Expert Opinion on Therapeutic Targets

ISSN: 1472-8222 (Print) 1744-7631 (Online) Journal homepage: https://www.tandfonline.com/loi/iett20

The Notch pathway: a novel therapeutic target for cardiovascular diseases?

Giorgio Aquila, Aleksandra Kostina, Francesco Vieceli Dalla Sega, Eugeniy Shlyakhto, Anna Kostareva, Luisa Marracino, Roberto Ferrari, Paola Rizzo & Anna Malaschicheva

To cite this article: Giorgio Aquila, Aleksandra Kostina, Francesco Vieceli Dalla Sega, Eugeniy Shlyakhto, Anna Kostareva, Luisa Marracino, Roberto Ferrari, Paola Rizzo & Anna Malaschicheva (2019) The Notch pathway: a novel therapeutic target for cardiovascular diseases?, Expert Opinion on Therapeutic Targets, 23:8, 695-710, DOI: 10.1080/14728222.2019.1641198

To link to this article: https://doi.org/10.1080/14728222.2019.1641198

Published online: 14 Jul 2019.

EXPERT OPINION ON THERAPEUTIC TARGETS
2019, VOL. 23, NO. 8, 695–710
https://doi.org/10.1080/14728222.2019.1641198

Taylor & Francis
Taylor & Francis Group

REVIEW

The Notch pathway: a novel therapeutic target for cardiovascular diseases?

Giorgio Aquila[a], Aleksandra Kostina[b,c*], Francesco Vieceli Dalla Sega[d], Eugeniy Shlyakhto[b], Anna Kostareva[b], Luisa Marracino[e], Roberto Ferrari[a,d], Paola Rizzo[d,e*] and Anna Malaschicheva [b,c,f*]

[a]Department of Medical Sciences, University of Ferrara, Ferrara, Italy; [b]Laboratory of Molecular Cardiology, Almazov National Medical Research Centre, St-Petersburg, Russia; [c]Laboratory of Regenerative Biomedicine, Institute of Cytology, Russian Academy of Sciences, St-Petersburg, Russia; [d]Maria Cecilia Hospital, GVM Care & Research, Cotignola, Italy; [e]Department of Morphology, Surgery and Experimental Medicine and Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy; [f]Department of Embryology, Faculty of Biology, Saint-Petersburg State University, St. Petersburg, Russia

ABSTRACT

Introduction: The Notch pathway is involved in determining cell fate during development and postnatally in continuously renewing tissues, such as the endothelium, the epithelium, and in the stem cells pool. The dysregulation of the Notch pathway is one of the causes of limited response, or resistance, to available cancer treatments and novel therapeutic strategies based on Notch inhibition are being investigated in preclinical and clinical studies in oncology. A large body of evidence now shows that the dysregulation of the Notch pathway is also involved in the pathophysiology of cardiovascular diseases (CVDs).

Areas covered: This review discusses the molecular mechanisms involving Notch which underlie heart failure, aortic valve calcification, and aortic aneurysm.

Expert opinion: Despite the existence of preventive, pharmacological and surgical interventions approaches, CVDs are the first causes of mortality worldwide. The Notch pathway is becoming increasingly recognized as being involved in heart failure, aortic aneurysm and aortic valve calcification, which are among the most common global causes of mortality due to CVDs. As already shown in cancer, the dissection of the biological processes and molecular mechanisms involving Notch should pave the way for new strategies to prevent and cure these diseases.

ARTICLE HISTORY
Received 26 March 2019
Accepted 4 July 2019

KEYWORDS
Notch; heart failure; aortic aneurysm; aortic valve calcification; endothelial dysfunction; cardiotoxicity; macrophages; Dll4; Notch1

1. Introduction

The Notch pathway, a mediator of the communication of molecular signals between adjacent cells, plays a pivotal role in the cardiovascular system, both during development and postnatal life. Whereas the role played by Notch during the development of the cardiovascular system has been deeply investigated, we are just now beginning to dissect the role of this signaling pathway in the molecular mechanisms involved in the postnatal homeostasis of the vasculature and of the heart and, consequently, in the pathophysiology of cardiovascular diseases.

The aim of this review is to provide an overview of the current knowledge on the role of the Notch pathway in the most common cardiovascular diseases, that is, aortic valve disease, aortic aneurysm and heart failure and, from a translational perspective, to focus on those areas of investigation close to the identification of novel therapeutic approaches targeting Notch. The challenges in this field, represented by the multiple, often opposite roles played by the Notch receptors in the cardiovascular system, and in general by the complexity of this signaling, will be discussed.

