Notch signaling in lung diseases: focus on Notch1 and Notch3

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Abstract: Notch signaling is an evolutionarily conserved cell–cell communication mechanism that plays a key role in lung homeostasis, injury and repair. The loss of regulation of Notch signaling, especially Notch1 and Notch3, has recently been linked to the pathogenesis of important lung diseases, in particular, chronic obstructive pulmonary disease (COPD), asthma, pulmonary fibrosis, pulmonary arterial hypertension (PAH), lung cancer and lung lesions in some congenital diseases. This review focuses on recent advances related to the mechanisms and the consequences of aberrant or absent Notch1/3 activity in the initiation and progression of lung diseases. Our increasing understanding of this signaling pathway offers great hope that manipulating Notch signaling may represent a promising alternative complementary therapeutic strategy in the future.

Keywords: lung disease, Notch1, Notch3, therapy, γ-secretase

Introduction

Notch is a highly conserved signaling pathway involved in the regulation of cell-fate acquisition and differentiation in several systems [Kopan and Ilagan, 2009]. It was initially discovered to be responsible for the specific phenotype displayed as ‘notches’ at the wing blades of Drosophila melanogaster. Nowadays, it is clear that the Notch-signaling pathway influences cell-fate decisions, such as survival or apoptosis, proliferation and differentiation, and maintains stem-cell quiescence and identity [Bi and Kuang, 2015]. To date, in mammals, there are four Notch receptors (Notch1–4) and five ligands named Jag1, Jag2 (homologs to Serrate), Delta-like (Dll)1, Dll3 and Dll4 [Fleming, 1998]. Both the receptors and the ligands are single-pass transmembrane proteins with extracellular domains, transmembrane and intracellular domains. The extracellular domain of the receptor is composed of a series of 29–36 epidermal growth-factor-like (EGFR-like) repeats. The EGFR-like domains are the ligand-interacting part of the Notch receptors. Following a single transmembrane domain, the intracellular domain consists of the RBPJ association module, the nuclear localization signal, ankyrin repeats and degradation signals (glutamine-rich repeat (OPA)/ proline/glutamic acid/serine/threonine-rich motifs (PEST) domain) [Bigas et al. 2013]. The pathway is initiated on the binding of a Notch receptor to a ligand located on a neighbor cell. Once receptor–ligand interactions occur, the Notch molecules in the target cells are processed by two successive proteolytic cleavages [Wakabayashi et al. 2015]. The first cleavage begins extracellularly, close to the transmembrane domain, and is mediated by metalloproteases of the ADAM family. The second cleavage proceeds within the transmembrane domain and is mediated by γ-secretase, which is a multiple protein complex consisting of Presenilin, Nicastrin, Aph1a (anterior pharynx defective 1 homolog) and Psen (presenilin enhancer 2 homolog) proteins. At the completion of this process, the Notch-intracellular domain (NICD) translocates into the nucleus and interacts with RBPJκ/CSL, a transcriptional repressor. Upon interaction with the NICD, RBPJκ/CSL is converted into a potent transcriptional activator [Ayaz and Osborne, 2014]. This transcriptionally active complex induces the expression of basic-helix-loop-helix (bHLH) family genes such as Hairy and enhancer of split (Hes) family genes (i.e. Hes1, 3, 5 and 7) and Hes-related with a YRPF motif (Hey) family genes (i.e. Hey1, Hey2 and HeyL) [Chen et al. 2014]. Both the Hes and Hey proteins execute most biological processes and partially underlie the target specificity of the
different Notch-receptor paralogs. The cell-cycle promoter CyclinD1, the proliferation-related gene c-Mycl, the antiapoptotic gene Bcl2, the gene for Notch-regulated ankyrin repeat protein, Delta1, the pre-T-cell receptor gene, p21cip1/waf1 and HER2 have also been identified as Notch target genes [Wakabayashi et al. 2015; Takebe et al. 2014].

The genomic sites at which Notch activates transcription vary from cell to cell and vary quite likely among different Notch paralogs. Thus, Notch signaling can occur in a variety of circumstances based on the presence of the five different ligands in the microenvironment, the expression of metalloproteinase and \(\gamma\)-secretase complex enzymes, as well as the expression of the four Notch receptors, yielding a large number of potential variations on the Notch signaling [Hernandez Tejada et al. 2014]. Little is known about how Notch ligands interact with various Notch receptors, but it does appear that preferences for certain Notch–ligand partnerships exist in vivo [Andrawes et al. 2013]. One of the best-described examples is that the Notch1–Dll4 interaction is a key regulator of angiogenesis [Hemmström et al. 2007]. However, a strong endothelial expression of the JAG1 ligand antagonises DLL4-Notch1 signaling during sprouting angiogenesis [Benedito et al. 2009]. DLL1 is an essential Notch ligand in the vascular endothelium of large arteries to activate Notch1 and maintains arterial identity [Sörensen et al. 2009]. The untypical ligand DLL3 can interact with, but does not activate, Notch [Schuster-Gossler et al. 2016]. The interaction of Dll1-Notch2 has been reported to play an important role in marginal zone B-cell development [Descatoire et al. 2014]. Notch2 and Jagged1 are necessary for appropriate bileduct development [Geisler et al. 2008]. In adult airways, Jag2-Notch3 has been reported to contribute to the expansion of p63+ cells in vivo [Mori et al. 2015]. A coordinated activation of Dll4/Notch4 plays a key role in the abnormal remodeling of tumor vessels [Zhang et al. 2016]. Despite the fact that these combinations of Notch receptors and ligands are common, it is still unclear whether these preferences are based solely on spatial and temporal differences in expression patterns or if there are underlying intrinsic differences in the affinity among various ligand–receptor complexes [Andrawes et al. 2013].

