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Notch in fibrosis and as a target of anti-fibrotic therapy

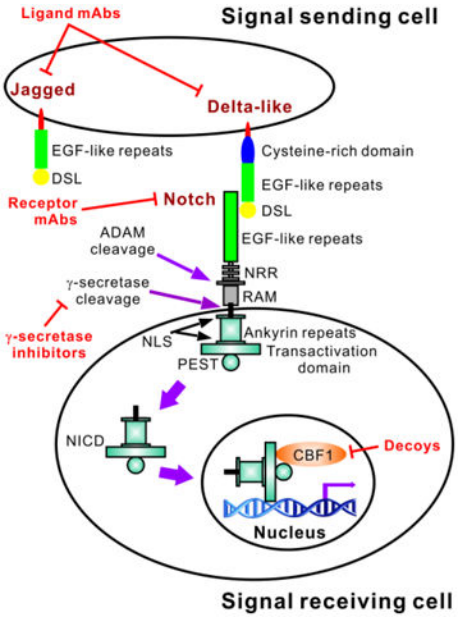
Biao Hu[*] and **Sem H. Phan**

Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan, 48109

Abstract

The Notch pathway represents a highly conserved signaling network with essential roles in regulation of key cellular processes and functions, many of which are critical for development. Accumulating evidence indicates that it is also essential for fibrosis and thus the pathogenesis of chronic fibroproliferative diseases in diverse organs and tissues. Different effects of Notch activation are observed depending on cellular and tissue context as well as in both physiologic and pathologic states. Close interactions of Notch signaling pathway with other signaling pathways have been identified. In this review, current knowledge on the role of the Notch signaling with special focus on fibrosis and its potential as a therapeutic target is summarized.

Graphical abstract



[*]To whom correspondence should be addressed: Dr. Biao Hu, Department of Pathology, University of Michigan Medical School, 109 Zina Pitcher place, Ann Arbor, MI 48109-2200, USA. Phone: 734-763-5731, Fax: 734-615-2331, biaohu@med.umich.edu.

Keywords

Notch; fibrosis; myofibroblast

Introduction

Fibrosis is characterized by excessive deposition of connective tissue often in conjunction with a reparative or reactive process [1]. Fibroblast proliferation, emergence of myofibroblasts, ECM deposition and tissue remodeling are additional key features [1]. Chronic progressive fibrosis can occur in virtually all organs including the lung[2], kidney[3], liver[4], skin[5] and heart[6]. It is commonly a result of excessive, prolonged or repeated injury with associated chronic inflammation [1-6]. Extensive research have uncovered complex mechanisms underlying fibrosis, which involve multiple cell types, factors, signaling pathways and genes[1-6]. In particular and germane to this review is recent mounting evidence from both animal model and human studies implicating the Notch signaling pathway in the pathogenesis of fibrosis [7-19].

Notch signaling

The Notch signaling network is an evolutionarily conserved intercellular signaling pathway that regulates interactions between physically adjacent cells [20]. Five ligands, namely Delta-like 1, Delta-like 3, Delta-like 4, Jagged-1 and Jagged-2 [21], were identified for the four notch receptor members Notch 1, Notch2, Notch3 and Notch4 in mammals[20-23]. The Notch receptors are single transmembrane polypeptides synthesized in the endoplasmic reticulum and transported to the cell surface through the trans-Golgi network [23]. They share structural elements containing an extracellular domain with multiple epidermal growth factor-like (EGF) repeats, transmembrane domain, and an intracellular domain with multiple subdomains[20-23]. The Notch proteins are cleaved in the trans-Golgi network, and presented on the cell surface as a heterodimer[20-23].

