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Notch, lipids, and endothelial cells

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Abstract

Purpose of review—Notch signaling is an evolutionary conserved pathway critical for cardiovascular development and angiogenesis. More recently, the contribution of Notch signaling to the homeostasis of the adult vasculature has emerged as an important novel paradigm, but much remains to be understood.

Recent findings—Recent findings shed light on the impact of Notch in vascular and immune responses to microenvironmental signals as well as on the onset of atherosclerosis. In the past year, studies in human and mice explored the role of Notch in the maintenance of a nonactivated endothelium. Novel pieces of evidence suggest that this pathway is sensitive to environmental factors, including inflammatory mediators and diet-derived by-products.

Summary—An emerging theme is the ability of Notch to respond to changes in the microenvironment, including glucose and lipid metabolites. In turn, alterations in Notch enable an important link between metabolism and transcriptional changes, thus this receptor appears to function as a metabolic sensor with direct implications to gene expression.

Keywords

atherosclerosis; dietary by-products; endothelium; inflammation; signaling pathway

INTRODUCTION

Endothelial cells provide a selective and highly responsive barrier that offers, under physiological conditions, an optimal ratio between vessel integrity and permeability. The endothelium also prevents thrombosis and regulates the trafficking of cells from the blood to adjacent tissues. Coordination of leukocyte trafficking in particular is of critical importance to inflammation and, in fact, the endothelium is the first line of regulatory control during the inflammatory response. In the absence of disease, endothelial cells maintain a closed, anti-inflammatory status, by preventing binding and extravasation of leukocytes from the

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Conflicts of interest

There are no conflicts of interest.

circulation. In contrast, during the response to inflammatory stimuli, endothelial cells become ‘activated’ by expressing a subset of leukocyte-adhesion molecules and facilitating the exit of leukocytes from the circulation into tissues. During this process, endothelial junctions become weakened promoting leakage of plasma proteins and solutes. Thus, the endothelium provides a critical barrier that regulates the inflammatory response and breakage of its homeostasis is a major determinant of vascular disorders, including hypertension, atherosclerosis, and thrombosis [1].

As a primary barrier between blood and tissue, the endothelium also differentially responds to hemodynamic patterns that define atherosusceptible versus atheroprotected sites of the arterial tree. In fact, laminar shear stress has been found to protect against the disease through the induction in endothelial cells of anti-inflammatory, antioxidant, and antithrombotic genes. In contrast endothelium exposed to disturbed blood flow exhibits biological changes such as increased permeability and chronic low-inflammation, which in the presence of additional risk factors favors the emergence of atherosclerosis lesions [2,3]. The activation of endothelial cells toward a prolonged proinflammatory and atherogenic phenotype could be driven by cell surface receptors in response to chemokines, like tumor necrosis factor (TNF) and IL-1β [4,5], and downstream signaling cascades that are fairly well understood. Importantly, endothelial activation can also be promoted and strengthened by a cohort of lipid mediators, a process that holds special significance to the onset and progression of atherosclerosis. In fact, it is fair to state that atherosclerosis is a lipid-driven, chronic inflammatory disease that develops as a result of lipid accumulation, followed by lipid modifications and subsequent growth of the intra-arterial atherosclerotic plaque. Oxidative modification of lipid products such as LDLs and derived phospholipids have been designated as a critical step in the initiation of atherosclerosis [6,7]. These oxidized lipid by-products trapped in the subendothelial space impact numerous cell types, including immune, smooth muscle, and endothelial cells. It has become clear that a large number of signaling pathways are altered in endothelial cells as the result of exposure to oxidized lipids, leading to endothelial activation, inflammation, and atherosclerosis [6,8]. Less known, however, are the intrinsic pathways essential for the maintenance of arterial endothelium integrity, involved in sensing lipid products, interpreting microenvironment cues, and transducing these readouts into transcriptional changes that counteract the effect of lipid by-products. Here, we are reviewing the recent literature that links deregulation of the Notch pathway to inflammatory processes and atherosclerosis, with a focus on its response to lipid by-products and subsequent pathophysiological impact.

