might result from the disparate function of Jagged1 and Dll-4 in the pathogenesis of allergic asthma (Huang et al., 2017).

Extracellular Matrix Production and Subepithelial Fibrosis. Subepithelial fibrosis, the most distinctive pathologic characteristic of asthmatic airway remodeling (Elias et al., 1999), is characterized by unnecessary deposition of extracellular matrix (ECM). Type-I collagen, tenascin-C (TN-C), periostin, and hyaluronan are the imperative components of ECM that serve as biomarkers of asthma and scaffolds for continuing airway remodeling. Type-I collagen, heterotrimer of two $\alpha 1$ (col1 $\alpha 1$) and one $\alpha 2$ collagen (col1 $\alpha 2$) subunits, becomes most abundant during subepithelial fibrosis. Recently, an in vitro study both in human (MRC-5 cells) and mice (L929 cells) lung fibroblast cell lines demonstrated that Notch signaling might regulate the expression of col1 $\alpha 1$ and col1 $\alpha 2$ via HES1-dependent mechanism (Hu et al., 2014). Subsequent in vivo study in asthmatic mouse models revealed excessive expression of type-I collagen, whereas KyoT2, a negative regulator of Notch signaling, alleviated the subepithelial fibrosis through HES-1-dependent mechanism (Hu et al., 2015).

TN-C is involved in the pathogenesis of bronchial asthma (Rogers et al., 2012), whereas its deficiency attenuates the allergen-induced bronchial asthma (Nakahara et al., 2006). Notch signaling regulates the endogenous TN-C expression via RBP-jk-induced target gene (Sivasankaran et al., 2009). Recently, Sarkar and colleagues (2017) have shown that integrin $\alpha 2 \beta 1$ might be a direct link between TN-C and Notch signaling. It will be important to study the involvement of TN-C regulation in Notch-deficient asthmatic mice to clarify the relationship between TN-C/Notch and asthmatic airway remodeling.

Hyaluronan, a polysaccharide of ECM, is found within the peribronchial and perialveolar spaces of the healthy lung. During inflammation, smaller fragments of hyaluronan that are proinflammatory in nature are produced; they affect other ECM components, including collagen I and II, and are potentially associated with airway remodeling, hyper-responsiveness, and clinical symptoms (Garantziotis et al., 2016). Hyaluronan regulates the expression of Notch genes because expression of Notch-1 mRNA decreases whereas

Notch-3 mRNA increases significantly compared with controls when normal human epidermal keratinocytes were incubated in dishes coated with sulfated hyaluronan (Nagira et al., 2007). Periostin is a multifunctional protein that is upregulated in response to IL-4 and IL-13 in airway epithelial cells and lung fibroblasts of asthmatics. Periostin is involved in many aspects of asthma, such as eosinophil recruitment, airway remodeling, development of a Th2 phenotype, and increased expression of inflammatory mediators (Li et al., 2015). Interestingly, Notch-1 is downregulated in periostin-deficient mice, suggesting that periostin could directly interact with Notch-1 precursor to stabilize the Notch-1 expression and subsequent signaling. Moreover, periostin directly interacts with type-I collagen (Norris et al., 2007), fibronectin, and TN-C (Kii et al., 2010). Through these interactions, periostin seems to induce airway remodeling via modulation of ECM production. To date, limited data are available to support the participation of Notch in EMC, its components and production, and airway epithelium and/or subepithelial fibrosis in asthmatics. Hence, more profound studies are needed.