Binding of ligands from the surface of neighboring cells to the receptor on the adjacent cell induces the conformational change of Notch, leading to the exposure of S2 site and triggers sequentially proteolytic cleavage by A Disintegrin and Metalloprotease (ADAM) and the γ secretase complex[20-24]. Cleavage by ADAM produces a substrate for the second cleavage by the presenilin-containing γ-secretase complex, releasing the Notch intracellular domain (NICD) [23, 24]. The cleaved NICD is then translocated to the nucleus where it binds with the transcription factor CBF1/Suppressor of hairless/Lag1 (CSL) and modulates gene expression [23, 24]. Without NICD, CBF1 (also known as RBPJ) protein binds to the consensus DNA sequence in association with SMART/HDACs complex, acting as a transcriptional repressor [25, 26]. Interaction between NICD and CBF1 displaces the SMART/HDACs corepressor complex, which is replaced with a co-activator complex (MAML1-3, EP300 and SNW1). This results in the transcriptional activation of the target genes primarily involving two families of helix-loop-helix transcription factors Hes (Hairy enhance of split) and Hey (Hairy/enhancer of spit related with YRPW motif) [25]. In addition to this canonical signaling pathway, non-canonical Notch signaling independent of

either CBF1 or γ-secretase cleavage, or both have been identified [20-22, 25]. Post-translational modifications including O-fucosylation and O-glycosylation via fringe proteins (lunatic, radical, and manic) regulate the specificity of Notch receptor-ligand binding, and are also critical for its function [27].

Termination of Notch signaling in the cell can occur naturally at or downstream of the Notch receptor[28-31]. The Notch receptor can undergo lysosomal degradation involving the ubiquitin ligase Itch/AIP4 or Nedd4, which act together with Numb [30] and Itch/AIP4 [28-30]. GSK3 controls NICD1 ubiquitination and proteasome-mediated degradation by phosphorylation of the NICD and regulates the NICD interaction with the E3 ubiquitin ligase CDC4/FBW7 [32, 33].

Notch and myofibroblast differentiation

Myofibroblasts are cells with phenotype between fibroblasts and smooth muscle cells [34, 35]. They express α-smooth muscle actin (ACTA2) and other general mesenchymal markers such as vimentin, and arise de novo in response to tissue injury [34, 35]. Myofibroblasts are the major extracellular matrix producing cell[34, 35]. They are enriched in injured tissue undergoing repair/remodeling and are thought to promote repair by contracting the edges of the wound[34, 35]. Additionally, myofibroblasts produce matrix to facilitate the the repair process [1, 34-36]. If they do not undergo apoptosis upon successful repair[37], excessive matrix production by persistent myofibroblasts can result in exuberant scar formation and fibrosis [1, 34, 35]. Thus chronic fibrotic lesions in diverse tissues are characterized by persistence of these myofibroblasts [1-6, 37]. Thus targeting this de novo genesis of the myofibroblast and/or its survival have been considered in therapeutic approaches for controlling chronic progressive fibrotic diseases.

Myofibroblasts have multiple origins based on their organ or tissue locations, and include resident fibroblasts, activated stellate cells, stromal tissue/mesenchymal progenitor/stem cells, epithelial/endothelial cells undergo epithelial/endothelial to mesenchymal transition (EMT/EndMT), as well as circulating mesenchymal precursors, fibrocytes, etc [34, 35, 37]. TGFβ and other fibrogenic cytokines/factors are known inducers of myofibroblast differentiation from these diverse precursors [38-40]. Recent evidence further indicates that the Notch signaling pathway is also involved in the regulation of myofibroblast differentiation in chronic fibrosis including in the lung [7, 15, 18, 41], kidney [42-46], liver [47-51], heart [12, 14] and skin [16, 52].