NOTCH SIGNALING PATHWAY

Notch signaling together with the WNT, sonic hedgehog, and bone morphogenetic protein/transforming growth factor β pathways are evolutionary conserved mechanisms involved in the development and homeostasis of most tissues. In mammals, expression of four Notch receptors (Notch1–4) and five canonical ligands [Delta-like ligand (Dll) 1, 3, 4 and Jagged (Jag)1, 2] coordinate activation of this signaling pathway. Canonical transactivation of the pathway occurs after binding of a receptor (signal-receiving cell) with a ligand presented on an adjacent cell (signal-sending cell). Endocytosis of the ligand exerts mechanical forces on the receptor that become accessible to proteases [9,10] enabling successive cleavage of

Notch extracellular domain and intramembrane domain by a-disintegrin and metalloprotease (ADAM) family members [11] and the γ-secretase complex, respectively [12,13]. These events ultimately release Notch intracellular domain (NICD) that is translocated to the nucleus where it interacts with a transcriptional complex, RBP-jκ (recombination signal-binding protein for immunoglobulin κ J region)/CSL (CBF1, suppressor of hairless, Lag-1), and MAML (mastermind-like), to induce target genes. In addition, Notch contributes to the regulation of cellular mechanisms through noncanonical pathways [14] (Fig. 1A).

The outcome of Notch activation is cell type and context dependent with multiple combinations of receptors and ligands that transduce different biological effects [15,16]. In addition, progress made in characterizing structural features of ligands and receptors has allowed us to understand the critical impact of post-translational modifications of the Notch receptors in ligand binding and activation [17,18■■,19■].

The biological relevance of Notch signaling to developmental processes is highlighted by the broad number of anomalies and disorders arising when the pathway is deregulated. This includes immune [20–22], skeletal [23–25], hepatic [26,27], vascular [28] defects and cardiac malformations [26,27,29]. More recently large studies examining genome-wide association study for coronary artery disease identified genetic signals enriched in Notch-related pathways [30,31■■] and polymorphisms near the *HEY2* gene (canonical target of Notch signaling) associated with Brugada syndrome [32].

Although the role of Notch has been extensively studied in the context of development and cancer [14,33,34], recent experiments using *in vitro* assays and mouse models also showed that changes in Notch activity can impact organ homeostasis in adults. Blocking Notch signaling is known to initiate sprouting angiogenesis [35–37], but this postulate should now be refined to include tissue-specific differences [38]. In endothelial cells, Notch signaling protects against apoptosis in a rat allograft model [39,40] and in response to laminar blood flow [41,42]. This review will focus on findings describing the impact of Notch deregulation in the initiation and progression of atherosclerosis.

NOTCH SIGNALING PATHWAY AND INFLAMMATION

Inflammation constitutes a major player in multiple steps of atherosclerosis. Intriguingly, Notch signaling has been shown to contribute to and be modulated by inflammatory signals. Importantly both pro and anti-inflammatory roles have been attributed to the pathway depending on the specific cell types.

In immune cells, including T lymphocytes and macrophages, blockade of Notch signaling often results in the repression of the inflammatory response [43] although differences are noted depending on the heterodimer engaged and the cell type studied [44,45]. Thus, during atherosclerosis, activated macrophages recruited to the vascular wall express the Notch ligand Dll4, which is increased in response to inflammatory stimuli. In particular, Dll4 participates to homotypic activation of Notch signaling and promotes expression of M1-type molecules [46]. Reduction of Notch signaling in macrophages with a broad spectrum inhibitor (γ-secretase inhibitor) or with antibodies against Dll4 attenuates atherosclerosis in

mice [47,48]. Blocking Dll4 also reduces the development of vein graft lesion in LDL receptor-null animals. Interestingly when using cell-type specific nanoparticles to deliver short hairpin RNA (siRNA) targeting Dll4 *in vivo*, the authors observed that the protective effect was exclusive to macrophages but was absent when endothelial cells were targeted [49■]. Although Dll4 is highly expressed in capillaries during development, mature arterial endothelium express Dll4 at low levels [50–52].