Myofibroblast Differentiation. All Notch receptors, except Notch-4, are accomplished with the regulation of myofibroblast differentiation. Notch-1/CSL activation induced the differentiation of myofibroblast through direct regulation of α -SMA (Nosedá et al., 2006), and α -SMA is normally used as a molecular marker for myofibroblasts. Knockout mice study has demonstrated that Notch-1 exhibited a distinct role in stimulating the α -SMA gene expression (Liu et al., 2009), whereas Notch-2 inhibited the TGF- β -induced collagen-I and α -SMA gene expression via downregulation of the Notch-3 in myoblasts (Ono et al., 2007). However, in 10T1/2 fibroblasts, overexpression of activated Notch-3 blocked the TGF- β 1-dependent smooth muscle-specific genes, such as α -SMA, by inhibiting the activation of Smad-3 and p38 mitogen-activated protein kinase (Kennard et al., 2008), whereas TGF- β 1 decreased the Notch-3 expression but unexpectedly *HES-1* was up-regulated. It has also been suggested that fibroblast proliferation is inhibited by Notch-1 and is mediated through Wnt-1-independent but Wnt-11-dependent Wnt-1-inducible signaling pathway protein 1 expression (Liu et al., 2012). In contrast, Notch-1 actively induced the differentiation of myofibroblast of alveolar epithelial cells through a TGF- β -Smad-3 pathway that activates α -SMA gene transcription in a TGF- β control element-dependent manner and SRF-binding site [CC(AT)6GG, also termed as CArG box]-dependent or CArG-dependent manner (Aoyagi-Ikeda et al., 2011). Interestingly, physiologic differentiation of myofibroblasts during lung alveogenesis required the involvement of *lfn*g-mediated Notch signaling (Xu et al., 2010). Notch in alveolar type 2 cells induced PDGF-A expression to expand the cell population of myofibroblasts (Tsao et al., 2016). Hence, the consequences of Notch signaling in fibrosis (Kavian et al., 2012) might be the result of activation of myofibroblast differentiation via endothelial-mesenchymal transition and epithelial-mesenchymal transition.

Epithelial-Mesenchymal Transition. Dysregulation of epithelial barrier function is a pathologic hallmark of asthmatic airway remodeling that leads to transdifferentiation of epithelial cells to attain mesenchymal characteristics, such as the production of extracellular matrix, α -SMA, and vimentin, and enhanced motility, in a process known as epithelial-mesenchymal

transition (EMT). Transcription factors including Snail-1, Slug (Snail-2), Twist-1/2, and zinc finger E-box-binding homeobox-1/2 (ZEB-1/2) serve as phenotypic markers of EMT. Slug, Snail, SIP1/ZEB, Twist, and E-47 negatively control E-cadherin expression. Notch crosstalks with numerous transcription and growth factors related to EMT, such as Snail, Slug, PDGF, fibroblast growth factor (FGF), and TGF- β (Gonzalez and Medici, 2014). Notch upregulated the Snail and Slug that subsequently led to transcriptional repressors of *CDH1* (E-cadherin gene), alleviation of the cell-cell junction, and destabilization of epithelial structure (primary step in EMT) (Leong et al., 2007). Notch-1 knockdown in intestinal epithelial cells contributed to decreased barrier function, a decline in the expression of intercellular tight junction protein Claudin-5, and increased intercellular permeability (Mathern et al., 2014). Overexpression of Notch alone upregulated Snail expression and downregulated E-cadherin, whereas inactivation of Notch decreased the Snail expression and attenuated the downregulation of E-cadherin expression, suggesting the participation of Notch signaling in EMT induction via Snail (Xie et al., 2012). Notch signaling-induced EMT could trigger the upregulation of mesenchymal markers (Snail, Slug) and downregulation of epithelial markers (E-cadherin) in the cardiac valve and cushion formation, human kidney epithelial cells, and non-small cell lung cancer cell lines. Importantly, Loffredo et al. (2017) recently identified Notch-2, among other recognized genes, as a novel marker central to dysregulation of epithelial-mesenchymal signaling in asthmatics and suggested that the EMT process, concerned with asthma pathogenesis, is a manifestation of an underlying profound and persistent suppression of epithelial differentiation, because significant suppression of epithelial differentiation was demonstrated in the scarcity of Notch signaling. Moreover, inactivation of Notch signaling by a γ -secretase inhibitor could reverse the EMT process, highlighting the direct involvement of Notch signaling in EMT induction, making Notch signaling attractive targets for new therapeutics.

