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Notch Pathway and its Role in Cardiovascular System: Review

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ABSTRACT
The Notch signaling pathway is a core signal transduction mechanism that control diverse cellular processes. Its activity is mandatory for normal embryonic development, as it control the transcription of genes implicated in cellular proliferation, differentiation, migration, and apoptosis. It is considered as a key regulator of neonatal cardiomyocyte development; thus, its abnormality is strongly correlated to cardiovascular congenital malformations. Despite, Notch pathway is considered relatively absent in adult heart; recent studies included evidence of the upregulation of Notch signaling pathway in response to some cardiac insults. In our review, we briefly discuss the possible role of Notch Pathway in adult cardiomyocytes and other cells involved in the cardiovascular health, and its possible contribution to cardiovascular diseases.

Keywords: Notch pathway, cardiovascular diseases, Notch receptors, Canonical Notch Activation

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INTRODUCTION
Recently, different studies reports that Notch pathway is being activated in injured stressed hearts, such as in coronary heart disease (1) and hypoxia/ reoxygenation (2). Contradictory results are also available considering the outcomes of Notch pathway upregulation in adult heart, whether it is cardioprotective (3), or may be one of the mechanisms that contributes to cardiac pathogenesis (4,5). This may be explained by the cell context in which Notch Pathway is upregulated, the diverse number of Notch receptors and ligands that confer different responses, the existence of both canonical and non-canonical activation mechanisms, and the widespread of different Notch receptors in different cells that are involved in cardiovascular pathogenesis; cardiomyocytes, endothelial cells, and vascular smooth muscle cells (VSMC) (6,7). Moreover, considering the role of Notch signaling in promoting inflammation and regulation of macrophages' activation and proliferation; which also contribute to the progression of considerable number of cardiovascular diseases; may help to further understand the cardiovascular influences of Notch Pathway activation (8,9).

The Notch Signaling Pathway
The Notch Pathway is an evolutionally pathway, that plays a well-defined key role during embryonic life, and in specific tissues in adults. However, in many tissues it is relatively absent in adults, and is being activated in response to specific insults (10). In the kidney, for example, the Notch pathway is activated during nephrgenesis, being inhibited in the neonatal and adult stages, and it is reactivated in situations of kidney damage and its activation correlated with the degree of renal damage (11). It is a fundamental pathway that seems to have a key role in many vital processes such as neurogenesis, gastric mucosal cells' proliferation, angiogenesis, and hematopoiesis (12–16), and its dysregulation is correlated to tissue abnormalities such as fibrogenesis and carcinogenesis (17,18). Obviously, it helps to determine cell fate, as it controls the transcription of genes involved in cellular proliferation and differentiation, as well as genes involved in fibrosis and apoptosis (12,19). The ultimate effect will depend largely on the cell context in which Notch Pathway is activated (17).

Receptors and Ligands
Up till now, a family of four receptors (Notch 1, 2, 3, and 4) and five ligands (Jagged 1, Jagged 2, and Delta-like-1, 3, and 4) have been identified in humans and mice. Canonical and noncanonical identified Ligands have been known for either agonistic or antagonistic signaling , which in turn might affect Notch signaling(20). Receptors and ligands are expressed on the cell surface and regulate adjacent cell-cell communication. They are composed of polypeptide chains with extracellular, transmembrane and functional intracellular domain (NICD). Both extracellular domain (NECD) and the single polypeptide chain of transmembrane fragment (TMF) are the polypeptides which encounter for the mature membrane-bound form of Notch (21,22).

