Review Article

Interventional Cardiology

Notch signalling pathway in atherogenesis: Effects in various cell types

Abstract

The Notch signaling pathway is one of the fundamental components of cellular signaling. This pathway regulates the choice of cell faith and can enhance proliferation, differentiation, and apoptosis. The role of Notch in cardiovascular system development and functioning is essential. Notch controls various aspects of normal cardiovascular maintenance and pathologies, among which there are pathological cardiac remodeling, ischemia, neoangiogenesis, and others. Atherosclerosis, which is a chronic inflammatory disease, is known for its complex pathogenesis and the variety of cell types involved. In every type of cell, Notch signaling has its specific features. Also, the growing body of evidence suggests that modulating different Notch elements, especially in T-cells, can be a promising strategy to counteract atherosclerosis. In this review, we summarized data on the Notch signaling in different cells during atherogenesis.

Keywords: Notch signaling •Atherosclerosis • CVD •Cell signaling • Cardiovascular disease

Anastasia V. Poznyak*, Vasily N. Sukhorukov, Ilya I. Eremin, Irina I. Nadelyaeva, Alexander N. Orekhov*

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow 125315, Russia

*Author for correspondence:

Anastasia V. Poznyak, Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow 125315, Russia, E-mail: tehhy_85@mail.ru;

Alexander N. Orekhov, Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow 125315, Russia, E-mail: a.h.opexob@gmail.com

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Introduction

The Notch signaling is an essential component of cell-to-cell communication. This pathway manages the choice of cell faith and adjusts proliferation, differentiation, apoptosis, and other key functions of the cell. There are four isoforms of Notch receptors (Notch 1-4), and five Notch ligands (Delta-Like Ligand (Dll 1, 3, and 4), and Jagged-1 and 2) expressing in mammals [1]. A single precursor is synthesized for Notch receptors, which moves to the Golgi apparatus, and there is cleaved by a furin-like protease into two subunits, extracellular and a transmembrane ones. After that, these subunits are transferred to the cell-membrane for the assembly [2]. The binding of a Notch ligand with its receptor launches the loss of extracellular part with the subsequent proteolytic cuts, the first by a disintegrin and metalloprotease (ADAM10 and/or 17) and the second by a γ-secretase, a multiprotein complex membrane protease, resulting in the release of the active form intracellular Notch (NICD) [3]. In the "canonical" variant of Notch pathway, NICD moves into the nucleus, where it binds to Recombinant Binding Protein for the immunoglobulin region κJ (RBPJ) of transcription factors and thus controls the transcription of target genes. This mediates the displacement of co-repressors and the recruitment of Mastermind proteins (MAML 1–3). Additional co-activators, such as p300 and PCAF are recruited to the NICD/RBPJ/MAML complex, which allows controlling the transcriptional expression of the genes manageable by Notch. Transcriptional repressors from HEY (Hairy and Enhancer of Split with YRPW) and HES (Hairy and Enhancer of Split) families turned out to be best investigated Notch target genes [4]. In "non-canonical" variant of Notch signaling, the activity of NICD can be independent from RBPJ. Also, the pathway can be triggered by the activation of γ-secretase without binding with canonical ligand,

or Notch signaling is activated in the absence of the cleavage of the γ-secretase complex. Non-canonical Notch signaling include interactions with other crucial pathways, such as mTORC2 (mammalian target of rapamycin complex 2)/Akt, Wnt/β-catenin, IKKα/β, and can also occur in mitochondria where Notch/PINK1 (PTEN-induced kinase 1) complexes modulate mitochondrial metabolism promoting cell survival by activating the mTORC2/Akt pathway [5,6].

Literature Review

Notch signaling in atherosclerosis

Atherogenesis: Atherosclerosis is a disease chronic inflammatory disease with complex pathogenesis, which is considered a precursor of a range of CVDs. Atherogenesis is characterized with the fatty streak formation on the inner vascular wall, which later develop into atherosclerotic plaques [7,8].