2. The Notch pathway

1.In mammals, the Notch family comprises four receptors (Notch 1–4) and five ligands (Delta-like ligand (Dll) 1, 3, 4 and Jagged (Serrate) 1, 2). The Notch receptors are type I single-pass transmembrane proteins with a large extracellular ligand binding region (Notch extracellular domain, NECD), a membrane-spanning and an intracellular domain (Notch intracellular domain, NICD). The NICD consists of a recombination signal binding protein-1 for J kappa (RBP-Jκ)-associated molecule (RAM) domain, seven ankyrin repeats (ANK), edged by two nuclear localization signals (NLS), a transcription factor scaffold domain or transactivation domain (TAD), present in Notch 1, 2, 3, and a region rich in proline-glutamate-serine-threonine (PEST) required for degradation of the protein [1]. The Notch ligands are divided into two general classes, depending on their homology to Drosophila prototypes Delta and Serrate, and are collectively referred to as DSL family (Delta/Serrate/LAG-2). They are type I transmembrane proteins with domain organization (Figure 1). Even though these Notch ligands are responsible for the majority of processes regulated by the Notch signaling, other structurally unrelated, non-canonical ligands, have also

CONTACT Paola Rizzo rzzpla@unife.it Department of Morphology, Surgery and Experimental Medicine and Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy; Anna Malaschicheva amalashicheva@gmail.com Almazov National Medical Research Centre, 2 Akkuratova Str., St-Petersburg 197341, Russia
*equal contribution

Article Highlights

- The Notch pathway plays pivotal roles in the cardiovascular system, both during the development and postnatal life
- The role of Notch in congenital cardiovascular diseases is well established
- Dysregulated Notch pathway is increasingly linked to heart failure, aortic aneurysm, and aortic valve calcification
- Cardiovascular diseases and cancer share risk factors and underlying molecular pathways, including the Notch signaling
- The dysregulation of Notch in solid tumors and leukemias has been long investigated and clinical trials targeting Notch in cancer are ongoing
- The accumulated experience of almost 30 years on the targeting of Notch for cancer therapy should expedite the development of novel Notch-based therapeutic approaches for cardiovascular diseases

This box summarizes key points contained in the article.

been identified [2]. A detailed description of Notch receptors and ligand and their function is available elsewhere [3].

The interaction between the Notch receptor on one cell and the ligand on the adjacent cell (trans-interaction) results in conformational changes of the receptor extracellular domain exposing a motif that is cleaved by ADAM (A Disintegrin And Metalloproteinase) (S2 cleavage site) [4]. The S2 cleavage creates membrane-tethered intermediate called Notch extracellular truncation (NEXT) that is substrate for γ-secretase protease complex, containing presenilin1, presenilin2, Pen-2, Aph-1 and nicastrin [5,6]. The γ-secretase cleaves the Notch receptors at the two distinct sites, S3/S4, and releases NICD, which translocates to the nucleus to regulate gene transcription [7]. Nuclear Notch signals cause changes in gene expression mediated by the transcription factor CSL (an acronym for CBF-1/RBP-Jκ in *Homo sapiens/Mus musculus*, respectively, Suppressor of Hairless in *Drosophila melanogaster*, Lag-1 in *Caenorhabditis elegans*). In the absence of NICD, CSL is bound by corepressor proteins, such as SMRT (NcoR) and SHRP (MINT/SPEN), and inhibits the transcription of target genes by recruiting histone deacetylases [8]. NICD/CSL binding displaces corepressor complexes and allows recruitment of the transcriptional coactivator Mastermind-Like-1 (MAML) and histone acetyltransferases such as p300 [9]. Formation of CSL/NICD/MAML complex results in direct transcriptional activation of target genes (Figure 1). Recent data also show a direct implication of NICD in chromatin remodeling [10,11]. In cancer and immune system, a Notch signaling active in the cytoplasm (referred to as 'non-canonical' Notch signaling to distinguish it from the canonical, nuclear Notch signaling) has been described [12]. Non-canonical Notch signaling occurs without CSL involvement and depends on interactions between the NICD and pathways such as mammalian target of the rapamycin 2 complex (mTORC2)/Akt, Wnt/β-catenin and Nuclear Factor kappa B (NF-κB)/AKK-α/AKK-β [12]. Non-canonical Notch signaling has also been observed in mitochondria where interactions between Notch and PTEN-induced kinase 1 (PINK1) promote cell survival by activating the mTORC2/Akt pathway. Notch signaling can be also triggered by ligands other than Jagged/Dll. These alternative ligands include F3/contactin, Delta-like 1–2 (DLK1-2) and epidermal growth factor domain 7 (EGFL7) and seem to influence Notch signaling by competing with Jagged/Dll for Notch