The Intracellular Cascade
Notch signaling can be activated via either canonically (more common) or noncanonically (17). During canonical Notch Pathway activation, the Notch receptors; that were synthesized in the endoplasmic reticulum and the Golgi apparatus as a single-chain; undergoes modification by glycosylation and proteolysis at S1, to produce the mature receptor that is being expressed on cell membrane (23). Notch signaling starts upon coupling of ligand and receptor coupling; expressed on the surface of two opposing cells; followed by endocytosis of the complex by ligand expressing cell. Subsequently, the receptor chain will be further cleaved by two more proteases at S2 and S3 leads to cleavage at the plasma membrane by two other proteases at S2 and S3 that removes the extracellular fragment of the heterodimer. Extracellular S2 cleavage is due to the tumor necrosis factor-alpha converting enzyme (TACE), a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), and (ADAM17). S3 cleavage is achieved by a ɤ-secretase, with the ultimate

release of the active NICD into the cytoplasm (Figure 1) (24).

NICD is a transcriptional regulator, it translocates to the nucleus and forms a complex with one of CBF1/Su(H)/Lag-1 (CSL) members; the recombinant signal binding protein 1 for Jk (RBPjk). In the case of the absence of Notch activation, CSL represses transcription of Notch target genes by recruiting corepressor proteins to form a multiprotein transcriptional repressor complex (25). NICD binding to CSL displaces corepressors proteins bound to CSL, and permits the binding of the Mastermind like protein (MAML); a transcriptional coactivator; to the complex, which thus culminates in activated expression of Notch specific genes (26) (Figure 2).

The most prominent Notch activated genes are the hairy and enhancer of split (Hes) and the hairy and enhancer of the split with YRPW motif (Hey) gene families, which are transcription regulators. Hes genes are believed to play important role in neural and endocrine functions, however, Hey genes are more involved in the cardiovascular system (27). Other Notch target genes include cyclin D1 (implicated in cell cycle progression), Bcl-2 (inhibitors of apoptosis), cyclin A, and Nuclear factor -kB (NF-kB) (28,29) (Figure 2).