The atherosclerosis development probably starts with the endothelial injury. Endothelial Cells (ECs) are very sensitive, and can perceive various stimuli, such as modified lipid particles, blood flow impairments, and others. In norm, stable/laminar shear stress protects the endothelium contributing to an anti-inflammatory, vasodilatory, antithrombotic, and non-proliferative phenotype [9,10]. Instead, low and disturbed/oscillatory shear stress stimulates endothelial dysfunction. This is implemented through the production of pro-inflammatory cytokines and enhanced pro-atherogenic genes transcription [11]. Low-Density Lipoprotein (LDL) particles are accumulated in the sub endothelial space, where they undergo the variety of modifications, such as oxidation, sialylation, and others. Among other effects, these modifications lead to an increased expression of adhesion molecules on the surface of ECs, such as Vascular Cell Adhesion Molecule-1 (VCAM-1), Intercellular Adhesion Molecule-1 (ICAM-1), and E-selectin, acting as chemioattractors for circulating monocytes and T lymphocytes [12,13]. After infiltration into sub-endothelium, the monocytes transform into macrophages, which, in turn, start to internalize modified LDL. By storing lipids, these cells become foam cells, which produce growth factors and cytokines. These molecules serve as an inflammatory response booster and recruit Extracellular Matrix (ECM) proteins- producing Vascular Smooth Muscle Cells (VSMCs). This results in the formation of fibrous, lipid-loaded plaques, which can become unstable and break away, leading to the formation of thrombus, and, subsequently, to myocardial infarction [14, 15].

The Notch pathway is involved in regulating the functioning of cells of all types that are implicated in atherogenesis. Stable/laminar shear stress upregulates Notch 1, which is required for transcription of genes that remain endothelial function [16]. Other investigations revealed that stable/laminar blood flow favors the induction of Notch 1, which promotes maintenance of endothelial barrier function and upregulates the anti-apoptotic protein Bcl-2, thus protecting ECs against apoptosis [17]. Also, the reduced expression of Notch 1 in response to circulating lipids and pro-inflammatory cytokines, such as Tumor Necrosis Factor alpha (TNF-α) and Interleukin (IL)-1β, was shown to cause inflammatory molecules and monocytes recruitment. The protective role of Notch 1 in the endothelium is supported by *in vitro* observations that demonstrated the induction of dysregulation of Notch signaling in ECs by inflammation, which results in to NF-kB activation and induction of ICAM-1, VCAM-1, and apoptosis [18]. The protective effect of 17-β-estradiol against TNF-α induced apoptosis requires Notch 1 activation was also shown by Fortini, et al [19]. However, the studies discussed so far are in contrast with other observations suggesting a pro-atherogenic and pro-inflammatory role of the Notch pathway in the contest of endothelium [20].

Intra-plaque hemorrhage is a marker of plaque instability, and it has been suggested that intra-plaque hemorrhage and rupture of the fibrous cap is associated with increased microvascular density. A complex interplay between Dll4/Jagged-1/Notch 1 signaling, inflammatory cytokines, and growth factors determines the extent of angiogenesis and, in particular, the number of new branches arising from pre-existing blood vessels. It follows that any factor that alters Notch activity in atherosclerotic plaques can influence plaque angiogenesis and hemorrhage [21].

Notch in endothelial cells

The following elements of Notch pathway are expressed in ECs: Notch 1, 2, and 4 receptors and Dll1, 4, Jagged 1, 2 ligands [22]. In various studies, Notch is shown to counteract the inflammatory cytokines-induced endothelial dysfunction. Both *in vivo* and *in vitro* studies are consistent with the finding that Notch signaling can be inhibited, and levels of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, as well as apoptosis, can be induced by inflammatory cytokines *via* mechanism involving Nf-kB [23]. It was also shown that the treatment of human vein ECs with serum from heart failure patients with increased levels of inflammatory markers inhibits Notch 1 and 4 activations. Recent studies have shown the anti-inflammatory role of Notch illustrated by the inhibition of miR155 synthesis by activation of Notch 1 in bone marrow-derived EC. This miRNA is involved in eNOS downregulation and NF-κB activation [24,25].