Notch and epithelial-mesenchymal transition

Epithelial-mesenchymal transition (EMT), as well as a similar transition occurring in vascular endothelial cells referred to as endothelial-mesenchymal transition (EndMT), are associated with the induction of transcription factors causing alterations in expression of genes that are involved in regulation of cell-cell adhesion, cytoskeletal dynamics[53]. These changes reflect the transition from epithelial/endothelial morphology and physiology to the mesenchymal phenotype [53]. In the case of epithelial cells there is gradual loss of E-cadherin expression and apical-basal polarity accompanied by reorganization of their

cytoskeleton to acquire a motile phenotype and eventual acquisition of the myofibroblast phenotype characterized by expression of ACTA2 [53]. While EMT has been well studied in embryonic development, it is suspected also to play some role in the genesis of new fibroblasts during the development of organ fibrosis in adult tissues[53, 54]. Indeed, in mature tissues, epithelium can undergo EMT following epithelial stress such as inflammation or wounding, leading to fibroblast proliferation and fibrogenesis [53, 54]. Transforming growth factor-β (TGF-β), one of the major profibrotic cytokines, induces EMT in vitro and has been associated with EMT in vivo[54]. However it remains unclear as to the level of contribution of EMT or EndMT to the overall fibroblast/myofibroblast population in tissues undergoing fibrosis relative to that from other cellular sources.

The role of Notch signaling in the regulation of EMT is suggested by indirect and direct studies. Thus Notch signaling molecules are reported to activate TGFβ in rat mesangial cells under hyperglycemic conditions [55]. Given the role of TGFβ in promotion of EMT, the potential significance of Notch signaling in this process is suggested [53, 55]. Since EMT is associated with chronic fibrosis in the kidney [54], lung [56-59], liver [49, 60] and heart[61-63] evidence for Notch signaling in EMT focused on epithelial cells derived these tissues. For example, a lung-related study used the rat alveolar epithelial cell line, RLE-6TN, to document Notch involvement[15]. In that study activation of Notch, either by ectopic expression of the NICD or by co-culture with Jagged1 expression cells, induces the expression of mesenchymal marker genes including ACTA2, collagen I and vimentin with concomitant reduction in the expression of epithelial marker genes such as E-cadherin, occludin, and zonula occludens-1[15]. In addition to these direct effects mediated by its intracellular domain, Notch can indirectly regulate EMT through other signaling pathways, including TGFβ [15],NF-κB [64] and β-catenin [65], and through the action of various regulatory miRNAs [66-70]. These mechanisms implicate Notch signaling in potential regulation of fibrosis by their impact on genesis of the fibroblast/myofibroblast. Thus therapeutic targeting of this pathway may represent a feasible approach for control of fibrosis in diverse tissues and chronic progressive fibroproliferative diseases.

Notch and Pulmonary Fibrosis

Notch signaling is required for lung development, and Notch receptors and ligands are present in both epithelial and mesenchymal compartments of the developing lung[71]. It is essential for cell differention and mobilization during lung alveogenesis [72], and in the mesenchyme, RBPJ is critical for recruitment and specification of arterial vascular smooth muscle cells, mesothelial epithelial-mesenchymal transition, and selection of Clara versus ciliated cell fate [73]. NOTCH1 is also essential for regeneration of Clara cells during repair of airway injury [74].

In the adult lung, activation of the Notch pathway is reported in patients with chronic obstructive pulmonary disease (COPD)[75], idiopathic pulmonary arterial hypertension [76] and idiopathic pulmonary fibrosis (IPF) [15]. Animal model studies confirmed activation of Notch signaling in pulmonary fibrosis, consistent with similar reports in lung specimens from patients with idiopathic interstitial pneumonias or IPF [7, 15, 18]. The majority of known Notch-related genes are expressed in human small airway epithelium [75] with

evidence of elevated Notch expression in lung myofibroblasts in the bleomycininduced model of pulmonary fibrosis and in lung specimens from patients with idiopathic interstitial pneumonias [15]. Mucus cells from patients with chronic obstructive pulmonary disease, idiopathic pulmonary artery hypertension or IPF express the Notch downstream transcription factor, HES1 [77]. Aberrantly sustained Notch activity in injured lungs results in an alveolar cyst architecture that is indicative of a fibrotic phenotype [78]. Moreover differential expression of miRNAs targeting the Notch signaling pathway is observed in rapidly or slowly progressive IPF patients compared to healthy controls, further implicating a role for Notch signaling in IPF pathogenesis [79]. Support is provided by evidence that Notch1 can upregulate type I collagen promoter activity through a Hes1-dependent mechanism [80]. Additionally in the bleomycin model, Jagged1/Notch-signaling is upregulated and found to be essential for myofibroblast differentiation induced by FIZZ-1, a profibrotic factor expressed mainly in IL-4/IL-13 activated alveolar epithelial cells [18]. Impairment of Notch signaling due to impaired fucosylation in FXKO mice inhibits myofibroblast differentiation and bleomycin induced fibrosis [18]. Additionally mesenchymal-specific conditional Notch1 deficient mice exhibited significant reduction of pulmonary fibrosis in the same bleomycin model [81].