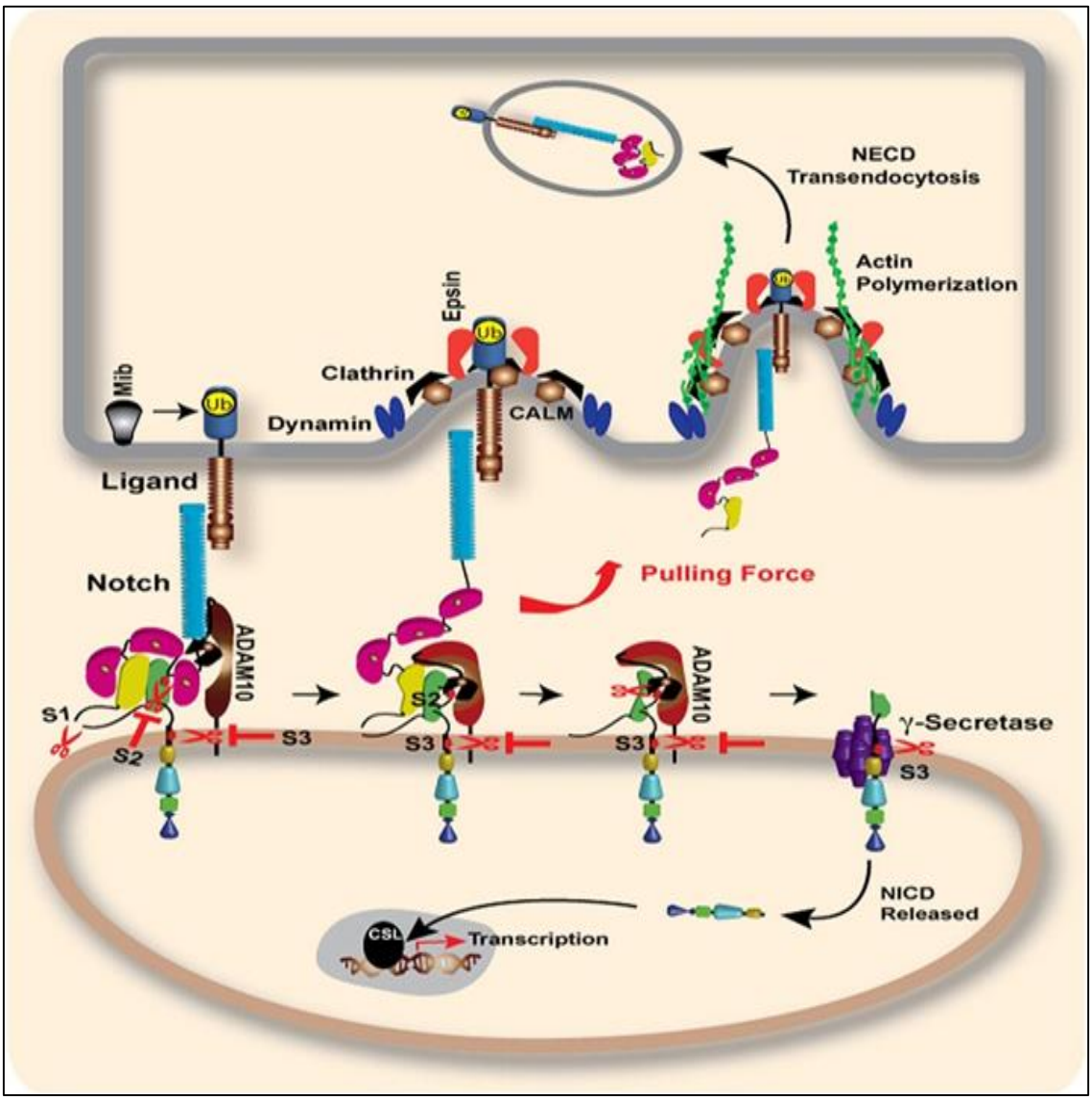

Figure 1: Activation of The Notch Receptor by Notch Ligand from A neighboring Cell.

Notch ligand is are located on the cell surface of signaling cell. The Notch receptor is located on the surface of the signal-receiving cell. Upon ligand binding, the receptor chin is processed at the S1 site by a furin protease, followed by 2 consecutive cleavages at S2 and S3, by ADAM and γ-secretase, respectively. Those cleavages free the NICD that translocates to the nucleus. and binds to CSL **(30)**. ADAM: A Disintegrin and Metalloprotease-containing protease, NICD: The Notch intracellular domain