In mammalian lungs, all Notch ligands and receptors are transcriptionally expressed [Post et al. 2000]. They were increasingly linked to a variety of lung diseases. Over the past decade, the role of Notch receptors in the pathogenesis of lung diseases has been subjected to extensive examination. Of them, two Notch receptors have been implicated: Notch1 and Notch3. Here, we mainly review the role of Notch1 and Notch3 in various lung diseases, such as chronic obstructive pulmonary disease (COPD), asthma, pulmonary fibrosis, pulmonary arterial hypertension (PAH), lung cancer and lung lesions in some congenital diseases.

The role of Notch1/Notch3 in chronic obstructive pulmonary disease

Notch1. Mucus hypersecretion has been established as a pathologic characteristic of smoking-related lung diseases, especially COPD [Wang et al. 2012b]. The altered balance of ciliated and secretory cells, particularly the increase in mucous (goblet) cells, contributes to the hypersecretion [Curran and Cohn, 2010]. Notch signaling has been identified as a major regulator of goblet-cell fate [Guseh et al. 2009; Boucherat et al. 2012]. In explanted embryonic lungs, the addition of a Notch ligand or an expression of a constitutively active form of a Notch1 receptor increased MUC5AC-containing mucous cells, whereas a \(\gamma\)-secretase inhibitor (GSI) reduced the mucous cells [Guseh et al. 2009]. Boucherat and colleagues reported that the expression levels of activated Notch1 and the effector gene Hey2 are enhanced in the areas of goblet-cell metaplasia along the airway epithelium and in the submucosal glands in COPD patients [Boucherat et al. 2012]. The in-vivo administration of a GSI attenuates goblet cell metaplasia in a Hoxa5 mutant mouse model. Conversely, in a postnatal mouse lung, Notch signaling directly repressed MUC5AC transcription in lung epithelial cells [Tsao et al. 2011] (Table 1). Moreover, disruptions of Notch signaling resulted in an aberrant postnatal airway phenotype characterized by marked goblet-cell metaplasia, decreased Clara cell number and increased ciliated cells. These studies indicate that different thresholds of activation of Notch signaling may determine whether a cell will become a secretory cell or a nonsecretory cell. This mechanism is likely to be in place not only in development but also in aberrant responses of the mature epithelium of the environmental agents that result in airway epithelial metaplasia [Shi et al. 2013]. The regulation of Notch signaling may become a potential therapeutic approach to restrain goblet-cell differentiation and mucus...
hyperproduction in the airways of patients with COPD.

Recent data described abnormal apoptotic events as one of the important mechanisms involved in the destruction of pulmonary tissue in COPD. Our previous research also confirmed that endothelial cell apoptosis was closely related to cigarette smoke [Peng et al. 2013; Yang et al. 2015; Kang et al. 2015; Chen et al. 2012a]. Over the past decade, Notch signaling has been highlighted in cell-fate determination, including apoptosis [Dang, 2012]. The genetic deletion of Notch1 results in abundant apoptotic cell death [Limbourg et al. 2005], whereas Notch1 overexpression protects cells from apoptosis [Qin et al. 2011]. Previous studies have suggested that oxidative stress can cause cellular apoptosis via both the extrinsic cell-death receptor pathway and the intrinsic mitochondrial-cell-death pathway [Sinha et al. 2013]. Thus, oxidative stress and apoptosis can interact and overlap with each other in the overall pathogenesis of COPD. Notch signaling has recently been reported to prevent the production of reactive oxygen species [Small et al. 2014; Cai et al. 2014]. Notch signaling maintains low oxidative stress in cells, and the inhibition of Notch using GSI results in the enhanced generation of reactive oxygen species. These results suggest that Notch signaling may be involved in cell apoptosis induced by cigarette smoke through regulating oxidative stress.

**Notch3.** A recent analysis of the airway transcriptome in human subjects has shown that all of the key functional components in the Notch signaling are widely expressed in the airway epithelium [Tilley et al. 2009]; and several of them, such as Notch3, Dll1, Hes, and Hey genes, are downregulated in healthy smokers and smokers with COPD. These changes raise the possibility that Notch signaling may contribute to the aberrant differentiation profile of the airway in COPD patients [Shi et al. 2009]. Notch3 plays a role in regulating the alveolar epithelium. The constitutive expression of Notch3 in the peripheral epithelium results in altered lung morphology, with a failure of the type I pneumocytes to differentiate from the type II pneumocytes [Dang et al. 2003] (Table 1). These observations may explain why, in emphysema, enlarged alveoli are mainly covered by type I-differentiated pneumocytes and why type II-pneumocyte proliferation is minimal [Chilosi et al. 2012]. However, how Notch3 exerts its function in COPD is still lacking evidence. Further studies should focus on the role of Notch in the pathogenesis and the progression of the disease.

| Table 1. Involvement of Notch1/Notch3 in chronic obstructive pulmonary disease. |
|---|
| **Reference** | **Specimen Source** | **Change of Notch1/Notch3** | **Biological function** |
| Guseh et al. [2009] | Lung tissue from Rosa-Notch1C-IRES-GFP mice | Notch1↑ | Increased mucous cells, decreased ciliated cells in the airway and prevented the differentiation of alveolar cell types |
| Boucherat et al. [2012] | Lung tissue from Hoxa5−/− mice | Notch1↑ | Induced goblet-cell differentiation and mucus overproduction |
| Tsao et al. [2011] | Lung tissue from Pofut1c−/− mice | Notch1↓ | Increased goblet cells and ciliated cells, decreased Clara cell number |
| Tilley et al. [2009] | Lung tissue from COPD patient | Notch3↓ | Notch3 downregulated in airway epithelium |
| Dang et al. [2003] | Lung tissue from SP-C-N3IC transgenic mice | Notch3↑ | Inhibited type I pneumocyte differentiation, induced abnormalities of lung morphogenesis and perinatal lethality |