Of note, EMT in non-small cell lung cancer is mediated by TGF- β , which promotes the interface of SMAD to the promoters of Snail. Crosstalk between Notch and TGF- β is imperative for induction of EMT, because Notch signaling is required to maintain TGF β -induced expression of HEY-1, and pharmacological inactivation of Notch and/or knockdown of HEY-1 or jag-1 could inhibit the TGF- β -induced EMT, demonstrating the key role of Notch signaling in TGF- β -induced EMT (Zavadil et al., 2004). Similarly, in alveolar epithelial cells, Notch-1 activated the α -SMA gene in a TGF- β control element-dependent manner and a CArG-dependent manner through TGF- β -Smad-3 pathway (Aoyagi-Ikeda et al., 2011). Meanwhile, pharmacological inhibition of Notch signaling could notably inhibit the TGF- β -induced expression of α -SMA, signifying Notch-induced EMT via TGF- β -Smad-3 pathway (Matsuno et al., 2012). Nevertheless, Notch prevented TGF- β -mediated EMT in cultured cells through Smad-7 induction (Tsai et al., 2014). Collectively, these studies indicate that the crosstalk between Notch signals and TGF- β play an insightful role in EMT, and more detailed studies are needed to evaluate the role of Notch signaling in EMT-induced asthmatic airway remodeling.

Pulmonary Vascular Remodeling. Pulmonary vascular remodeling, including increased subepithelial vascularity and microvascular leakage, is significantly increased in

asthmatics with the severity of asthma (Shifren et al., 2012). Multiple factors are involved, including inflammatory mediators (pro-inflammatory mediators such as IL-6 and -8, TNF- α , TGF- β), extracellular matrix proteins, bioactive extracellular matrix fragments (matrikines), growth factors (FGF family, PDGF, TGF- β , TNF- α , and IL-1 family), and proteases (matrix metalloproteinases and ADAMs) (Harkness et al., 2015). The enhanced vasculogenic effect in pulmonary tissue is determined by the Th-1- and Th-2-dependent selection predisposition in T-cells, which act as chemotactic substances for endothelial precursor cells, such as VEGF-A, to induce Th-2-type inflammatory responses that are proinflammatory and lead to the angiogenic switch.

Notch-3/Jagged controls the differentiation and expansion of Treg (Anastasi et al., 2003), and Treg exhibits the angioregulatory response (Asosingh et al., 2007). So, Notch signaling demonstrates a crucial role in angiogenesis and vasculogenesis (Iso et al., 2003). Activation of Notch-HES-1 axis via TGF- β exhibited Treg-mediated immunosuppression (Ostroukhova et al., 2006). Injection of sensitized Treg cells in a chronic mouse asthma model alleviated the airway remodeling by reducing the pulmonary vasculature via induction of apoptosis through a Dll-4/Notch signaling pathway (Huang et al., 2009). Dll-4 and Jag-1 surprisingly demonstrated the different responses in asthma models (Huang et al., 2017). Dll-4 alleviated the airway hyper-responsiveness by suppressing neovascular airway remodeling via Treg (Huang et al., 2009), and Jag-1 initiated allergic asthma by stimulating the Th-2 differentiation and IL-4 production, leading to airway inflammation, airway hyper-responsiveness, and asthmatic airway remodeling (Okamoto et al., 2009).