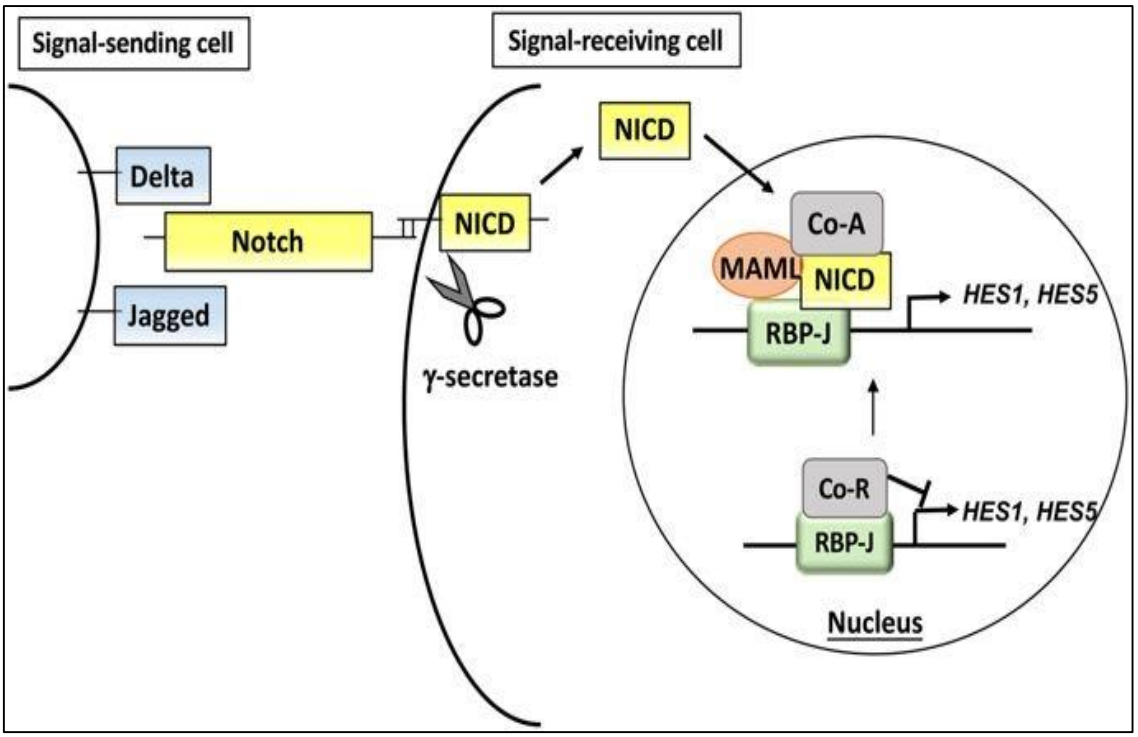

Figure 2: A Model of Canonical Notch Activation.

In the nucleus, NICD binds to (RBP-j) one of CSL members, the binding of NICD results in displacement of transcriptional repressors (Co-R) by coactivators (Co-A), in presence of the transcriptional coactivator Mastermind (MAML). The results is activation of transcription of Notch target genes (e.g., Hes1, Hes5) (31).

Noncanonical Notch Signaling Pathways

In addition to canonical activation of Notch Pathway, it was found that Notch can also be activated by a wide variety of noncanonical ligands; this may explain the variable and missing information about Notch activation outcomes (30).

This pathway is even variable, including Notch pathway effects that progress independently of CSL, without S3 cleavage, those initiated without ligand binding, or signals that activate CSL-genes without NICD release. Among suggested mechanisms of noncanonical Notch signaling are induction of non-CSL transcriptional factors, including β-catenin, hypoxia-inducible factor-1α, NF-κB, and estrogen receptor (ERα). For example, Notch can activate through R-Ras to promote cell adhesion. Alternatively, Notch may interact with IKKa in the NF-kB pathway or lymphoid enhancer-binding factor 1 in the Wnt pathway. Collectively, our knowledge about the role of the noncanonical Notch signaling in biology and pathophysiology remains primitive (Figure 3) (28).

The effect of Notch ligands and feedback loop on Notch signaling activity

From the above mentioned, it had been reported that the pleiotropic nature of Notch pathway has been accounted by the canonical and non-canonical Notch ligands, transmembrane proteins, which might act as agonists or antagonists through a specific molecular transduction mechanisms (20). With respect to the canonical DSL (Delta, Serrate, Lag2) ligands, the nature of the ligands might affect being either amplifying or repressing the Notch signaling. Parks and coworkers identified the structure-function analysis of these ligands and found that the DSL domain together with the flanking N-terminal (NT) domain and first two Epidermal Growth Factor (EGF) repeats are required for DSL ligands to bind Notch (32). DSL ligands can either activate or inhibit the Notch signaling depending on the nature of cell surface interaction (20). For instance, a Trans-interaction between the DSL ligand on one cell (signal-sending cell) and Notch receptor on the opposing cell (signal- receiving cell) is required to amplify the Notch signaling (32). On the other hand, DSL ligand can inhibit the Notch signaling when interacts with the Notch receptor within the same cell, Cis –interactions (33,34). In addition, DSL ligands activity are also regulated by glycosylation of Notch through modulating the ligand-binding properties (35). Nevertheless, the molecular basis of cis-interaction and the exact mechanism by which it could affect Notch are not fully understood. In addition to the cis- and trans-interactions, it had been thought by some that Notch ligands might have a concentration dependent inhibitory effect on Notch signaling activity. High levels of ligand causes an inhibitory effect while low levels activate the Notch signaling (36)

Another molecular explanation for the pleiotropic nature of Notch signaling is the non-canonical Notch ligands such as Delta-like 1 (Dlk-1) (37). Indeed, Dlk-1 can only support cis-interaction with the Notch resulting in cis-inhibition and not trans-activation of Notch signaling (20,38).