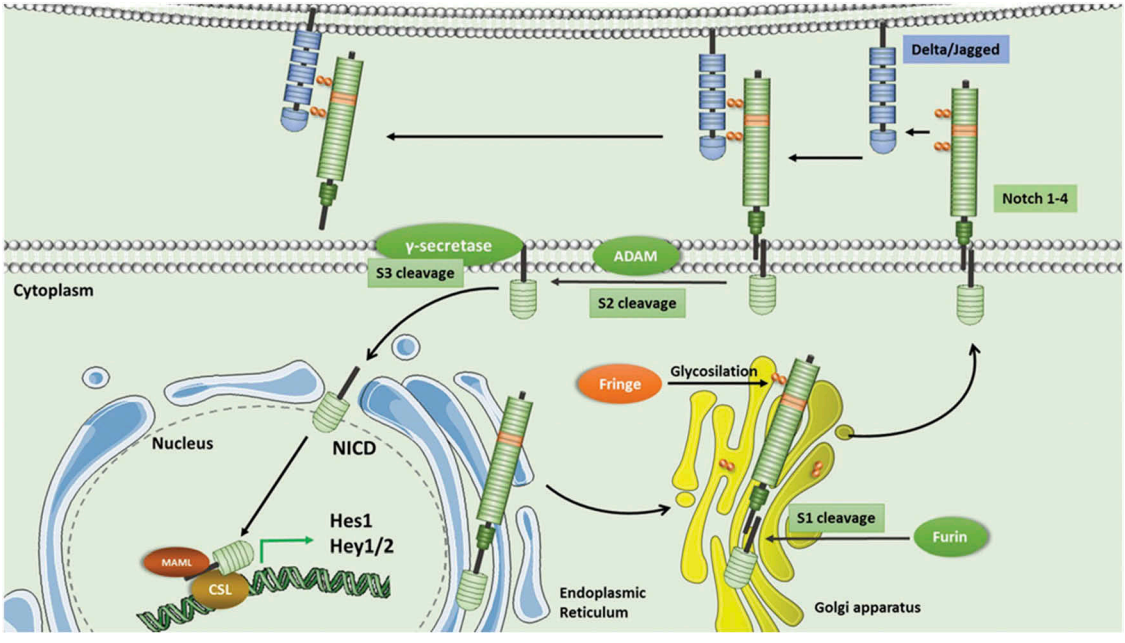

Figure 1. The Notch signaling. The Notch signaling pathway is mediated by four Notch transmembrane receptors (Notch 1–4 and five transmembrane ligands (Delta-like 1, Delta-like 3 and Delta-like 4, Jagged1 and Jagged2). Notch receptors are synthesized as single-chain precursors that are cleaved, in the Golgi by a Furin-like convertase, into an extracellular and a transmembrane subunit and modified by glycosyltransferases Fringe. Notch signaling is triggered after ligand-receptor interaction which leads to two sequential proteolytic cleavages by A Disintegrin And Metalloproteinases (ADAM) to remove the extracellular subunit, and then, by a multisubunit protease γ-secretase, which catalyzes the second proteolytic cleavage that liberates Notch intracellular domain (NICD). NICD translocates into the nucleus where it binds to the transcription factor CSL (CBF1 in humans, Suppressor of Hairless in Drosophila and LAG in C. elegans) and coactivators, including Mastermind-like proteins (MAML1). The activated complex upregulates the expression of target genes, such as Hes1 and Hey1/2. This last sequence of events is defined canonical Notch signaling. For a description of the alternative non-canonical Notch signaling the reader is referred to the main text.

receptor binding [12,13]. Notch activation, by either mechanisms described above, influences cell proliferation, apoptosis, and differentiation [13]. More recently, a role of Notch in the regulation of autophagy has been reported in cancer [14] and immune cells [15].