COPD, chronic obstructive pulmonary disease.
forms of asthma [Kallinich et al. 2007]. The Notch pathway is confirmed to be a signaling mechanism involved in the development, differentiation and activation of T cells [Zhou et al. 2015; Zhang et al. 2013]. The introduction of an activated allele of Notch1 into CD4+ T cells led to the specific and direct upregulation of a developmentally regulated Gata3 transcript, which acts in concert with Notch signaling to synergistically activate the IL-4 expression and the Th2 cell responses [Fang et al. 2007]. Consistent with this, the GSI treatment of bronchoalveolar lavage cells stimulated via T-cell receptor (TCR) or non-TCR pathways led to a decrease in Th2 cytokine production with a concomitant increase in Th1 cytokine secretion [Kang et al. 2009]. These studies suggest that blocked Notch1 signaling may benefit diseases associated with the excessive production of Th2 cytokines. However, a different opinion has been proposed; that Notch1 can influence T-bet, a Th1-specific T box transcription factor, by regulating Tbx21. The administration of GSI substantially impeded the Th1 polarization both in vivo and in vitro [Minter et al. 2005] (Table 2). Although the evidence supporting a role for Notch in the Th1-cell differentiation cannot be discounted, the evidence so far has been more convincing for a role of Notch in the Th2-cell differentiation than for a role in Th1-cell differentiation [Amsen et al. 2009]. Further studies are needed to clarify the exact role of Notch1 in the pathogenesis of asthma and explore whether or not the interference of Notch1 signaling is a feasible treatment option for asthma.

Eosinophils are key effector cells in the pathogenesis of allergic disease and are recruited from the circulation to inflammatory tissues in response to allergic stimuli [Zhang et al. 2015].

Table 2. Involvement of Notch1/Notch3 in asthma.

| Reference      | Specimen Source                          | Change of Notch1/Notch3 | Biological function                                      |
|----------------|------------------------------------------|-------------------------|----------------------------------------------------------|
| Zhou et al. [2015] | Active lung T cells from asthmatic mouse model | Inhibit Notch1 with GSI | Decreased IL-4 and IL-5 expression and increased IFN-γ expression |
| Fang et al. [2007] | CD4+ T cells from DNAMAMLf/fDo11.10 (B10.D2) mice | Forced expression of N1ICD | Promoted IL-4 expression                                  |
| Kang et al. [2009] | Bronchoalveolar lavage cells from asthmatic mouse model | Inhibit Notch1 with GSI | Decreased in Th2 cytokine production and increased in Th1 cytokine secretion |
| Minter et al. [2005] | CD4+ T cells from C57Bl/6 mice | Inhibit Notch1 with GSI | Inhibited IFN-γ and Tbx21 expression                        |
| Radke et al. [2009] | Eosinophils | Inhibit Notch with GSI | Enhanced viability, decreased actin polarization, and diminished chemokinesis of eosinophils |
| Kang et al. [2005] | Umbilical cord blood cells | Inhibit Notch with GSI | Induced eosinophil differentiation                        |
| Kang et al. [2007] | BAL cells from OVA exposed mice | Inhibit Notch1 with GSI | Inhibited eosinophil accumulation within allergic airways |
| Anastasi et al. [2003] | Spleen, lymph nodes, and pancreas from Notch3-transgenic mice | Notch3↑ | Enhanced generation of Treg cells                        |
| Kared et al. [2006] | CD4+CD25+ cells from NOD mice | Activate Notch3 by mobilized Lin–Sca-1+c-kit+HPC | Promoted the expansion of Treg Cells both in vivo and in vitro |
| Maekawa et al. [2003] | CD4+ T cell from BALB/c mice | Forced expression of N3ICD | Promoted naïve T cells differentiation toward the Th1 phenotype |

GSI, γ-secretase inhibitor; N1ICD, Notch1 intracellular domain; N3ICD, Notch3 intracellular domain; HPC, hematopoietic progenitor cell, BAL, bronchoalveolar lavage; OVA, ovalbumin; IL, interleukin.
asthmatic patients have shown that eosinophil numbers are significantly increased in bronchoalveolar lavage fluid, sputum and endobronchial biopsies in response to airway hyperresponsiveness [Gaurav et al. 2014]. Evidence of Notch-receptor activation and the subsequent transcription of the Notch-responsive gene Hes1 were observed in granulocyte-macrophage colony-stimulating factor (GM-CSF)-stimulated eosinophils [Radke et al. 2009]. Notch signaling regulates the terminal differentiation and subsequent effector phenotypes of eosinophils, partly through the modulation of the extracellular signal-regulated kinase pathway [Kang et al. 2005, 2007]. GSI treatment induces the differentiation of eosinophils lacking effector functions in vitro. In mice in vivo, the eosinophil accumulation within allergic airways was impaired following the systemic treatment with GSI or the adoptive transfer of eosinophils treated ex vivo with a Notch inhibitor [Liu et al. 2015] (Table 2). In summary, the remarkable effect of GSI on eosinophil differentiation implied a multipronged therapeutic value of this protein in the treatment of asthma. The continued attention to the study of Notch signaling in asthma will be crucial for generating new ideas for asthma prevention and treatment.