As discussed, Notch signaling is imperative for regulation of proximodistal cell fates, pseudostratified epithelium differentiation, pulmonary neuroendocrine cell differentiation, pulmonary goblet cell fate, and alveolar and pulmonary vasculature development. Deletion and/or deterioration of Notch signaling during early lung development leads to abnormal enlargement of alveolar spaces, disturbance of the integrity of bronchial and epithelial smooth muscle layers (Tsao et al., 2016), widespread cellular death (Swiatek et al., 1994; Hamada et al., 1999), aberrant maturation of vascular smooth muscular cells (Krebs et al., 2000), vascular remodeling defects (Gale et al., 2004; Roca and Adams, 2007), hypoplastic lungs with attenuated Club cells count (Tsao et al., 2009; Morimoto et al., 2010), and goblet cell metaplasia (Tsao et al., 2011; Danahay et al., 2015; Lafkas et al., 2015). These pathologic changes elaborate the underlying causes for abnormal lung functioning. Moreover, Notch genes participating in fetal lung development may clarify the molecular processes involved in lung function impairment, but clinical data to support the role of Notch signaling in linking lung development and asthmatic airway remodeling is yet missing. However, murine model studies suggest that abnormal in utero expression of Notch genes, concerned with healthy lung development, can result in impaired lung physiology after birth. Review of all data suggests that Notch signaling exhibits a distinct but mysterious role in linking lung development and asthmatic airway remodeling, therefore detailed experimental and genetic studies are needed to investigate the underlying philosophy.

Notch Signaling as a Potential Therapeutic Target

As Notch signaling exhibits a potential role in the regulation of asthmatic airway remodeling, direct or indirect targeting of Notch pathways at signal sending–signal receiving sites, intracellular signaling levels, transcriptional/post-transcriptional levels, specific cofactor action sites, and crosstalk with other pathways would facilitate development of new effective therapeutic approaches to control/reverse asthmatic airway remodeling (Fig. 3).

Targeting Notch Signaling via γ -Secretase Inhibitors. γ -Secretase inhibitors, which are nonspecific Notch pathway inhibitors, inhibit the S3 cleavage that subsequently inhibits the production of NICD from membrane-tethered Notch receptors and transcription of target genes. γ -Secretase inhibitors are divided into three main classes: peptide isosteres, azepines, and sulfonamides. L685458, MW167 (gamma-secretase inhibitor II), and DAPT (also known as GSI-IX and Compound 3) are reversible peptide-based γ -secretase inhibitors. From DAPT, LY-411575 (compound 5; 100-fold stronger than DAPT), LY-450139 (compound 6) and RO-4929097 (Roche, Nutley, NJ) have been developed that are even more effective. DBZ (dibenzazepine or YO-01027) and JLK6 are irreversible azepine-based γ -secretase inhibitors. MK-0752 and compound 18 are sulfonamide-based γ -secretase inhibitors. Various clinical trials are ongoing to explore how and whether targeting Notch signaling via γ -secretase inhibitors can alleviate different ailments, particularly in oncology, and in the case of asthmatic airway remodeling, few approaches have been performed via antagonizing γ -secretase.

In an asthma model, L685458 alleviated allergic asthma phenotypes via downregulation of Th-2 cytokine production (Kang et al., 2009) and antagonized the differentiation of Th-17 cells (Zhang et al., 2015a). L685458 vitiated the airway goblet cell metaplasia in a *Hoxa-5* mutant mouse model

(Boucherat et al., 2012). In asthma models, MW167 attenuated the Th-2 polarization, airway inflammation, and the levels of IL-4 and IL-5 in BALF and serum compared with PBS-treated (Zhou et al., 2015). Furthermore, DAPT reversed the pathologic remodeling of airway epithelium (Gomi et al., 2015) and attenuated the accumulation of eosinophils in allergic airways (Liu et al., 2015). In human airway epithelial cells, DBZ attenuated the IL-13-induced airway mucous metaplasia either independently of STAT-6 or downstream of the STAT-6 pathway (Guseh et al., 2009). Treating CD4⁺ T cells with DBZ resulted in inhibition of Th-2 differentiation, IL-4 production, airway hyper-responsiveness, and eosinophilic airway inflammation (Okamoto et al., 2009). Importantly, like other key growth factors and signaling pathways, appropriate Notch level is needed for normal airway development and homeostasis. Nonspecific inhibition of Notch pathway via γ -secretase inhibitors may cause many unpredictable negative effects. Therefore, more specific γ -secretase inhibitors for a bench-to-bedside therapeutic application need to be developed.