Notch signaling is extremely dose-sensitive, due to the lack of a signal amplification step or utilization of secondary messengers to transmit the signal from the cell surface to the nucleus [16]. For this reason, Notch activity is fine tuned by post-translational regulation [17] and the stability of the active form of Notch is tightly regulated, mainly by phosphorylation and ubiquitination [18]. Another peculiarity of this signalling pathway is that, by altering the profile of ligands and receptors expressed in the cells and/or the affinity of the receptors for a specific ligand, through glycosylation mediated by a class of enzymes called Fringe [19] (Figure 1), numerous scenarios of Notch activation patterns can be generated in a specific cell or tissue [20]. Of interest in this context, binding of receptor and ligand in the same cell (cis-interaction) can lead to the inhibition of signaling [21]. Additionally, interplays with other signaling pathways are of crucial importance in Notch-mediated regulation of several physiological processes, both during development and postnatally [13]. Specifically, interactions between Notch and Wnt/β-catenin control arterial specification of endothelial cells and regulate epithelial-mesenchymal transition that initiates myogenesis during embryogenesis [13]. Notch and Bone Morphogenetic Protein (BMP) signaling crosstalk is implicated in cardiac valve formation, regulates blood vessel branching, and controls chondrocytes proliferation during the formation of the cartilage [13]. Crosstalk between Notch and hypoxia-inducible factors (HIF) regulates hypoxic responses, in which low oxygen levels result in the increase of NICD that in turn stabilizes HIF-1α and HIF-2α [13]. Interactions between Notch and NF-κB pathway are crucial for the regulation of cellular immune responses and inflammation [22]. Furthermore, in recent times, it has been shown that the interactions between estrogen receptors and the Notch pathway regulate several processes underlying cardiovascular homeostasis [23].

As a result of all these interactions, the outcome of Notch activation is cell type- and context- dependent with multiple combinations of receptors and ligands transducing different biological effects [10,24].

2.1. Notch in cardiovascular development

The sequential expression of components of the Notch pathways and related genes is indispensable during the development of heart and vessels. The role of Notch in the cardiovascular development has been deeply investigated and its discussion is beyond the scope of this review: we give here a brief overview of the field, to set the stage for our discussion of the role of Notch in cardiovascular disease, and refer the reader to several excellent reviews for details [25–27]. Notch ligands and receptors are sequentially expressed in the developing heart, thus ensuring proper heart development. Notch ligand Jagged1 is expressed very early during heart development, labeling the presumptive valve area of the atrioventricular channel (AVC) and the trabecular myocardium, while ligand Dll4 and receptors Notch2 and Notch4 are expressed in the endocardium. Then, Dll4 expression decreases whereas Jagged1 expression is maintained in the endocardium and is activated in the compacted myocardium [25,28]. This sequential expression of Notch genes *Jagged1, Dll4, Notch2 and Notch4* supports myocardial patterning, maturation and compaction and cardiac trabeculae formation [25,28]. The Notch pathway plays an important role in the development of outflow tract (OFT) of the heart which starts with endothelial-to-mesenchymal transition (EMT) in the endocardial cells leading to the formation of cardiac valves. The involvement of Notch1 in this context is indicated by the expression of this receptor in prospective valve endocardium at the beginning of EMT [29]. Consistently with the importance of Notch in the developing heart, mice lacking Notch target genes *Hey1* and *Hey2* die during embryogenesis due to severe cardiovascular malformations, including impaired development of EMT [30].

The Notch pathway is crucial for vasculature development by determining arterial-venous specification mainly through the endothelial expression of Dll4 as demonstrated by severe vascular defects and lack of arterial markers in *Dll4*-deficient embryos as well as in *Rbpj* mutants and *Hey1/Hey2* double mutant embryos [30,31]. Notch gain-of-function mouse embryos also develop arteriovenous malformations [32] and ectopic Notch4 and Notch1 expression in endothelial cells results in the development of arteriovenous malformations and embryonic vascular remodeling defects [33]. Dll4 expression is required for vascular stabilization and differentiation of the emerging vascular tree. Specifically, the decision to either form a new sprout or widen the original vessel relies on differential expression patterns of Dll4, BMP and vascular endothelial growth factor (VEGF) between endothelial cells [34].

Thus, Notch pathway is indispensable for embryonic development and postnatal maintenance of cardiac and vascular tissues (Table1): its role in postnatal life and the consequent contribution of its dysregulation to cardiovascular disease will be thoroughly discussed in the next paragraphs.