**Notch3.** Recent reports have suggested that other CD4+ T-cell subsets may play a role in asthma, including Th17 cells and CD4+CD25+ Treg cells [Shi et al. 2011]. Reduction with or without defects in Treg cells have been detected in asthma patients [Xu et al. 2012]. The administration of Treg cells can reduce existing inflammation and prevent the subsequent development of airway remodeling [Kearley et al. 2008], suggesting that Treg-cell-mediated immunological regulation plays a protective role in asthma. A constitutively active Notch3 intracellular domain (Notch3-ICD) in transgenic mice enhances the generation of Treg cells [Anastasi et al. 2003]. In addition, Treg-cell expansion required cell-to-cell contact and Notch3 signaling, which was mediated selectively through the Notch ligand Jag2 expressed by the multipotent hematopoietic progenitor-cell subset [Kared et al. 2006]. Thus, Notch3 signaling may be involved in the development of asthma through regulating the generation and the expansion of Treg cells. Besides Treg cells, the overexpression of Notch3-ICD in activated CD4+T cells also promoted Th1, which is associated with the enhanced expression of T-bet [Maekawa et al. 2003] (Table 2). This is further confirmed that the enhanced Notch3 level is beneficial to the development of asthma and that the manipulation of this pathway may be particularly effective in the treatment of asthma.

**The role of Notch1/Notch3 in pulmonary fibrosis**

**Notch1.** Pulmonary fibrosis is characterized by epithelial-cell dysfunctions, the accumulation of fibroblasts and myofibroblasts and the relentless deposition of an extracellular matrix [Loomis-King et al. 2013]. The differentiation of fibroblasts into α-smooth-muscle actin-(α-SMA) expressing myofibroblasts represents a critical step in the pathogenesis of idiopathic pulmonary fibrosis [Garrison et al. 2013]. The overexpression of Notch has been shown to facilitate the myofibroblast differentiation from lung fibroblasts [Liu et al. 2009], suggesting a potential role of Notch1 in pulmonary fibrosis. Epithelial–mesenchymal transition (EMT), a process during which epithelial cells are converted to mesenchymal cells (such as myofibroblasts), is considered to contribute to pulmonary fibrosis [Chapman, 2011]. It was recently reported that the activation of Notch1 signaling induced EMT, whereas Notch1 silencing reversed the EMT process both in vitro and in vivo [Namba et al. 2010; Shao et al. 2015] (Table 3). These results support implication that Notch1 plays an active role in the pathogenesis of pulmonary fibrosis.

**Notch3.** A possible role of Notch3 in myofibroblast differentiation was postulated in studies in which the knockdown of Notch3 using small interfering RNA (siRNA) effectively reduced the expression of SMA [Chen et al. 2012b]. Along this line of evidence, it has been demonstrated that the transforming growth factor β (TGF-β)-induced differentiation of C2C12 cells into myofibroblasts was enhanced by Notch3 [Ono et al. 2007]. In vivo, myofibroblast differentiation was impaired in Notch2-/-/Notch3-/- compound-mutant embryos but not in single mutants, suggesting that these receptors function redundantly to induce myofibroblast differentiation [Xu et al. 2010]. Similar to Notch1, the role of Notch3 in myofibroblast differentiation is also controversial. Kennard and colleagues showed us that the Notch3 overexpression blocked the TGF-β-induced differentiation of 10T1/2 fibroblasts into myofibroblasts (Table 3) [Kennard et al. 2008]. These findings suggest that the effect of Notch on myofibroblast differentiation can be either stimulatory or inhibitory, depending on the cell of
origin, the inducer and the specific Notch receptor involved [Xu et al. 2010].

The role of Notch3 in pulmonary arterial hypertension

PAH is a disease that affects small pulmonary arteries. The proliferation of smooth-muscle cells in the small peripheral pulmonary arteries is a common characteristic in all forms of PAH [Montani et al. 2013]. Notch3 is expressed only in arterial smooth-muscle cells in human vasculature [Thistlethwaite et al. 2010]. Several studies have shown that Notch3 was involved in vascular smooth-muscle-cell differentiation and proliferation [Xia et al. 2012; Campos et al. 2002] and that Notch3 knockout mice displayed vascular smooth-muscle defects associated with postnatal maturation and arterial specification [Domenga et al. 2004]. Thus, it is not difficult to understand that Notch3 plays a role in the development of PAH. Recently, Notch3 has been studied in PAH in humans, as well as in rodents, by Li and colleagues [Li et al. 2009]. They found that elevated levels of Notch3 expression were found in the lung tissues of the PAH group compared with the control group and the severity of the disease was correlated with the amount of Notch3 protein in the lung. Mice with homozygous deletion of Notch3 did not develop PAH in response to hypoxic stimulation, and PAH could be successfully treated in mice by the administration of GSI. In addition, when exposed to chronic hypobaric hypoxia, C-C chemokine ligand type-2 receptor(CCR2)-deficient mice display a more severe PAH phenotype than wild-type mice with an increased expression of Notch3, implying that the absence of CCR2 results in spontaneous PAH, most likely via the dysregulation of Notch3 signaling [Yu et al. 2013] (Table 4). Above all, the inhibition of Notch3 signaling might be a novel strategy in the intervention of pulmonary hypertension.