Modulation of Notch signaling by inflammatory signals also occurs in endothelial cells from various vascular beds. In human umbilical vein endothelial cells (HUVEC), a pulse of TNFα was shown to increase JAG1 through activation of NF-κB but a concurrent decrease of Notch4 and target genes Hairy and Enhancer of Split 1 (HES1) and Hairy and Enhancer of Split-Related 1 (HESR1) were also observed [53] resulting from a possible negative regulatory loop [54,55]. In human aortic endothelial cells (HAEC), *JAG1* expression was upregulated by a short treatment with inflammatory cytokines, including IL-1β and TNFα, whereas *NOTCH1* and targets *HES1*, Hairy/Enhancer-of-Split Related with YRPW motif-Like (*HEYL*) were strongly repressed through a mechanism involving Signal Transducer and Activator of Transcription 3 (STAT3) activation [56■■]. Therefore, despite the increase in ligand, the overall signaling pathway was also suppressed upon exposure to inflammatory mediators. Others also reported that in endothelial cells *in vitro* and from small coronary vessels in a model of heart transplant in rat, inflammatory stimuli differentially impact Notch2 and Notch4, with the first being upregulated, whereas the latest was repressed [40,57].

In addition to a direct impact of inflammatory cytokines on Notch signaling, repression of the Notch pathway promotes endothelial cell activation. For example, repression of endothelial Notch4 triggered an increase in Vascular Cell Adhesion Molecule 1 at the cell surface [40]. In endothelial cells from the bone marrow, the canonical effector of Notch signaling RBP-jκ inhibits MicroRNA-155, NF-κB activation and subsequent production of proinflammatory cytokines [58].

Inflammatory activation of the endothelium is also linked to disturbed hemodynamic shear stress in the arterial tree. Although relevant mechanosensors have been investigated, the Notch signaling pathway has emerged as both sensitive to and a mediator of shear stress. In fact, data collected *in vitro* and *in vivo* have shown that the pathway could be activated by shear stress [54,59–63] while it is required for downstream biological processes such as endothelial cells alignment [64], arterial specification [59–61], repair of the endothelium [65] and repression of inflammatory and osteogenic genes [63,66■■]. In particular in aortic valve leaflet, Notch activity levels are lower in the aortic side compared with the ventricular side, the first being more prone to the emergence of calcific and inflammatory events [67].

Consistent with a molecular impact of differential Notch activity in endothelial cells, work by Theodoris and colleagues have recently uncovered that mutations in *NOTCH1*, that have been known to cause aortic valve disease [29], were also responsible for profound changes in epigenetic landscape. The authors showed that *NOTCH1* haploinsufficiency in aortic valve endothelial cells disrupts antiosteogenic and anti-inflammatory networks normally induced by protective hemodynamic shear stress [66■■]. Although mechanical dysfunction

was proposed to be a strong determinant of the disease, their findings shed light on a more direct role for Notch1 in the maintenance of endothelial cell fate and its critical contribution in repressing intrinsic expression of inflammatory mediators. In agreement with these findings knockdown of *NOTCH1* in HAEC led to the upregulation of pro-inflammatory and proatherogenic molecules that promote binding of monocytes *in vitro*. These results using human cells were corroborated in mice carrying heterozygous deletion of endothelial *Notch1*. Inactivation of a single allele in the endothelium resulted in the accumulation of C-X-C Motif Chemokine Ligand 1 (CXCL1) in the luminal side of the aorta and significant recruitment of CD45 Positive (immune) cells. Furthermore, inducible endothelial deletion of *Notch1*, even in adult uninjured arteries, supported a critical role for Notch1 in the maintenance of a nonactivated endothelium [56■■]. Finally, Notch3 expressed by vascular smooth muscle cells is essential to protect against their inflammatory activation and transdifferentiation induced by inflammatory stimuli, such as IL-1β [68,69].