Targeting Notch Signaling Other than γ -Secretase Inhibitors. Fringe (fucose- β 1, 3-*N*-acetyl glucosaminyl transferases) regulates Notch signaling via ligand-receptor binding, and Notch signaling regulates the development and differentiation of T-helper cells. An OVA-sensitized asthmatic rat model has shown significantly low expression of both *manic fringe (mfng)* and *lunatic fringe (lfng)* compared with *radical fringe (rfng)* (Gu et al., 2012). Overexpression of *lfng* in CD4⁺ T cells in asthmatic rats, using *lfng* plasmid, inhibited the Th-2 cytokine production in a Notch-dependent manner (Gu et al., 2012), suggesting that overexpression of *lfng* may be helpful in treating Th-2 cytokine-associated asthma. In contrast, an increased expression of *lfng* has been reported in respiratory syncytial virus (RSV)-induced asthma models. Accordingly, STAT-5-dependent *lfng* expression in Th-2 development augmented the Dll4-Notch-mediated IL-4 release

Fig. 3. Therapeutically effective components of Notch pathway to treat asthmatic airway remodeling. There are several therapeutically effective nodes in the Notch signaling pathway, some are putative targets for new drug intervention. Cleavage by γ -secretase (a), ADAM secretase (b), and furin (c) can be targeted pharmacologically and are under preclinical studies to treat asthmatic airway remodeling. Interfering with the interaction between NICD and MAML (d) or CSL (or RBP-j κ) (e) nullify Notch signaling. Interfering with fucosylation, glycosylation (f), or other EGF-specific modifications (g) by inhibiting fringe activity may modulate specific Notch pathways. Similarly, Notch antibodies targeting Notch ligands (h) or receptors (i) would exclusively target individual receptor pathways. Finally, Notch ligand fusion peptides (j) can be used as an effective target. dnMAML, dominant-negative MAML.

during RSV-induced allergic asthma, whereas genetic deletion of *lfrng* inhibited the Dll4-mediated Notch activation, Th-2 cytokine production, and IL-4 release (Mukherjee et al., 2014). These studies suggest that fringe could be a new curative approach for the impediment of allergic asthma and airway remodeling, so it cannot be discounted. Therefore, more studies are needed to elucidate the exact role of fringe in the pathogenesis of asthma and to explore whether interference with fringe is a feasible treatment.

Dll-4 expressed on dendritic cells influence the differentiation of T-helper cells toward Th-1 and Th-2 or Treg (Amsen et al., 2009). Dll-4 neutralization exacerbated the IL-5 and IL-13 production, Th-2 cytokine production, and airway hyper-responsiveness during RSV infection (Schaller et al., 2007; Jang et al., 2010; Ting et al., 2017), whereas Dll-4 activation during differentiation sustained Treg cell phenotype and function to control RSV infection (Ting et al., 2017). Moreover, intravenous administration of OVA-pulsed plus Dll4-pretreated DCs in Th-2-dominant asthmatic murine models notably increased the production of IL-10, reduced the production of proinflammatory cytokines, decreased the severity of airway hyper-responsiveness, and alleviated the expression levels of OVA-specific IgE, indicating that Dll-4 altered the DC activation (Huang et al., 2013). In contrast, in vivo neutralization of Dll-4 remarkably decreased the Th-2 cytokines production, mucus production, and airway hyper-reactivity, whereas Dll4-mediated Notch activation during viral exacerbation further augmented the *lfrng*-dependent Th-2 cell activation (Mukherjee et al., 2014). It would be reasonable to hypothesize that allergen-induced Th-2 inflammatory surroundings are used by virus to further intensify the allergic circumstance most probably owing to RSV-induced pro-Th-2 factors (IL-25) (Kaiko et al., 2010) and thymus and activation-regulated chemokine (Monick et al., 2007) that ultimately amplify the development and recruitment of Dll4-mediated Th-2 cytokine in the lung. Hence, these observations suggest that Dll-4 could be an effective therapeutic target for modulating airway hyper-responsiveness and chronic asthma, but it is difficult to justify the exact role of Dll-4-mediated Notch signaling because of either the complexity of Notch receptor-ligand interactions or participation of noncanonical Notch pathways. Nonetheless, continuous attention is required to explore the exact role of Dll4-mediated Notch signaling to treat asthmatic airway remodeling.