The role of Notch1/Notch3 in lung cancer

Notch1. Lung cancer, a major killer cancer that accounts for millions of deaths every year worldwide, is a heterogeneous disease group, divided into two major categories: non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC) [Agalioti et al. 2014]. NSCLC makes up approximately 85% of lung cancers. SCLC comprises small parts of the total of lung cancer cases [Zhou, 2014]. Recently, there has been an increasing interest in Notch in lung cancer research. Abnormalities in the Notch-signaling system are considered to play a role in the tumorigenesis of bronchiogenic carcinoma [Zhou et al. 2013]. A recent meta-analysis conducted by Yuan and
colleagues indicated that Notch signaling is a valuable biomarker for predicting the progression of NSCLC, and that the higher expression of Notch signaling (mainly Notch1 and Notch3) was associated with a greater possibility of lymph node metastasis, higher tumor-node metastasis (TNM) stages and poor survival of NSCLC patients [Yuan et al. 2015]. However, Notch1 function in lung cancer exhibits properties suggesting both tumor promotion and inhibition depending on the tumor cell type and the survival environment. For example, Notch1 is suspected to have a growth-promoting function on NSCLC, but it plays a tumor-suppressive role in SCLC [Eliasz et al. 2010; Sriuranpong et al. 2001]. Notch1 was detected to suppress tumor proliferation under normoxia; however, under hypoxia (a condition that more closely reflects tumor physiology), it had a converse role in tumor promotion [Chen et al. 2007].

The expression of Notch1 protein in the lung adenocarcinoma group and squamous cell carcinoma was significantly higher compared with the normal lung group [Zhou et al. 2013]. The high level of Notch1 in NSCLC may be explained by a recent study that the loss of NUMB, an inhibitor of Notch signaling, was detected in about 30% of NSCLC cases, which leads to increased Notch activity [Westhoff et al. 2009]. In the same study, a Notch1-activating mutation was found in about 10% of the clinical NSCLC cases. The expression of activated N1ICD in the pulmonary epithelium of mice induced lung adenomas, which progressed to generate adenocarcinoma when combined with the overexpression of MyC, which also develops lung tumors following a prolonged latency period [Allen et al. 2011]. The activation of Notch1 by ADAM17 and the subsequent regulation of the EGFR expression are required for the tumorigenicity of NSCLC cells [Baumgart et al. 2010]. After Notch1 ablation in vivo, there is a dramatic decrease in tumor initiation and burden in a mouse model of lung adenocarcinoma, demonstrating that Notch1 is implicated in the initiation, proliferation and survival of NSCLC models in preclinical studies [Licciulli et al. 2013]. The overexpression of Notch1 has been shown to inhibit apoptosis in lung adenocarcinoma [Wael et al. 2014]. Inhibiting Notch signaling by GSI could induce apoptosis in lung squamous cell carcinoma cells through a caspase-dependent and caspase-independent manner [Cao et al. 2012]. The downregulation of the Notch pathway was correlated with the upregulation of miR-34a, which can inhibit NSCLC cell proliferation, induce apoptosis and inhibit the invasion in NSCLC [Ji et al. 2012]. Notch signaling not only activates cell proliferation and antagonises apoptosis, but it is also involved in the invasion and metastasis of lung

| Reference       | Specimen Source                                      | Change of Notch1/Notch3 | Biological function                                                                 |
|-----------------|------------------------------------------------------|-------------------------|-------------------------------------------------------------------------------------|
| Xia et al. [2012] | Primary human coronary artery smooth muscle cells   | Activate Notch3 by cocultured with human coronary artery endothelial cells | Induced the expression of α-SMA and calponin                                       |
| Campos et al. [2002] | Vascular smooth muscle cells                       | Forced expression of N3ICD | The growth rate of the cells was retarded during the subconfluent phase and failed to decelerate at postconfluence |
| Domena et al. [2004] | Tissue from Notch3−/− mice                        | Notch3↓                  | vSMC coat was thinner than in wild-type arteries; Arterial myogenic responses are defective; Postnatal maturation stage of vSMC is deficient |
| Li et al. [2009]  | Lung tissue from PAH patient Lung tissue from Notch3−/− mice | Notch3↑ Notch3↓          | The severity of PAH correlated with the amount of Notch3 protein. Notch3 knockout mice were resistant to the development of PAH. |
| Yu et al. [2013]  | Lung tissue from Ccr2−/− mice                      | Notch3↑                  | Displayed a more severe PAH phenotype than wild-type mice                           |

α-SMA, α-smooth muscle actin; N3ICD, Notch3 intracellular domain; vSMC, vascular smooth-muscle cells; PAH, pulmonary arterial hypertension.
cancer. EMT is an important process leading to cancer cell metastasis [Thiery et al. 2009]. The present study investigates the hypothesis that EMT could be induced by Notch activation via the mediating expression of various EMT-related genes, which are associated with cancer cell resistance to therapy and metastasis [Matsuno et al. 2012]. Meanwhile, the inactivation of Notch signaling by a GSI could reverse the EMT process [Xie et al. 2012]. In addition, the silencing of Notch using siRNA resulted in a mesenchymal–epithelial transition, which was associated with the impaired invasion and the anchorage-independent growth of NSCLC [Xie et al. 2013]. Notch1 can also promote the invasion of lung cancer cells by regulating the MMP9 expression. The elevated expression of MMP9 induced by DLL1, an important factor associated with tumor invasion, could be significantly decreased by inhibiting Notch signaling using GSI [Li et al. 2014] (Table 5).