Vascular regions with the altered blood flow and low shear stress are more vulnerable for atherosclerosis lesion formation. In accordance with other studies of a shear stress Notch modulation, the decreased expression of Notch pathway components was found in atheroprone regions of mouse aortic arch [26]. This finding contributes to the suggestion that disturbed blood flow could prepossess those areas to atherosclerosis *via* influencing Notch

signaling. Thus, the treatments with a pure heart rate inhibitor ivabradine, which can potentially modulate shear stress, up regulates genes of the Notch pathway and reduce the expression of pro-apoptotic and pro-inflammatory genes in the endothelium of aortic arch of Apo E-deficient mice [27]. Also, the reduced expression of miRNA126-5p was shown in atheroprone regions of aortic arch of Apo E deficient mice. This links the shear stress and Notch regulation, contributing to the crucial role of a functional Notch signaling for the repair of the endothelium. Downregulation of miRNA126-5p by low shear stress leads to up regulation of Dlk1 which, by inhibiting Notch 1 signaling, interferes with ECs proliferation needed for the repair of endothelium damages caused by dyslipidaemia [28,29].

Notch in vascular smooth muscle cells

Vascular Smooth Muscle Cells (VSMCs) are a major cell type in the vascular media. In VSMCs, the Notch signaling is mediated by Notch 1, 2, 3, and the ligand Jagged-1. Due to the insufficient differentiation of VSMCs they can switch their phenotype under the effect of various circumstances, such as inflammation, in response to which the contractile/quiescent can be changed to a secretory/proliferative [30]. After this transition, VSMCs start to produce pro-inflammatory molecules. This is a hallmark of atherosclerotic lesions formation. However, the molecular mechanisms underlying this phenotypic switch. *In vitro* and *in vivo* studies revealed the interaction between Jagged-1-mediated Notch activation and the trans differentiation of VSMCs induced by IL1β [31-33]. Other studies suggest that the Jagged1/Notch 3 axis promotes the VSMCs contractile phenotype and NF-kB-mediated inhibition of Notch 3 favors the transition from a contractile to a secretory, pro-inflammatory phenotype [34]. An inhibition of VSMCs proliferation in humans through *via* cell-cycle arrest, as well as the localization of high levels of Notch 2 to the non-proliferative zone of injured arteries was demonstrated to be caused by Jagged-1 mediated activation of Notch 2 [35]. Also, Aquilla et al. have shown the reduction of contractile phenotype and the induction of pro-inflammatory markers in association with low levels of Jagged-1 and high levels of Dll4 with cholesterol accumulation in VSMCs of rat aorta [31]. Moreover, active Notch inhibits apoptosis of VSMCs. Notch 1 is potentially involved in proliferation and cell survival in the context of vascular injury. Thus, it was also shown that perivascular delivery of siRNA for Notch1 inhibited neointimal formation and VSMCs migration and proliferation [36].

Notch in macrophages

Activated macrophages are extremely important for the atherosclerosis development. Notch signaling triggers a pro-inflammatory phenotype in macrophages. Treatment of macrophages with IL-1β stimulates Dll4-mediated Notch 3 signaling resulting in the transcription of genes coding pro-inflammatory factors that may enhance plaque burden, progression, and thrombogenicity. It was also shown that Dll4-dependent activation of Notch signaling in macrophages leads to an increased inflammatory response [37,38]. Treatment of with pro-inflammatory stimuli, e.g., lipopolysaccharide, which stimulates the M1 pro-inflammatory phenotype, and triggers the transcription of Dll4, Jagged-1, and Notch 1. Conversely, Notch inhibition appears to increase the polarization of macrophages toward an anti-inflammatory M2 phenotype [39].