To note, because Notch is involved in close range cell–cell communication heterotypic activation in addition to homotypic activation must be considered. For example, in the liver, Dlls expressed by synovial endothelial cells activate Notch signaling in Th1 lymphocytes, inducing the expression of IL-10 to blunt the inflammatory response [70■]. Interactions between macrophage Notch and Dll4 expressed on tip-cells are important for retinal sprouting angiogenesis [71] and recently an *in vitro* model of angiogenesis integrated Notch/ligand interactions in macrophages, mural cells, and endothelial cells [72■]. Finally, Notch activation in smooth muscle cells, essential for their fate decision and maintenance, is driven in part by ligands expressed by endothelial cells [73] (Fig. 1B).

REGULATION OF NOTCH SIGNALING PATHWAY BY LIPID PRODUCTS

Lipid by-products are major contributors to atherosclerosis as they promote inflammatory-related events as well as endothelial dysfunction. For example, in endothelial cells, Notch signaling is blocked by oxidized lipids. A follow-up consequence of Notch suppression is the emergence of a pro-inflammatory transcriptional signature that includes upregulation of CXCL1, IL-8 and E-Selectin [56■■]. *In vivo*, exposure of wild-type mice to high-fat diet led to a decrease in endothelial Notch1 expression and activity, which was rescued when circulating cholesterol levels were reduced. The findings demonstrate that by-products of high-fat diet, currently used to induce atherosclerosis in mice, rapidly impact endothelial Notch pathway and promote inflammation. Consistent with this observation, NOTCH1 expression and activity were also repressed by high dose of oxidized 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylc holine (Ox-PAPC) in HAEC, through a mechanism involving the activation of STAT3 [56■■]. Ox-PAPC also triggers a strong and rapid downregulation of *JAG1* and target genes *HES1* and *HEYL*. Repression of NOTCH1 by Ox-PAPC participated in endothelial cell activation downstream of oxidized phospholipids. Importantly, the degree of *NOTCH1* suppression by Ox-PAPC was variable across 147 donors and associated with specific polymorphisms [56■■] previously linked to HDL levels in approximately 100 000 individuals [74,75]. Therefore, endothelial Notch signaling appears to be a sensor of oxidized-PAPC.

Data from different systems support such a sensor function of Notch and provide potential mechanisms, including modulation of plasma membrane lipid composition and protein post-translational modification. Oxidized phospholipids, in particular Ox-PAPC bind to and activate ADAM10 [76]. ADAM10 contributes to the cleavage of Notch receptor; this protease is also able to shave the ligand JAG1 from the membrane [77] an event that is expected to affect close range ligand-dependent activation of the pathway that requires transcytosis of Notch extracellular domain by the ligand expressing cells [10]. However, the net effect of excessive ADAM10 activity on Notch signaling remains to be explored, as the biological impact of soluble Notch ligand is unclear [78] and this enzyme also participates in the cleavage of the receptor itself. Another critical enzyme in Notch activation is γ-secretase, a complex of transmembrane proteins, which cleaves the intramembrane domain of Notch receptor (among other substrates) to release its transcriptionally active form. Interestingly, the activity of γ-secretase is highly sensitive to local membrane lipid composition. In fact, cholesterol raft-like membrane structures were proposed to be optimal to sustain high activity [79–82]. Although the precise mechanisms are not yet elucidated, changes in membrane lipid composition and fluidity occur in response to oxidized phospholipids such as oxidized LDL [83], an event that may affect γ-secretase function and Notch activity. In tumor cell lines, exosomal lipids were shown to affect Notch1 signaling likely through changes in membrane lipid microenvironment [84]. In addition, a recent study in HUVEC suggested that the protective effect of epigallocatechin gallate, a natural polyphenol that can inhibit metalloproteases, toward ox-LDL was mediated by Jag1/Notch signaling [85]. Thus lipid by-products might also affect endothelial Notch signaling through changes in membrane composition and regulation of protease activity, but more direct validation is needed to support these conclusions.