Regarding SCLC, the overexpression of Notch1 resulted in the inhibition of SCLC growth and the suppression of the neuroendocrine (NE) tumor phenotype [Sriuranpong et al. 2001]. Thus, Notch1 signaling is suppressed in SCLC. Inactivating mutations in Notch family genes has been observed in 25% of human SCLC cases [George et al. 2015]. The activation of Notch signaling in a preclinical SCLC mouse model strikingly reduced the tumor number, abrogated the neuroendocrine gene expression and extended the survival of the mutant mice [George et al. 2015], suggesting a tumor-suppressor role of Notch in SCLC. Moreover, Notch1 can affect the invasion and metastasis of SCLC by controlling EMT. The induction of Notch1 in SCLC cells resulted in the suppression of EMT markers and inhibited the expression of gamma-laminin 2-chain alpha, which contributes to cell motility and invasion [Hassan et al. 2014] (Table 5).

Notch1 exerting its biological effect on lung cancer depends on oxygen concentrations. Recently, Notch1 has been reported as markedly upregulated under hypoxic conditions [Chen et al. 2007]. The inhibition of Notch1 signaling, either using a GSI or through Notch1-RNA interference, led to NSCLC cell death, specifically under hypoxia. The reintroduction of active Notch1 rescued the pro-apoptotic effects of GSI. On the other hand, Notch inhibition in normoxic lung adenocarcinoma cells had no effect on lung adenocarcinoma cell survival. These results suggest that the survival of NSCLC cells under hypoxia is highly dependent upon Notch1 signaling. Donnem and colleagues showed that Notch1 is an independent prognostic factor in resected NSCLC through its correlation with the vascular endothelial growth-factor A and that the mutual overexpression could well reflect a higher level of hypoxia in these neoplasms [Donnem et al. 2010]. Moreover, in the subset of NSCLC patients without TP53 mutations, the level of activated Notch1 correlates with poor clinical outcomes [Westhoff et al. 2009]. This may be explained by the observation that Notch1 can suppress p53-mediated NSCLC cells’ apoptosis [Licculli et al. 2013] (Table 5). Notch1 ablation induces p53-dependent apoptosis as a consequence of increased p53 stability. Thus, high Notch1 activation in NSCLC may result in a worse prognosis and treatment resistance. These results suggest a potential role for inhibiting Notch1 activity as a new therapeutic approach for NSCLC.

Immune therapy is already established as a central component of many cancer-treatment regimens, including lung cancer, for its low toxicity [Dougan and Dranoff, 2009]. Given that T cells can recognize specific antigens with their large repertoire of TCRs, it is proven to be an effective and safe adoptive immunotherapy [Wang et al. 2012a]. Notch signaling is confirmed to play a role in the modulation of T-cell differentiation and immune responses. Activated DLL1–Notch signaling can induce robust tumor antigen-specific T-cell effector and memory responses, enhance T-cell infiltration into the tumor, while decreasing Treg differentiation, and dramatically slow tumor growth [Biktasova et al. 2015; Huang et al. 2011]. These results suggest that the stimulation of DLL1–Notch signaling may be a potential therapeutic utility in cancer-treatment settings. An approach to generally inactivate Notch signaling via the inhibition of γ-secretase is currently being evaluated as a possible anticancer strategy for tumors with an acquired Notch gain of function [Egloff and Grandis, 2012]. GSIs have numerous possible targets, but their antineoplastic effects are thought to be mostly due to Notch inhibition, primarily Notch1, observed in several studies [Nguyen et al. 2015]. GSI administration after radiation significantly improved the radiation resistance induced by Notch activity in Notch-expressing lung cancer [Mizugaki et al. 2012] (Table 5). However, there is still a lack of studies about GSI in the treatment of NSCLC. It follows that a broad number...
Table 5. Involvement of Notch1/Notch3 in lung cancer.