Besides the Notch ligands modulatory effects, Notch-mediated lateral inhibition is one mechanism that control the fate of two adjacent cells into opposite fates, amplification of suppression, although having the same differentiation (39,40). One explanation is that suppression of Notch signaling occurred in the winner cell, which produces more Delta ligands while amplification of Notch signaling is usually happened in the loser cell which downregulates Delta production. Nevertheless, the mechanisms that activate the Notch signaling in the loser

cell are the same which terminates Notch signaling in the winner cell (39,41).

The Role of Notch Pathway in the Cardiovascular System

The Notch pathway plays a magnificent role in the cardiovascular system. It is a key regulator in multiple processes during embryonic cardiovascular system development and maintenance (41), such as the proliferation and differentiation of vascular cells and cardiomyocytes. Sequentially, Notch dysregulation is believed to contribute largely to congenital cardiovascular diseases and strong evidence grow about a possible role of Notch in the etiology of many cardiovascular complications in adults, as well (42). However, like many other signaling pathways, its effect is complexed interactions with other pathways, in addition to being itself including many different receptors and ligands, which makes its effect difficult to be predicted (43).

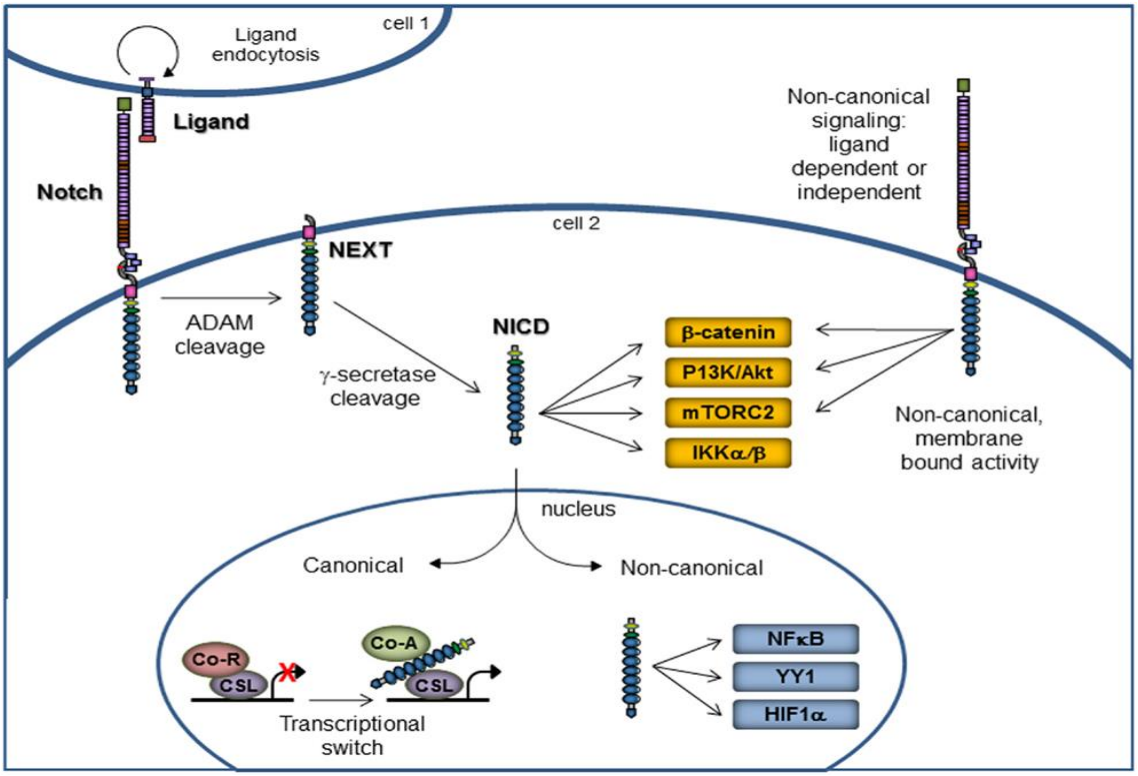

Figure 3: Notch canonical versus non-canonical signaling.

Canonical Notch pathway is illustrated on the left side of the figure. Notch receptor is activated by ligand on a neighboring cell, with subsequent proteolytic cleavages releasing the NICD. The non-canonical signaling pathway is illustrated on the right side of the figure and it illustrates that non-canonical Notch activation may occur with or without ligand, with or without receptor cleavage. In Non-canonical Notch signaling is independent of CSL, and interaction with many different pathways can occur, either in the cytoplasm or in the nucleus. Abbreviations: ADAM, a disintegrin and metalloprotease; NEXT, Notch extracellular truncation; NICD, Notch intracellular domain; Co-R, corepressor; Co-A, coactivator **(17)**.

The Role of Notch Pathway in Cardiomyocytes