In addition to the regulation of myofibroblast differentiation from fibroblasts in the mesenchymal compartment, Notch also regulates EMT in the lung [15]. The pharmacologic inhibition of Notch signaling significantly inhibits TGF-β-induced expression of ACTA2. Furthermore Notch induces the expression of TGF-β family members as well as the phosphorylation of Smad3, an important mediator for TGFβ signal [15]. A771726, an active metabolite of leflunomide known to induce ILD stimulates the expression of Jagged-1, 2, Dll-1 and Notch-1, 3, 4 mRNAs in a dose-dependent manner accompanied by increased NICD in the nuclear extract. This effect was diminished by N-[N-(3,5-difluorophenacetyl-L-alanyl)]-Sphenylglycine t-butyl ester (DAPT), an inhibitor of γ-secretase [82].

In addition to direct regulation of myofibroblast differentiation and EMT, Notch can also interact with other signal pathways to regulate pulmonary fibrosis. In animal model studies lung injury is accompanied with suppression of CXCR7 expression and recruitment of vascular endothelial growth factor receptor 1 (VEGFR1) expressing perivascular macrophages [83]. This recruitment stimulates Wnt/β-catenin-dependent increase of Jagged1 expression in pulmonary capillary endothelial cells, which in turn stimulates Notch signaling in fibroblasts and enhances fibrosis [83]. Treatment with a CXCR7 agonist or pulmonary capillary endothelial cell targeted Jag1 shRNA after lung injury promotes alveolar repair and reduces fibrosis [83]. Notch signaling can also stimulate IGF1R expression to induce AKT phosphorylation and cooperate with HIF1α to promote pulmonary fibrosis [84].

Chronic Kidney Disease

Renal fibrosis is characterized by the increased deposition of collagen and extracellular matrix, de novo genesis of myofibroblasts, migration of leukocytes, dysfunctions of epithelial cells and loss of capillaries [85]. Both Notch1 and Notch2 are expressed during kidney development [86, 87]. But unlike in the lung, Notch activity is not detectable in

mature human and rodent kidneys [88]. It appears that in various renal disorders in humans and in animal models Notch signaling is reactivated. In patients with diverse fibrotic kidney diseases, cleaved Notch1, Notch2, and Jagged1 are expressed on podocytes and their level of expression correlates with the amount of proteinuria across all disease groups [89]. Notch3 receptor activation drives inflammation and fibrosis following tubulointerstitial kidney injury [90]. The degree of glomerulosclerosis correlated with podocyte expression of cleaved Notch1, while the severity of tubulointerstitial fibrosis and the estimated glomerular filtration rate correlated with expression of cleaved Notch1 in the tubulointerstitium [89]. Renal tubular Notch signaling triggers a prosenescent state after acute kidney injury [91]. Mutation of NOTCH3 is related to the kidney fibrosis found in patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a disease mainly characterized by nonspecific neointimal fibrosis and hyalinosis of the arterial wall [92]. Increased Jag1/Notch1/HeyL expression in a folic acid–induced kidney injury model and in kidneys of tubulointerstitial fibrosis/chronic kidney disease patients have been reported [45]. Epithelial Notch signaling regulates interstitial fibrosis development in the kidneys of mice and humans [45].