3. Congenital heart disease directly associated with Notch pathway mutations

Mutations in the genes of the Notch family cause a wide range of congenital defects affecting the heart and vessel development. These mutations are often characterized by incomplete penetrance and variable expression. Mutations in human *NOTCH1* gene were first described in association with bicuspid aortic valve (BAV), a congenital heart defect (CHD) in which the aortic valve has two leaflets instead of three, and this state is most commonly associated with pathologic calcification of the aortic valve [35] and dilations and aneurysms of the aorta [35,36]. Recently, we and others have described *NOTCH1* gene variants and mutations in aortic stenosis patients with normal tricuspid valve [37–39]. Besides BAV, Preuss at al. studied families with a history of left-ventricular OFT obstructions and revealed protein-altering mutations clustering predominantly in genes *NOTCH1, ARHGAP31, MAML1, SMARCA4, JARID2* and *JAGGED1*, all belonging to the Notch signaling cascade [36].

NOTCH1 and *JAG1* mutations have been associated with Tetralogy of Fallot (TOF), a severe developmental CHD [40],

Table 1. The role of Notch pathway in the cardiovascular system under physiological and pathological conditions.

Region/structure	Physiological conditions	Ref(s)	Pathologic conditions	Ref(s)
MYOCARDIUM	Jagged1/Dll4 -Notch1 control heart development	[25,28]	NOTCH1 and JAGGED1 mutations are associated with TOF	[40]
			NOTCH1 mutations are associated with hypoplastic left heart syndrome	[42]
			NOTCH1, RBPJ and DLL4 mutations cause AOS	[43]
	Jagged1/Notch1 promotes proliferation of immature cardiomyocytes . Notch1 is turned off in mature cardiomyocytes because of epigenetic regulation	[74,118]	JAGGED1 and NOTCH2 mutations cause Alagille syndrome	[46]
			Dll1 levels are increased in the serum of patients with heart failure and dilated cardiomyopathy	[75,76]
			Dll4 inhbition with anti-Dll4 antibodies causes in heart failure	[78,79]
			After infarction, or in overloaded myocardium, Notch1 reactivation reduces cardiomyocyte death, hypertrophy and fibrosis	[74,128,129]
	Notch1 is active in CPCs where it maintains an undifferentiated proliferative state and favors myocyte lineage specification	[130]	Notch1 reduces fibrotic response by a Jagged1-mediated differentiation of CPCs into cardiomyocytes in heart undergoing pressure overload	[131]
			Notch1 in the infarcted myocardium leads to increased angiogenesis and improved cardiac function	[142]
	Notch1 mediates the protective effect of blood shear stress on the valve endothelium blocking the expression of inflammatory genes	[53]	Notch1 is involved in osteogenic transition and valvular calcification	[51–55]
AORTIC VALVES	Notch1 orchestrates EMT necessary to heart valves formation	[29]	NOTCH1 mutations are associated to BAV and CAVD	[35]
ARTERIES/AORTA	Notch1 is necessary for transduction of the positive effects of shear stress and for the normal function of the endothelium	[53]	Notch1 downregulation – induced by inflammation, dyslipidemia, and low levels of estrogens – causes endothelial dysfunction in regions characterized by turbulent blood flow.	[23]
	Notch1 is necessary to preserve endothelial integrity and barrier function	[84,85]		
	Notch controls SMCs differentiation and in necessary for the maintenance of wall integrity	[60–63]	NOTCH1 loss-of-function is linked to dilations and aneurysms of the aorta	[35,36,56]
			Dll4/Jagged1/Notch1/Notch4 determine arterial-venous specification during development and regulates postnatal angiogenesis	[80,141]
	Jagged1/Notch3 interactions promotes SMCs survival and the maintenance of non- proliferative and contractile phenotype	[61,94–96]	NOTCH3 mutations causes CADASIL	[47]
			Jagged1 and Hey2 reduction and Dll4 increase are associated with inflammation and decreased SMCs contractility induced by cholesterol	[20]
IMMUNE CELLS	Notch1, Notch2, Notch3, Dll4, Dll1, Jagged 1 controls immune cells differentiation and function	[92,115]	Notch1 activation in macrophages promotes M1 inflammatory phenotype while inhibiting M2 polarization	[102,103]
	Notch1 and Notch2 promotes macrophages activity during wound repair processes	[111]	Endothelial Dll4 promotes macrophages M1 polarization and the production of inflammatory ctyokines and inhibits IL4-induced M2 polarization	[106–109]
			Endothelial Dll1 regulates Notch2-mediated conversion of Ly6Chi monocytes into Ly6Clo monocytes facilitating the repair of ischemic tissues	[112]