Cardiomyocytes' stem cell precursors express considerable levels of Notch1 receptor, and Notch activation is mandatory for their proliferation. But the role of the Notch pathway in cardiomyocytes after birth is still not clear. Rat neonatal cardiomyocytes were found to actively proliferate, and their proliferation is correlated with high Notch expression. However, those cells then lose their proliferative power, correlated also with negligible Notch expression (41,44).

Recently, evidence of the reactivation of the Notch pathway in response to cardiac injury has been elucidated; e.g. the expression of Notch receptors has been detected in myocardial biopsies of patients with heart failure (45). However, the specific role and impact of Notch reactivation are still not well characterized. Studies showed that Notch 1 is reactivated in cardiomyocytes in ischemic heart and post myocardial infarction in adult rats, and it inhibited cardiomyocytes apoptosis; probably by activating the Akt pathway. These findings suggest that Notch pathway may reactivate in adult cardiomyocytes, and aids in cardiomyocytes conservation (3). Kratsios's results were also in agreement with this postulation, as he found that transgenic mice overexpressing Jagged1 (a Notch ligand) on cardiomyocytes showed better cardiac performance and less fibrosis as compared to wild animals (46). Notch mediated cardioprotection can be explained in terms of increasing cardiomyocyte survival, antiapoptotic effects, promoting angiogenesis, and stimulation cardiac stem cells proliferation (41).

Contrarily, Campa *et al*. have shown that activation of Notch in mature cardiomyocytes is correlated with apoptosis and concluded that prolonged Notch activation can thus contribute to cardiac pathogenesis (47). Similarly, Zheng *et al*. concluded that elevated expression of Notch1 was positively correlated to that of NF-κB in coronary heart disease rat cardiomyocytes, and that Notch1 activation plays a role in the development of coronary heart disease (48). So it is believed that the effect of active Notch signaling in adult cardiomyocytes will largely depend on the timing and dosing of the activation,

thus whether Notch pathway will induce cardiomyocytes apoptosis and therefore cardiotoxicity, or Notch will be unregulated in advance to protect cardiomyocytes against toxic insults (47,48).