In vivo experiments with genetically modified mice and inhibitors of Notch signaling confirm the role of Notch signal in kidney fibrosis. Genetic deletion of the Notch pathway in tubular epithelial cells reduced renal fibrosis [45]. Notch3 deficient mice exhibit less proteinuria, uremia, and inflammatory infiltration [93]. Tubular epithelial cell-specific expression of active Notch1 caused rapid development of tubulo-interstitial fibrosis [45]. Transgenic mice expressing the active Notch1 in developing podocytes showed severe proteinuria and progressive glomerulosclerosis at 2 weeks after birth [94]. Over-expression of NICD in proximal tubules results in aggravated tubular damage and a fibrotic phenotype [94]. In contrast, pharmacologic inhibition of γ-secretase using DAPT reduces tubulointerstitial fibrosis and peritoneal dialysis fluid-induced peritoneal fibrosis in rats [95].

In vitro, activation of Notch signaling with Delta-like 4 caused prosenescent changes in tubular cells while inhibition with DAPT attenuated these changes. Activation of the Notch3 receptor in the glomeruli induces phenotypic changes in podocytes promoting renal inflammation and fibrosis and leading to disease progression [93]. Cross talk between TGFβ and Notch signaling has also been reported in the kidney. Thus inhibition of γ-secretase ameliorates kidney fibrosis via inhibition of TGFβ/Smad2/3 signaling pathway activation [96]. In addition, TGFβ induces renal epithelial Jagged-1 expression in fibrosis [46], while Notch mediated EMT is associated with increased expression of the Snail transcription factor [17]. Depletion of Notch4 in shear-stimulated proximal tubule epithelial cells attenuates collagen accumulation via effects on TGFβ signaling [97]. The vitamin D analogue, paricalcitol attenuates TGF-β1 induced Smad2 phosphorylation and upregulation of the Notch ligand Jagged-1, ACTA2 and thrombospondin-1 and prevented the TGF-β1-mediated loss of E-Cadherin [98]. Additionally, miRNA regulation of Notch signaling affects EMT in tubular epithelial cells [99].

Notch and Skin fibrosis

Constitutive expression of the NICD is reported in normal keratinocytes of the epidermis, hair follicles, sebaceous gland endothelial cells, and immune cells [100-103]. Notch signaling regulates late-stage epidermal differentiation and maintains postnatal hair cycle homeostasis [101-103]. In fibroproliferative skin diseases, the NICD is highly expressed in fibroblasts of keloids and moderately to highly expressed in hypertrophic scars and dermatofibromas, whereas low or no expression is detected in the fibroblasts of normal skin specimens and morpheas [101]. In hypertrophic scar, more Notch1 and Jagged1 positive keratinocytes were found than in normal skin [100]. The Notch pathway was hyperactivated in skin biopsy samples from patients with scleroderma and animal models[104]. Increased expression of ADAM-17, a proteinase involved in Notch activation, is also overexpressed in these skin samples [104]. Accumulation of the NICD and increased HES-1 transcription are noted in animal models of scleroderma [52, 104].

Inhibition of Notch signaling using the γ-secretase inhibitor DAPT or with Notch-1 antisense strategy reduces fibrosis [16, 52]. In addition to prevention of fibrosis, delayed targeting of Notch signaling is also effective in causing almost complete regression of established experimental fibrosis [52]. In systemic sclerosis, activation of Notch signaling is detected in lesional skin as well as in cultured fibroblasts isolated from the skin samples [16]. This systemic sclerosis dermal fibroblast phenotype resembles that of healthy dermal fibroblasts with Jagged-1 induced Notch activation [16].

Therapeutic targeting of Notch signaling, alone or in combination with other interacting signaling pathways has shown some success in animal model translational studies. In one study targeting multiple morphogen (Notch/Hedgehog/Wnt) pathways is shown to have additive antifibrotic effects with improved tolerability [105]. Their results indicate that inhibition of Hedgehog, Wnt and Notch signaling dose-dependently ameliorated animal models of fibrosis. Combination therapies with low doses of Hedgehog/Wnt inhibitors or Hedgehog/Notch inhibitors demonstrate additive anti-fibrotic effects in preventive as well as in therapeutic regimens. In contrast to high dose monotherapies, combination therapies are well tolerated and may help to overcome dose-limiting toxicity of Hedgehog, Wnt and Notch signalling.