| Notch receptor | Reference | Specimen Source | Change of Notch1/Notch3 | Biological function |
|---------------|-----------|----------------|-------------------------|---------------------|
| Notch1        | Westhoff et al. [2009] | Cancerous tissue from NSCLC patient | Notch1↑ | Involved in the pathogenesis of NSCLC and correlated with poor clinical outcomes in the NSCLC patients without TP53 mutations |
|               | Allen et al. [2011] | Lung tissue from transgenic mice | Forced expression of N1ICD | Induced lung adenomas and generated adenocarcinoma when combined with overexpression of MyC |
|               | Baumgart et al. [2010] | NCI-H520, NCI-H292, NCI-H358, NCI-1650, NCI-1975 and NCI-2170 cells | Activated Notch1 by ADAM17 | Involved in the pathogenesis of NSCLC |
|               | Licciulli et al. [2013] | Lung tissue from Notch1fllox/fllox mice and A549, H460, H522, H441, H727 cells | Notch1↓ in vivo; Inhibit Notch1 with siRNA in vitro | Notch1 downregulation inhibited the initiation, proliferation and survival of NSCLC and induced p53-dependent apoptosis |
|               | Xie et al. [2014] | H69, H69AR, H1668, A549, H2170 and SBC-3 | Inhibit Notch1 with shRNA or forced expression of N1ICD | Notch1 has an inhibitory effect on cell growth and NE differentiation in SCLC, and has a tumor inhibitory effect on ADC cells, but not SCC cells. |
|               | Cao et al. [2012] | Cancerous tissue from NSCLC patient and human lung SCC cell line | Notch1↑ in vivo; Inhibit Notch1 with GSI in vitro | Notch 1, 2 are positively correlated with lymph node metastasis; Notch1 inhibition induced cell apoptosis |
|               | Ji et al. [2012] | A549 and H1650 cells | Inhibit Notch1 by miR-34a | Inhibit NSCLC cell proliferation, induce apoptosis and inhibit invasion |
|               | Xie et al. [2012] | PC9 cells and PC9/AB2 cells | Inhibit Notch1 with siRNA or forced expression of N1ICD | Notch1 activation promoted EMT in PC9 cells; Notch1 inhibition reversed EMT and restored sensitivity to gefitinib in PC9/AB2 cells. |
|               | Xie et al. [2013] | PC9, NCI-H1650, and gefitinib-acquired resistant PC9/AB2 and NCI-H1650 cell lines | Forced expression of N1ICD; Inhibit Notch1 with siRNA or GSI | Notch1 activation promoted EMT in gefitinib-acquired resistant PC9/AB2 and NCI-H1650 cell lines; Notch1 inhibition resulted MET and restored sensitivity to gefitinib in PC9/AB2 and NCI-H1650 cell lines. |
|               | Li et al. [2014] | H520, H1299 and A549 cell lines | Activated Notch1 by delta-like 1 homolog | Involved in the invasion of lung cancer |
|               | Sriuranpong et al. [2001] | DMS53 and NCI-H209 cells | Forced expression of N1ICD | Inhibited SCLC cell growth and hASH1 expression |
|               | George et al. [2015] | Cancerous tissue from SCLC patient and preclinical SCLC mouse | Forced expression of N1ICD | Inactivating mutations in Notch family genes has been observed in 25% of human SCLC; Notch1 activation inhibited the proliferation of SCLC tumours and the expression of neuroendocrine gene. |
|               | Hassan et al. [2014] | H69AR, SBC3, H69 and H1688 cells | Inhibit Notch1 with siRNA or forced expression of N1ICD | Notch1 activation inhibited EMT and invasion of SCLC. |
|               | Chen et al. [2007] | A549 and H1755 cells | Activated Notch1 by hypoxia | Involved in the pathogenesis of lung adenocarcinoma |
|               | Donnem et al. [2010] | Cancerous tissue from NSCLC patient | Notch1↑ | Notch-1 expression was independently associated with poor prognosis in adenocarcinomas; Coexpression of Notch-1 and VEGF-A indicated a particularly poor prognosis in NSCLC. |
|               | Biktasova et al. [2015] | H157, H460, HCC15, HCC1437, HCC1264, HCC2469, Lewis lung carcinoma cells and D459 cells | Activated Notch by Dll1 | Increased T-cell infiltration into tumors, elevated tumor antigen-specific T-cell effector and memory responses, decreased the number of regulatory T cells and limited tumor vascularization |
|               | Huang et al. [2011] | Lewis lung carcinoma cells and D459 cells | Activated Notch by Dll1 | Augmented T cell function and dramatically slowed tumor growth |
|               | Mizugaki et al. [2012] | HCC2429, H460, A549 and H1395 cells | Inhibit Notch1 with GSI | Inhibited tumor growth, induced cell apoptosis and prevented Notch-induced radiation resistance |

[Continued]
of drugs with sufficient specificity and affinity for the inhibition of Notch receptor could be discovered for lung-cancer therapy.

**Notch3.** In lung cancers, Notch signaling was originally implicated in an epithelial tumor by the discovery of chromosome 19 translocation causing a massive overexpression of Notch3 [Dang et al. 2000]. Notch3 had a stronger positive degree of expression in NSCLC compared with the corresponding nontumor tissue [Zhou et al. 2013]. It has been shown that Notch3 is overexpressed in 39% of resected NSCLC cases [Haruki et al. 2005]. In a genetically engineered murine model of NSCLC, tumor cells with an induced expression of Notch3 had an increased tumorigenicity [Zheng et al. 2013]. Manic Fringe plays a role in tumor suppression in the context of lung cancer [Yi et al. 2013]. The reintroduction of Manic Fringe in lung cancer cells can decrease Notch3 protein stability and reduce cell proliferation and tumor growth. Cancer stem cells have been identified in a number of solid tumors, including breast cancer, brain tumors, lung cancer, colon cancer and melanoma [Dawood et al. 2014]. ALDH activity was identified as a marker for NSCLC, non-small cell lung carcinoma; N1ICD, Notch1 intracellular domain; NE, neuroendocrine cell; SCLC, small cell lung carcinoma; ADC, adenocarcinoma; SCC, squamous-cell carcinoma; GSI, γ-secretase inhibitor; EMT, epithelial-mesenchymal transition; VEGF-A, vascular endothelial growth factor-A; Dll1, Delta-like 1; TNM, tumor-node metastasis; MyC, proliferation-related gene; ALDH, aldehyde dehydrogenase; SCLC, small cell lung cancer; EMT, epithelial–mesenchymal transition; OMP, olfactory marker protein; Fc, cell-surface protein.