The Role of Notch Signaling in Vascular Endothelial Cells

The Notch signaling is an important regulator of endothelial cell proliferation. It works in harmony and cooperates with different pathways to orchestrate the function of endothelial cells. Therefore, Notch receptors; 1, 2, and 4; as well as its ligands; Jagged 1 and 2 and DLL 1 and 4 are normally expressed in the endothelium of adults (41). It has been shown that Notch activating induces endothelial cell proliferation (49). Moreover, It also contributes to maintaining the integrity of the endothelium in stressful conditions such as ischemia and inflammation, mechanism may include antiapoptotic activity via increasing Bcl-2, in addition to modulating angiogenesis (50,51). Accordingly, it has been demonstrated that Notch inhibition leads to the induction of endothelial cells apoptosis and non-functional angiogenesis (52). The Notch endothelial protective activity was more imminent in cardiac allograft vessels with dysfunctional Notch 4 expression, in which endothelial dysfunction was prominent (50). Moreover, Notch signaling, by controlling the function of endothelial cells and maintaining endothelium integrity, plays a significant role in the formation of atherosclerotic plaques. Notch inhibition was found to worsen atherosclerosis by enhancing endothelial cells dysfunctions (41).

However, on the other hand, Liu *et al*. reported that Notch signaling regulated genes to be significantly upregulated in atherosclerotic loci (53). Similarly, Nus *et al*. found a correlation between endothelial Jag1–RBPJ complex and inflammation, via upregulating NF-κB and vascular cell adhesion molecule (54). Also, Quillard *et al*. have shown that increased Notch activity was associated with increased endothelial cells apoptosis, in response to inflammation (50). These contradictory results can be explained by the wide variability of receptors and ligands, and both the canonical and non-canonical signaling mechanisms (55).

The Role of Notch Pathway in the Vascular Smooth Muscle Cells

Notch pathway was also found to be a key regulator maintaining vascular smooth muscle cells (VSMCs) structure and function. The VSMCs expresses Notch receptors; 2 and 3; and jag 1 as the main ligand), and Notch 2 and Notch 3 mutations was found to induce defects in VSMCs development, giving a strong evidence of the implication of Notch pathway in the regulation of VSMCs differentiation, maturation, and recruitment during angiogenesis. It is also believed that Notch receptors mediate endothelial cells to VSMCs communication, and affect the phenotype of VSMCs (27,41,55,56). Experiments have shown that activation of Notch signaling not only increases proliferation but also prevents apoptosis of VSMCs. Recently, the Notch pathway has been strongly correlated to the pathogenesis of pulmonary hypertension and cerebral autosomal dominant arteriopathy via affecting VSMCs (43,57).

The Role of Notch Signaling in Macrophages

Although macrophages are not part of the cardiovascular system, but it is well-established that macrophages play an important role in cardiovascular pathogenesis, e.g. atherosclerosis and ischemia. M1 macrophages exaggerate post-myocardial infarction injury by inducing inflammation and scaring, while the anti-inflammatory M2 macrophages infiltration promotes tissue repair, thus the ratio of M1/M2 is of great importance (58). Notch pathway plays a significant role in inflammation by mainly affecting macrophages activity. Macrophages are divided into high-inflammatory M1; secreting proinflammatory cytokines such as IL-6 and TNF-α; and an anti-inflammatory M2; secreting anti-inflammatory cytokines such as IL-4 and IL-10 (59,60). It was reported that Notch1 induces the differentiation of M1 macrophages, increasing the M1/M2 ratio, and induces inflammation by increasing synthesis of IL-6 and TNF-α. Accordingly, Notch1 inhibition culminates in reduced M1/M2 ration, with increased secretion of Il-4 and IL-10 (Figure 4) (5,6,61,62).