Notch and Liver fibrosis

The first evidence for a role of the Notch signaling pathway in hepatic disease was the finding that mutation of the Notch ligand, Jagged1, result in Alagille syndrome (AGS) which correlated with a variety of pediatric disorders including biliary atresia, congenital hepatic fibrosis, sclerosing cholangitis, cystic fibrosis, fulminant hepatic failure, tyrosinemia, and chronic rejection [106]. Jagged1 deficiency results in absence of reactive ductular cells and accumulation of hepatobiliary cells lacking the biliary-specific transcription factor, hepatic nuclear factor 1β, which prevents the switch to a biliary phenotype [107]. In contrast to undetectable expression of Jagged-2, Delta like-1, and Delta like-3, Jagged-1 and Delta like-4 are expressed in normal and diseased liver tissue with primary biliary cirrhosis, primary sclerosing cholangitis, or alcoholic liver disease [108]. Jagged-1 expression is

significantly up-regulated in diseased liver tissue [108]. In primary liver cell isolates, Jagged-1 is expressed in all cell types, while Delta like-4 expression is localized to biliary epithelial and liver endothelial cells, but not in hepatocytes [108]. Genomic analysis of differentially expressed genes in liver and biliary epithelial cells reveals overexpression of Notch transcripts in patients with primary biliary cirrhosis liver [109] or hepatocellular carcinoma[110, 111]. Notch3 and Notch4 are not expressed in normal liver [50, 112] but Notch4 is expressed by hepatocytes at the edge of regenerative nodules and in cell planes adjacent to fibrous septa [112], While Notch3 is significantly up-regulated in fibrotic liver tissues from patients with chronic active hepatitis [50]. Expression of Notch1 and Hes1 is reduced in activated hepatic stellate cells (HSCs) [9] but hepatitis B virus (HBV) infection drives increased Notch1 expression in intrahepatic T cells in cirrhosis [113]. Notch 2, Notch 3, Hey2 and HeyL expression increases significantly during activation of quiescent HSCs to myofibroblastic HSCs [49]. High activation of Notch signaling is also observed in hepatic progenitor cells isolated from tissue with primary biliary cirrhosis [114]. Moreover the numbers of Notch1, Notch3, and Notch4 positive cells are significantly increased in fibrotic areas in the CCl_4 injured rats [115]. Notch signaling is also highly activated in a rat model of liver fibrosis induced by CCl_4 [116] while TGFβ treated hepatic stellate cell line (HSC-T6) [116] exhibited elevated expression of Jagged1, Notch3, and Hes1 [116]. In the ethanol-induced zebrafish fibrosis model, a subset of Notch-responsive HPCs retains its capacity to regenerate as hepatocytes is identified [117]. More direct evidence documenting a role for Notch signaling in liver fibrosis is revealed by studies in a variety of mouse models. The $AlbCre^{+/-}$ transgenic mice were crossed with HNF-6 (*HNF-6*$^{flox/flox}$, HNF-6 KO)[118], RBPJ (*RBP*$^{flox/flox}$, RBP KO)[119], or both HNF-6 and RBPJ (DKO) mice to knocked out HNF-6 or RBPJ alone or simultaneously in the bipotential hepatoblast progenitor cell [120]. While fibrosis is not observed when HNF-6 or RBPJ is knocked out individually in the bipotential hepatoblast progenitor cell, simultaneous knockout of both these genes results in cholestasis, hepatic necrosis, and fibrosis [120]. In contrast, myeloid-specific disruption of RBPJ attenuates fibrosis in CCl_4 induced hepatic fibrosis along with reduction in inflammation, HSC activation and expression of inflammatory and profibrotic factors including platelet-derived growth factor (PDGF)-B and TGFβ1 [121].