Notch as a target

Example of cancer: The understanding of the involvement of Notch signaling into the pathogenesis of atherosclerosis is insufficient. The good example of detailed knowledge of role of the Notch signaling is cancerogenesis. The main aspect of Notch inhibition in cancer were considered Gamma Secretase Inhibitors (GSIs), which are a heterogeneous group of small molecules able to avert the γ-secretase enzymatic complex-mediated Notch cleavage [40]. At first, GSIs were developed on the basis of their effect on Notch 1, but it can affect the cleavage of other Notch paralogs or interfere with other pathways. Several GSIs are currently involved into Phase I and/or II clinical trials in cancer patients. Targeting Notch in the context of atherosclerosis could result to be more complex compared with the oncology setting in which Notch inhibition interferes with growth of every solid tumour and leukaemia studied so far [41,42].

Atherosclerosis: *In vivo* studies have shown the ability of GSIs to interrupt the atherogenesis process. Systemic administration of GSI (LY411,575 0.2, and 1.0 mg/kg/day for 8-weeks) inhibited Notch signaling in ApoE-deficient mice on a high fat diet and decreased total plaque areas in the aortic sinus [43]. Moreover, lowered levels of ICAM-1 and migration ability were shown in macrophages obtained from these mice. Effects appeared to be dose-dependent: 0.2 mg/kg/day did not cause loss weight and alterations of intestine and thymus, while 1 mg/kg/day dose caused intestinal and immunologic toxicity [43]. This allows suggesting that only low doses of GSI could be used long term without adverse effects. Thus, the use of GSI for atherosclerosis treatment can weaken the inflammatory activities of macrophages. The resulting effect on atherosclerosis progression would depend on how the treatment affects the complex interplay between acquired and innate immunity, and thus, the balance between pro- or anti-atherosclerotic T cells [44]. As for macrophages, mesoporous silica nanoparticles containing GSIs could be used to specifically deliver these molecules to these cells. Also, a specific approach to block Notch and inflammation in macrophages may be realized by cell-specific delivery to macrophages of Notch inhibitors miRNAs using siRNA loaded exosomes [45]. GSIs-coated stents could be

used to prevent re-occlusion in some patients, after percutaneous intervention, since Notch could be also involved in restenosis due to its effect on promotion of vascular smooth muscle cells proliferation [46].

Interesting observation was made on the sulindac, a non-steroidal anti-inflammatory drug, which prevents the development of with triple negative breast cancer through the Notch suppression in cancer stem cells without inhibiting Notch expression or cleavage in murine T-cells. This data contributes to the potential beneficial effect of sulindac on the atherosclerosis, especially on the regulation of Notch signaling in macrophages [47].

The use of blocking antibodies can be a promising approach, but for developing distinct targeting it is necessary to refine our knowledge of the role of each receptor and ligand in each exact cell of both innate and adaptive immune system in atherogenesis [48]. For now, blocking antibodies against Dll4, Notch 1, Notch 2, or Notch 3 have been already tested in clinical trials (phase I) in patients suffering from cancer. Also, this can be important for patients with Peripheral Artery Disease (PAD), whose intraplaque levels of Dll4 mRNA is potentially linked to the disease progression [49]. The doubts on such treatment safety were resolved due to the common expression of Dll4 in the vasculature and the immune system. After 12 weeks of agents blocking Dll4 administration no toxicity was observed in one study on murine model, but other authors admitted adverse effects in the liver [50,51]. Heart failure was observed in some cancer patients in response to administration of anti-Dll4 antibody [52,53]. Anti-Jagged-1 immunotherapy was shown to suppress myeloid-derived suppressor cells and overcome tumor-induced tolerance by activating T-cell, thus proposing a protective role of Jagged-1-mediated signaling in atherosclerosis [54]. This is also supported with the observed association between slower progression of disease in PAD patients and high levels of Jagged-1 mRNA intraplaque [55]. Current understanding was formed almost on the studies on animal models or cellular culture. Even in humans, the status of Notch signaling has been investigated only in a small number of carotid arteries.