Another mechanism by which lipids may interfere with Notch signaling relate to potential lipid–ligand interactions. In fact, a C2 phospholipid recognition domain in N-terminal region of Jag1 was recently uncovered. This domain also present in Dll1 does not impact dimerization with the receptor but it regulates levels of Notch activation [86]. More recently, ligand-independent induction of Notch was observed in response to shingosine-1-phosphate (S1P) and S1P receptor 3 engagement in cancer stem cells [87]. A protective role for S1P bound to HDL has been previously shown in the onset of atherosclerosis [88] with a recent study uncovering molecular mechanisms involved in their anti-inflammatory and antiatherogenic function in endothelial cells [89■■]. Although the molecular events are likely to differ from cancer stem cells, it would be interesting to determine whether regulation of Notch pathway by S1P may also occur in adult aortic endothelial cells.

Together, the findings converge on the idea that this evolutionary conserved pathway may be considered as a signaling hub between circulating factors and cell homeostasis. A role for Notch in ‘sensing’ the systemic metabolic status is further supported by elegant studies in different systems. In the developing mouse heart, hyperglycemia abolishes left–right axis formation and affects heart morphogenesis. The resulting condition that resembles congenital heart defects associated with pregestational diabetes was shown to be secondary to a reduction in Notch pathway activity [90■■]. In the nematodes Notch [abnormal Germ Line Proliferation (GLP-1] signaling is modulated by nucleotide levels and proposed to be part of a sensing mechanism to adapt their reproductive program to environmental and

nutritional clues [91■]. Finally, recent advances in the characterization of structural features of the oligomerization of Notch and its ligands through O-fucose and O-glucose provided additional clues supporting that the activity of the pathway may reflect the metabolic state of the cell [18■■] (Fig. 1C).

CONCLUSION

Progress have been made in understanding the role of Notch signaling in the adult vasculature and current studies converge toward a protective role of the pathway in endothelial cells from large vessels. In addition, evidence from different models support that the Notch activity may be considered as a component of the cell 'sensing' machinery, transducing microenvironment and metabolic clues to transcriptional changes. This includes shear stress, inflammatory signals, and dietary by-products but also close range activation through homo and heterotypic contact with cells residing in blood vessels. Although the current studies show a rapid impact of these stimuli on endothelial Notch, the long-term effect of chronic exposure to inflammatory mediators and lipid byproducts together with heterotypic communication within the plaque deserves additional investigations. Despite the complexity of the plaque microenvironment this will contribute to improve our understanding of the role and regulation of endothelial Notch in the disease progression and stabilization.

As several therapeutic strategies aim at inhibiting Notch signaling, for example, to reduce tumor angiogenesis, a better characterization of this pathway in adult vessels is important. Although most studies on endothelial Notch are focused on cancer setting and cardiovascular diseases, it is also critical to consider its potential role in the microvasculature of highly metabolic organs that are constantly challenged by inflammatory molecules and metabolic by-products.

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KEY POINTS

- Notch signaling is critical for the maintenance of vascular homeostasis.
- Notch contributes to and is modulated by inflammation in various cell types.
- Notch activity is impaired by dietary by-products, including oxidized lipids.
- Repression of Notch signaling in arterial endothelial cells unlocks proinflammatory and proatherogenic signals that contribute to the initiation of atherosclerosis.