Natural regulatory T cells (nTreg; CD4⁺CD25⁺) or induced regulatory T cells (iTreg) can effectively attenuate the features of allergic airway inflammation and inhibit the development of airway remodeling (Kearley et al., 2008; Lan et al., 2012), and Notch/Jagged controls the differentiation and expansion of Treg cells (Hoyne et al., 2000); in particular, constitutive Notch-3 activation enhanced the Treg cell generation (Anastasi et al., 2003). Adoptive transfer of iTreg (Xu et al., 2012) and Dll4-expressing Treg (Huang et al., 2009) to an OVA-sensitized chronic asthma mouse model effectively alleviated the airway remodeling. Moreover, administration of Dll4-expressing antigen-presenting cells in OVA-induced murine asthma model ameliorated allergic asthma via induction of Treg-mediated regulatory pathway, whereas blockage of Dll-4 impaired the Treg differentiation, and resulted in exaggerated asthma phenotypes (Huang et al., 2017). Hence, Treg needs to be further investigated in established airway remodeling to determine the importance of this therapeutic approach.

The pseudostratified airway epithelium is layered by the balanced proportion of secretory and ciliated cells, accompanied by undifferentiated basal cells, which differentiate to basal stem cells (goblet cell hyperplasia) during asthma. An adult trachea study has shown that persistent activation of Notch impeded ciliogenesis and endorsed the luminal differentiation of the basal cell into secretory cells (Rock et al., 2011). Consistent with this, STAT-3 activated by IL-6 produced in response to injury, promoted multiciliogenesis and regeneration of airway multiciliated cells from basal cells through upregulation of ciliogenesis genes, such as *Foxj1* and *Mcidas*, and through downregulation of Notch-1 (Tadokoro et al., 2014). Interestingly, signaling pathway between Toll-like receptor 4 and Notch-1 might coordinate with STAT-3, because expression levels of Toll-like receptor 4 and Notch-1, and phosphorylation of STAT-3 in asthma models was increased but decreased after Cornuside, a natural secoiridoid glucoside, treatment (Zheng, 2016). Hence, modulation of ciliogenesis by IL-6/STAT-3, via direct regulation of *Notch-1*, might prove helpful to prevent/treat the asthmatic airway remodeling.

RBP-jk along another transcriptional complex (NICD-CBF-1/RBP-jk-MAML) induces the transcription of target genes, whereas targeting/inactivation of RBP-jk inhibits the RBP-jk-mediated transcription and Notch pathway. KyoT2, the negative regulator of Notch pathway, inhibits RBP-jk-mediated transcriptional activation (Collins et al., 2014). Hu et al. (2015) showed that KyoT2 downregulated asthmatic airway remodeling by alleviating the subepithelial fibrosis and airway hyper-responsiveness through the HES1-dependent mechanism as well as improved lung function, suggesting that targeting/inactivation of RBP-jk might act as a potential clinical treatment strategy for asthmatic airway remodeling in future.