In vitro studies with primary isolated cells or immortalized cell lines further confirm the role of Notch in myofibroblast differentiation. In HSC-T6 cells, an immortalized rat liver stellate cell line, Notch1 inhibits but Hes1 stimulates the promoter activities of α-SMA, COL1α1 and COL1α2 [9]. Co-culture of bone marrow-derived mesenchymal stem cells with HSCs inhibits HSC proliferation that requires cell-cell contact and is partially mediated by Notch pathway activation [51]. Notch signaling stimulates Sox9b expression, which is required to maintain Notch signaling in intrahepatic biliary cells [122].

Based on the ample evidence implicating Notch signaling in liver fibrosis, a number of approaches targeting this pathway have been attempted to control the fibrotic response and/or enhance regeneration. A number of Wnt and Notch antagonists have been identified to facilitate hepatocyte regeneration in the fibrotic liver by chemical screens [117]. The Wnt agonists attenuate Notch signaling by inducing Numb, a membrane-associated protein that inhibits Notch signaling [117]. On the anti-fibrotic side, blocking Notch signaling activation by a γ-secretase inhibitor, DAPT, significantly attenuates rat liver fibrosis induced by

injection of CCl_4, accompanied by suppression of EMT [116]. Additionally DAPT treatment does not affect hepatocyte proliferation in vivo but affords some protection from hepatocyte apoptosis [116]. Interestingly inhibition of Notch signaling in activated HSCs or myofibroblasts induces a mesenchymal-to-epithelial-like transition[116]. In a rat model of cholestatic liver fibrosis, inhibition of Notch signaling with DAPT is also effective in reducing fibrosis [48]. Treatment with another Notch inhibitor, RO4929097 reduces liver fibrosis partly through its effects on hepatic cell differentiation [123]. Another selective γ-secretase inhibitor, Avagacestat, is found to inhibit TGFβ-induced HSC activation and contractility [124]. Therapeutic use of this inhibitor in vivo significantly attenuates fibrogenesis in the CCl_4-induced mouse liver fibrosis model [124]. Transfection of Notch3 shRNA in a rat CCl_4 induced liver fibrosis model reverses EMT in fibrotic livers by reducing TGFβ1 expression [125]. Consistent with the Notch inhibitor study[48, 116, 123, 124], the inhibition of Notch3 has no effect on hepatocyte proliferation but reduces hepatocyte apoptosis [125]. Inhibiting RBPJ function with a synthetic decoy oligodeoxynucleotide (ODN) in the 3,5-Diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet mouse fibrosis model is also effective in reducing fibrosis [47]. Finally, resveratrol (3,5,4'-trihydroxy-trans-stilbene), a phytoalexin produced naturally by several plants in response to injury or when the plant is under attack by pathogens[126], protects from liver fibrosis by decreasing lipid peroxidation and suppression of Notch1 and Notch3 gene expression [115]. Further studies are necessary to evaluate the therapeutic potential of these agents used in the animal model and in vitro studies.

Conclusion remarks

There is ample evidence for the role of the Notch signaling pathway in regulation of key cellular processes and functions essential for the pathogenesis of fibrosis. However, different effects of Notch activation are observed depending on cellular and tissue context as well as in both physiologic and pathologic states. In addition, Notch interacts closely with other signaling pathways, indicating that combination therapies targeting the other signaling (for example Hedgehog) pathways may be required for effective treatment of diseases with chronic progressive fibrosis. Therefore, investigation of the molecular mechanisms regulating Notch activation in a specific cell context and the complex interplay with additional signaling partners involved is important for a successful therapeutic strategy. Although clinical trials targeting the Notch signaling pathway have not been conducted for treating chronic fibrotic diseases, many have been conducted for treating cancers. The compounds and strategies developed in these cancer studies may be useful and applicable for future studies in developing novel therapies for chronic fibrotic diseases that currently have no effective therapy.