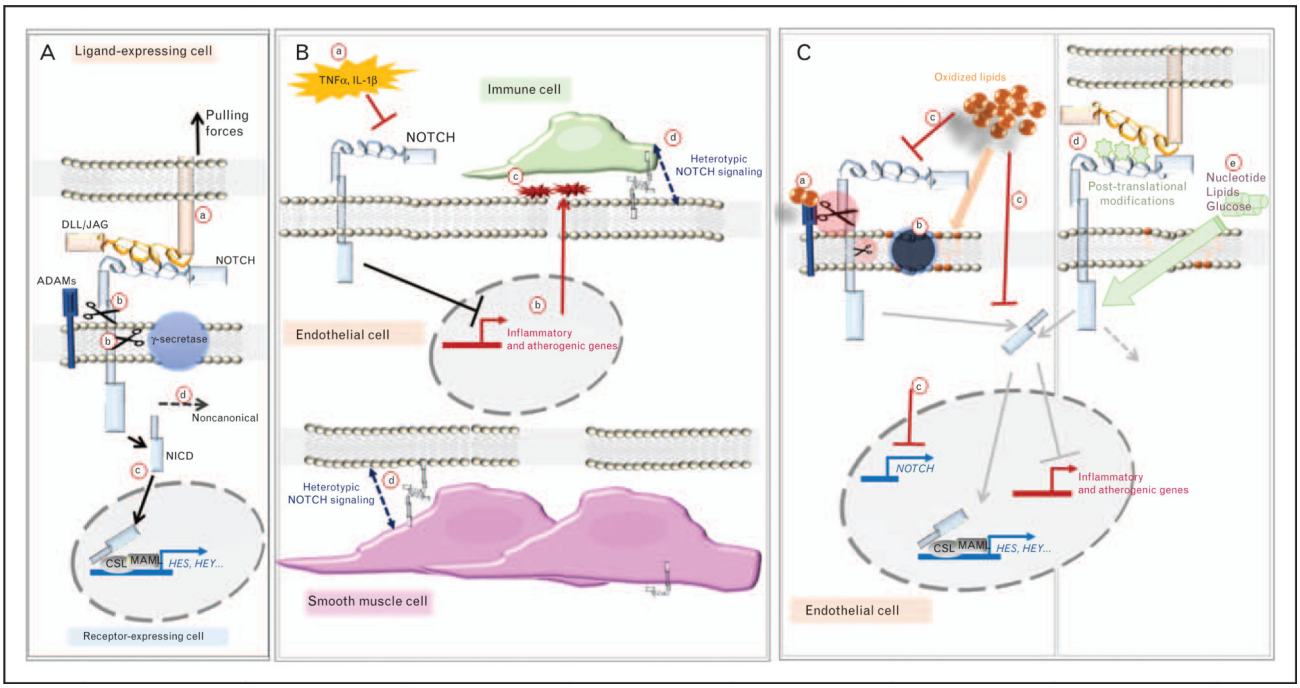

FIGURE 1. Endothelial Notch signaling pathway and interactions with the microenvironment

(A) After dimerization of a NOTCH receptor with a DLL/JAG ligand (a), proteolytic cleavage of NOTCH by ADAM family proteases and the γ-secretase complex occurs (b), releasing NOTCH intracellular domain (NICD) that translocates to the nucleus, interacts with the MAML/CSL complex to induce the transcription of target genes (c; HES, HEY family). Non-canonical effects of NOTCH have also been described (d). (B) Inflammatory stimuli suppress NOTCH expression and activity in endothelial cells (a); this results in the expression of inflammatory and atherogenic mediators (b) favoring the recruitment of immune cells (c). Notch signaling can involve bi-directional heterotypic communication between endothelial cells and immune or smooth muscle cells (d). (C) Changes in protease activity secondary to oxidized lipids exposure that can (a) bind to and increase activity of ADAM proteases and (b) change the lipid microenvironment altering γ-secretase activity. Oxidized phospholipids repress NOTCH expression and activity, a mechanism that promotes endothelial activation (c). NOTCH signaling is regulated by post-translational changes that may reflect biosynthetic activity of the cell (d). Nutrients impact NOTCH expression and activity (e). ADAM, a-disintegrin and metalloprotease; CSL, CBF1, Suppressor of Hairless, Lag-1; Dll, Delta-like ligand; MAML, mastermind-like; NICD, Notch intracellular domain.