The epigenetic study revealed that asthma is linked with epigenetic changes in the Notch-1 promoters, such as abnormal histone acetylation and methylation (Cui et al., 2013). Cui and colleagues investigated the histone modification of asthmatic lung CD4⁺ T cells and revealed that histone acetylation and trimethylation levels of Notch-1 gene promoter P300 and pCAF activities were remarkably elevated compared with control. Although histone acetyltransferase activity, expression levels of Notch-1 and HES-1, and asthmatic parameters such as IL-4, IL-5, and IL-13 were significantly decreased after the intervention of asthmatic lung CD4⁺ T cells with garcinol, a potent natural inhibitor of histone acetyltransferases, p300, and pCAF (Cui et al., 2013), suggesting that targeting histone acetylation might be a new therapeutic strategy for the appropriate regulation of the Notch signaling pathway and for the treatment of asthmatic airway remodeling.

In summary, our ever-increasing understanding of the Notch signaling pathway offers great hope that specific targeting of Notch signaling may represent a promising alternative complementary therapeutic strategy for treatment of asthmatic airway remodeling in future.

Side Effects Associated with Notch Pathway Inhibition

Although preclinical studies, on Alzheimer's disease but not on asthmatic airway remodeling, have shown that long-term interference in Notch signaling by γ -secretase inhibitors led to severe gastrointestinal toxicity, such as vomiting, nausea,

diarrhea, decreased appetite, and inflammation owing to full inhibition of Notch pathway (Hsu et al., 2013). Importantly, Notch drives gastrointestinal precursor cells toward an epithelial fate and away from a secretory cell fate, whereas Notch inhibition results in an imbalance of this process and leads to secretory goblet cell hyperplasia (van Es et al., 2005). Additionally, inhibition of the Notch pathway leads to skin cancer (Demehri et al., 2009) and disruption of the immune system (Wong et al., 2004). Consequently, a dose-dependent effect of LY-411575 (γ -secretase inhibitor) on the intestine, spleen, and thymus of mice has been reported (Wong et al., 2004), because LY-411575 at high- and low-dose concentrations attenuated and enhanced the β -amyloid, respectively, by modulating γ -secretase complex at different binding sites. These toxicities might be challenging for any Notch inhibitor. Therefore, some γ -secretase modulators sparing the Notch cleavage have been developed (Chávez-Gutiérrez et al., 2012). Further, NSAIDs as therapeutic agents for reducing inflammation by targeting γ -secretase are under study (Saura, 2010). To minimize these side effects, two possible strategies have been proposed: the coadministration of γ -secretase inhibitors with glucocorticoids such as dexamethasone (Real et al., 2009) and recurrent dosing plans of γ -secretase inhibitors (Purow, 2012). Both strategies have been shown to spare the gut of toxicity for the most part but maintain the desired effect.

Concluding Remarks

Although major research has been conducted to understand the role of Notch signaling in malignancies and fibroproliferative disorders, clinical data to support the role of Notch signaling in asthmatic subjects are still lacking. Evidence obtained from animal models support the crucial role of Notch signaling in embryonic lung development, initiation and progression of asthmatic airway remodeling, and linking impaired fetal lung development either with asthmatic airway remodeling directly or through other pathways. Moreover, currently identified Notch targets can be counted on a few fingers, but even in those cases, we have little knowledge. γ -secretase inhibitors and other described approaches might be effective to overcome both aberrant expression and overproduction of Notch signaling, but they are associated with unwanted effects owing to the pleiotropic nature of Notch signaling; therefore, further detailed genome-wide association studies are needed to develop more specific γ -secretase inhibitors and modulators that could easily differentiate between wellness and illness. In this regard, a better understanding of the communications of canonical and non-canonical Notch signaling, consideration of how and why different target genes are activated according to cell type and time, exploration of Notch signaling crosstalk with other pathways, and apposite targeting of Notch signaling will open a new therapeutic era. In particular, future studies aimed at delineating the precise cellular and molecular mechanisms underpinning Notch-mediated asthmatic airway remodeling suppression will be important for developing potential therapeutic strategies.

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