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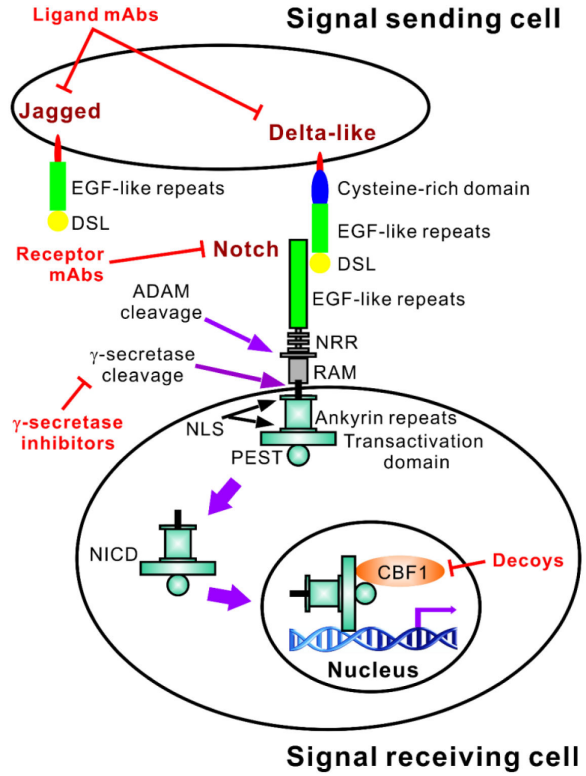

Figure 1.

Brief overview of the Notch pathway with indication of sites for targeted control of the Notch signaling pathway. Notch receptors have an extracellular domain comprised of epidermal growth factor (EGF)-like repeats and three Lin-12 Notch repeats (LNR). Ligand binding triggers sequential receptor cleavages involving ADAM family metalloproteases and the γ-secretase complex, ultimately leading to the cytoplasmic release of the intracellular domain (NICD). This comprises a RAM domain, six ankyrin repeats between two nuclear localization sequences (NLS) and a transactivation domain. A PEST sequence is also present at the C-terminus of all four Notch receptors, with a transactivation domain present in Notch1 and 2. After cytoplasmic release, the NICD translocates (solid purple arrows) into the nucleus where it exerts its transcriptional activity to regulate target genes, such as *ACTA2*. Both Delta-like and Jagged ligands contain Delta-serrate-Lag2 (DSL) domains followed by a variable number of EGF-like repeats (8 for Delta-like1 and 4, and 6 for Delta-like3). An additional cysteine-rich domain is present in Jagged ligands, which also have 18 EGF-like repeats. The sites currently targeted for control of the Notch signal pathway in fibrosis are indicated in red.

Table 1

Current strategies used in control of the Notch pathway in fibrosis. (1) blocking the activation of Notch receptors by γ-secretase inhibitors, (2) targeting the ligand or (3) targeting NICD activity, and (4) inhibiting signal transduction.

Reagent	Organ/Tissue	Species	Reference
(1)Targeting Notch receptor activation*			
DAPT	Liver, Kidney, Skin, Peritoneum	Rat, Mouse and Human	[52, 91, 95, 116, 127, 128]
(2)Targeting ligand*			
Jagged1 shRNA	Lung	Mouse	[83]
(3)Targeting NICD			
Notch1 anti-sense construct	Skin	Mouse	[52]
Notch3 anti-sense oligodeoxynucleotides	Kidney	Mouse	[93]
Notch3 shRNA	Liver	Rat	[125]
mir-148a	Liver	Mouse	[123]
mir-34a	Kidney	Human	[99]
astragaloside	Liver	Rat	[129]
astragalus	Lung	Rat	[130]
(4)Targeting signal transduction			
RBPj decoy oligodeoxynucleotide	Liver	Mouse	[47]
resveratrol	Liver	Rat	[115]
scutellarin	Heart	Rat	[131]
Dibenzazepine	Kidney	Mouse	[96]
olmesartan	Heart	Mouse	[128]

*Targeting the ligand and receptor using monoclonal antibodies has also been used in other studies but not in fibrosis.