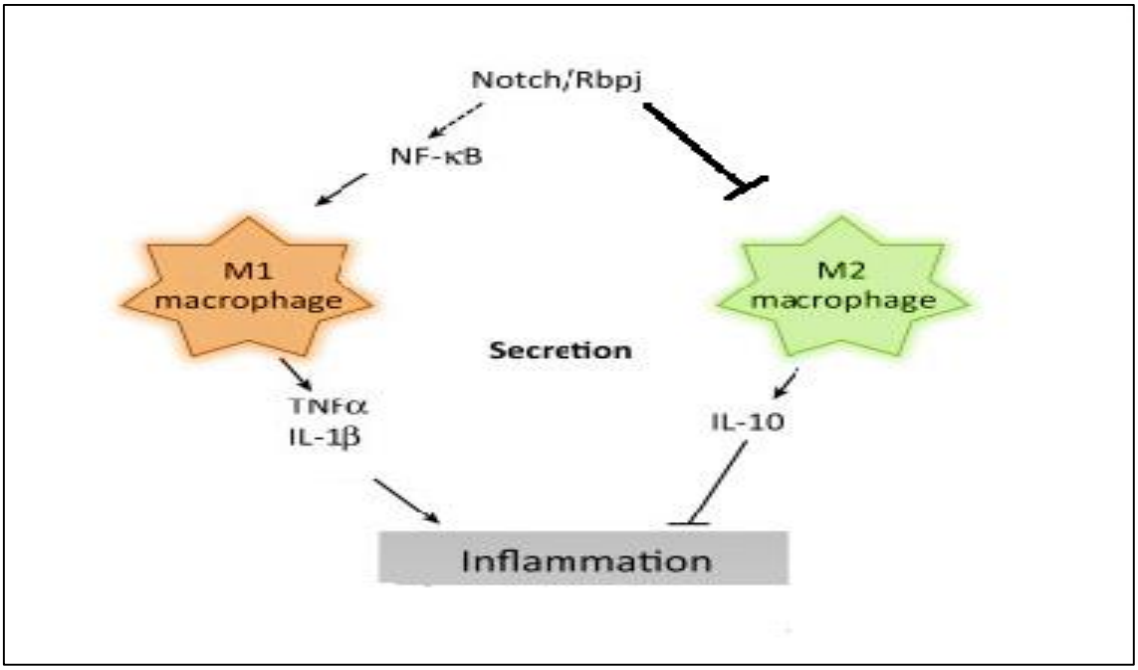

Figure 4: Notch Pathway in Inflammation.

Notch signaling affects inflammation by M1 macrophage activation via NF-κB. M1 secretes TNF-α and IL-1β that promotes inflammation. On the other hand, Notch activation inhibits M2 polarization this reducing IL-10, that inhibits inflammatory process (63).

In addition to affecting macrophage differentiation, Notch is also implicated in their migration and secretion of inflammatory mediators. Aoyama *et al*. concluded that Notch inhibition reduces macrophages migration and infiltration into atherosclerotic plaques. Other studies showed that proinflammatory mediators, such as lipopolysaccharides and IL-1β, induce the expression of Notch Dll4 receptor in macrophages. Dll4 induces further release of proinflammatory cytokines, thereby initiating a positive feedback loop. *Besides,* incubation of macrophages with Dll4 stimulates the transcription of proinflammatory genes, e.g., iNOS, and activates Akt, and NF-κB. Collectively, inhibition of canonical Notch Pathway is followed by inhibition of macrophages inflammatory responses (64,65).

In the heart, Yin *et al*. found that M1 macrophages infiltrating the infarct area express mainly Notch 1, and their infiltration was inhibited by the use of a Notch inhibitor, associated with increased M2 macrophages and better cardiac function following myocardial infarction (9). It was also found that DLL 4 accelerate the inflammation of the heart vessels, and that antibody to DLL 4 suppresses M1 macrophages (66). That from these data, it can be concluded that Notch activation in macrophages induces inflammation, mainly by increasing M1/M2 ratio, with DII4 and Notch1 are considered the main keyplayers (67).

CONCLUSION

There is a growing evidence regarding the implication of Notch Pathway in cardiovascular diseases' development. Better understanding of the diversity and contradictory effects of Notch signaling needs further research, as it may help in ameliorating or even preventing the development of different cardiac insults.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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