The most serious obstacles of targeting Notch in atherosclerosis are the variety of Notch implications in different cell types. An active Notch signaling is crucial for endothelial protection endothelium from dysfunction driven by inflammation and for maintaining the non-proliferative/contractile state of VSMCs [56,57]. However, Notch activation within macrophages in plaques is linked to the inflammatory and unstable plaque phenotype. This can be overcome by the specific targeting of each Notch signaling component consistent with the cell type. So, the identification of the exact role of each components of the Notch pathway in the pathophysiology of atherosclerosis is an essential step for the development of novel therapeutic strategies targeting Notch.

Discussion

Atherosclerosis is a chronic inflammatory disease with a complex pathogenesis. In every stage of this disease, Notch signaling acts in different ways in various cell types. Notch activation stimulates a pro-inflammatory M1 phenotype in macrophages at the expense of the M2 anti-inflammatory subtype, and thus induces atherogenesis. However, there are still big gaps in our understanding of particular molecular mechanisms, but strong evidences revealed that the Dll4/Notch 1 axis is pivotal in favoring M1 polarization, while blocking M2 immunosuppressive macrophages and their cytokines [4,39]. T-cells have two opposite effects, they are able to protect from atherosclerosis and promote the development of the disease as well. The differentiation of T-cells is regulated by the interaction of Notch ligands Dll1, Dll4, Jagged-1, Jagged-2 on APCS with Notch receptors on T-cells. APCs expressing Dll1 or Dll4 induce the differentiation toward pro-atherosclerotic Th1 whereas Jagged ligands instruct T-cells toward the less inflammatory Th2 subtype [58,59]. Also, Jagged mediates the inhibitory activity of MDSCs on CD4 and CD8 T-cells. Considering Tregs, Notch signaling is required for their differentiation from naïve T-cells, whereas in already established Tregs Notch mediates the differentiation toward a Th1-like inflammatory phenotype [60]. However, as we mentioned above, the direct mechanisms of the particular role of Notch in these transitions remains unclear. It can be proposed that Notch potentially promote atherogenesis through stimulation of Th and CD8 cells formation. All this leads us to the conclusion that modulation of Notch pathway could be a promising strategy to prevent. Thus, for example inhibition of the Notch pathway can be beneficial because of the consequent lowering of inflammation of the vascular wall *via* interfering with the production of cytokines from M1 macrophages and with Th1 cells infiltration in the plaque [61]. In principle, this strategy could have the advantage of increasing the immunomodulatory activity of M2 macrophages without depleting anti-inflammatory Tregs in the plaque. Tregs participates in the range of ongoing clinical trials on type I diabetes, graft transplantation, and others. Mostly, the naturally occurring FoxP3+Tregs from patients, followed by *in vitro* expansion and reinfusion are taken for such trials [62]. What is important for our exact topic, is an observation made on murine models stated that adoptive transfer of Tregs have reduced atherosclerosis [63, 64].

Conclusion

This proposes the beneficial effect of the same approach in patients. Another promising strategy consists of preventing a reduction of Notch 1 caused by turbulent shear stress or dyslipidemia or low estrogen conditions. This way, endothelial dysfunction can be reduced, and, consequently, plaque formation in atheroprone areas of the aortic endothelium can be lowered, too. Heart rate reducing

drugs, miRNA, or specific estrogen receptor agonist could be used to prevent Notch 1 downregulation in these areas. To sum up, we can conclude that targeting elements of Notch signaling pathway can be a promising strategy to counteract atherosclerosis, but there are still some obstacles have to be overcome.

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Conflicts of Interest

The authors declare no conflict of interest.

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