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Invited review

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

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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.

Contents

1. 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].

2. 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

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(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].

3. 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 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].

4. 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. 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.

5. Notch and pulmonary fibrosis

Notch signaling is required for lung development. 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-