**Table 5.** (Continued)

| Notch receptor | Reference | Specimen Source | Change of Notch1/Notch3 | Biological function |
|---------------|-----------|-----------------|-------------------------|---------------------|
| Notch3        | Dang et al. [2000] | 44 lung cancer cell lines (including HCC2429) | Notch3↑ | Notch3 overexpression is associated with a translocation involving 19p, and overexpression is frequent in NSCLC |
|               | Zhou et al. [2013] | Cancerous tissue from lung cancer patient | Notch3↑ in NSCLC Notch3↓ in SCLC | Involved in the pathogenesis of bronchogenic carcinoma |
|               | Zheng et al. [2013] | CD24+ITGB4+Notch hi cells from KrasG12D, Trp53R172H, eYFP mice and primary human NSCLC cells isolated from patient samples | Inhibited Notch3 with shRNA | Attenuate self-renewal and tumor propagation in NSCLC cell lines and primary patient tumors. |
|               | Yi et al. [2013] | HCC2429, H460 cells and lung tissue from tumor xenograft model | Inhibited Notch3 by Manic fringe | Inhibited lung cancer cell proliferation and tumorigenesis |
|               | Sullivan et al. [2010] | 45 NSCLC lines, 7 SCLC lines and cancerous tissue from lung cancer patient | Inhibited Notch3 with shRNA or GSI | Reduced ALDH+ lung cancer cells, commensurate with a reduction in tumor-cell proliferation and clonogenicity. |
|               | Konishi et al. [2007] | HCC2429, HCC461, HCC193, HCC95, HCC15, HCC827, HCC44 and HCC78 cells | Inhibited Notch3 with GSI | Reduced tumor cell proliferation, inhibited serum independence, and induced apoptosis |
|               | Haruki et al. [2005] | Cancerous tissue from lung cancer patient and HCC2429, H460, BEAS-2B cells | Notch3↑ in vivo; Inhibited Notch3 by dominant-negative receptor in vitro | Notch3 is overexpressed in 39% of resected NSCLCs; Notch3 inhibition dramatically reduced soft agar colony formation, increased apoptosis, and increased the tumor’s dependency on exogenous growth factors |
|               | Shi et al. [2014] | Cancerous tissue from NSCLC patient and H292, A549, Calu-3 cells | Notch3↑ in vivo; Inhibited Notch3 with siRNA in vitro | Patients with high Notch3 expression had a poorer prognosis; Notch3 inhibition dramatically suppressed the proliferation, migration, invasiveness abilities and prompted apoptosis in NSCLC cells |
|               | Ye et al. [2013] | Cancerous tissue from NSCLC patient | Notch3↑ | Notch3 overexpression was significantly correlated with TNM stage, lymph node metastasis and shorter overall survival |
|               | Yen et al. [2015] | Small cell lung xenograft tumors from mice | Inhibited Notch2/3 by OMP-59R5 | Reduced cancer stem cells frequency |
|               | Lin et al. [2010] | HCC2429 cells | Inhibited Notch2/3 by Notch3 recombinant Fc-fusion proteins | Induced apoptosis and suppressed tumor growth |
Notch1/3 genes. The role of Notch1/3 in lung lesions in congenital diseases

Lung development occurs in the embryonic period and can be regulated by various molecules and signaling pathways. If there is something wrong in these molecules and signaling pathways, the lung development may be affected, and the survivors may suffer from additional morbidities of lung diseases. Mutations in Notch-signaling pathway members cause developmental phenotypes that affect the development of many organ systems. Here, we briefly review some lung lesions seen in congenital diseases related to mutant Notch1/3 genes.

**Notch1.** Adams–Oliver syndrome (AOS) is a rare syndrome characterized by aplasia cutis congenita of the scalp and terminal transverse-limb defects. Pulmonary vascular abnormalities have been described in AOS, including PAH, pulmonary vein stenosis, hypoplastic pulmonary arteries and pulmonary arterio-venous malformation [Lehman et al. 2016]. Recently, Stittrich and colleagues found mutations of the NOTCH1 gene in a proportion of an AOS cohort [Stittrich et al. 2014]. Southgate and colleagues also identified loss of function or haploinsufficiency of NOTCH1 as the primary cause of AOS and an important genetic factor in AOS with associated cardiovascular complications [Southgate et al. 2015]. Mutant NOTCH1 expression was associated with the downregulation of the Notch target genes Hey1 and Hes1, indicating that NOTCH1-related AOS arises through dysregulation of the Notch-signaling pathway.

**Notch3.** Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a dominantly inherited small-artery disease that leads to dementia and disability in midlife. CADASIL is caused by mutations in the extracellular domain of NOTCH3, resulting from the gain or loss of cysteine residues in the EGFR-like repeats [Penton et al. 2012]. Recently, it has been reported that
multiple neoplastic lesions were observed in a 62-year-old man who was diagnosed with CADA-SIL. In the lungs of the patient, carcinoid tumorlet and foci of NE cell proliferation were seen, which were related to the activation of Notch3 [Hassan et al. 2015]. This case displays a striking correlation between Notch3 and pulmonary NE neoplasms, highlighting the importance of Notch3 signaling in multiple developmental processes of CADA-SIL, but the exact mechanism needs to be studied further.

Conclusion
The present investigation indicates that Notch signaling has clearly emerged as a critical pathway in diverse lung disorders. It has been plainly appreciated that aberrant Notch signaling, especially Notch1 and Notch3, contributes to the pathophysiology of human pulmonary disease, such as COPD, asthma, pulmonary fibrosis, PAH, lung cancer and lung lesions in some congenital diseases. In different pulmonary diseases, the change of Notch signaling is inconsistent. For instance, Notch was reported to be downregulated in COPD and SCLC, whereas it was increased in other lung diseases. Moreover, Notch signaling may exert completely opposite effects on lung cancer for the promotion or inhibition, depending on the cell type. Notch signaling was activated in NSCLC, which was closely related to the initiation and survival of the tumor. Conversely, Notch signaling is suppressed in SCLC, and the overexpression of Notch signaling resulted in the inhibition of SCLC growth. Notch signaling could be targeted for the treatment of selected pulmonary malignancies, but the results from the necessary clinical trials to establish the safety and efficacy of this approach are lacking. A number of interventions are already proceeding, and in the years to come, Notch will undoubtedly be an important tool for understanding and treating many pulmonary diseases. Thus, a better understanding of Notch signaling in the lung is likely to be important and provide information central to new treatment approaches.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Natural Science Foundation of China (nos.: 81170036, 81370143).

Conflict of interest statement
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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