Abbreviations: CPC, cardiac precursor cell; SMC, smooth muscle cell; EMT, endothelial to mesenchymal transition; TOF, Tetralogy of Fallot; AOS, Adams-Oliver syndrome; BAV, bicuspid aortic valve; CAVD, calcific aortic valve disease; CADASIL; Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy

characterized by stenosis of right ventricular OFT, ventricular septal defect, dextraposition of aorta and right ventricular hypertrophy. A recent study of whole exome sequencing in 829 TOF patients has shown that the *NOTCH1* locus is the most frequent site of genetic variants predisposing to nonsyndromic TOF [41]. Mutations in *NOTCH1* are also associated with hypoplastic left heart syndrome, a defect in which the left side of the heart is underdeveloped [42] and with the Adams-Oliver syndrome (AOS), a rare congenital disease characterized by cardiac, vascular and neurological symptoms, including valvular and ventricular abnormalities, atrial septal defect, and TOF [43]. In AOS have been also found mutations in *RBPJ* and *DLL4* [43] and in *EOGT*, encoding an EGF domain-specific O-linked N-acetylglucosamine transferase which presumably could regulate Notch receptors [44].

A recent study of a cohort of 428 patients with a spectrum of diseases affecting aortic development such as aortic valve stenosis, a bicuspid aortic valve, aortic valve insufficiency coarctation of the aorta, and hypoplastic left heart syndrome, subvalvular or supravalvular aortic stenosis, hypoplastic aortic arch, interruption of the aorta, and mitral valve anomalies clearly demonstrates that the phenotypic spectrum of *NOTCH1* mutations includes a wide variety of pathologies affecting the whole conotruncus of the heart [45]. This is in agreement with the described role of the Notch pathway in determining the fate of neural crest–derived cells. Alagille syndrome (ALGS), a congenital disease that mainly affects liver ducts and heart development, in the vast majority (up to 96%) of patients, is caused by mutations in *JAGGED1* and *NOTCH2* (in 1–2% of the cases) [46].

Lastly, CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy), a hereditary autosomal dominant disease, which affects the small cerebral arteries, thus causing subcortical infarcts and damages to the white matter (leukoencephalopathy), is associated with mutations in *NOTCH3* [47].

4. Cardiovascular disease not always directly associated with defined mutations

As Notch is important for cardiovascular development, it is not surprising that mutations in genes of the Notch family lead to various types of cardiac and vascular disorders. However, there is accumulating evidence that a wider spectrum of cardiovascular diseases is associated with dysregulation of the Notch signaling pathway, even without obvious mutations in Notch-related genes.

4.1. Calcific aortic valve disease

Calcific aortic valve disease (CAVD) is a frequent heart valve disease characterized by progressive mineralization of the valvular tissue. Both endothelial and interstitial cells, which form the aortic valve, contribute to its calcification [48]. To some extent, mineralization of the aortic valve shares similarities with bone ossification, for which Notch is considered as one of the most important pathways. The exact role of Notch in aortic valve calcification remains unknown and the existing evidence is controversial. Acharya et al. demonstrated, through chemical inhibition of Notch by γ-secretase inhibitor DAPT, that inhibition of Notch1 activity resulted in accelerated calcification while stimulation of Notch signaling attenuated the calcific process [49]. Similarly, Nigam et al. showed that Notch1 in aortic valve cells represses Bmp2 and prevents the progression of osteogenic calcification [50]. Furthermore, calcific aortic valve disease has been associated with higher expression levels of lncRNA H19, which interferes with the expression of *NOTCH1* [51]. Contrary to these findings, Zeng et al. showed that Notch1 actually promotes osteogenic calcification in human valve interstitial cells (VIC) [52]. Recent *in vitro* work, using induced pluripotent stem cell (iPSC)-derived endothelial cells, showed that *NOTCH1* haploinsufficiency disrupts endothelial cell response to shear stress and unlocks pro-osteogenic and inflammatory network [53]. Whether activation of Notch is pro- or anti-osteogenic is unclear. Most probably, the described discrepancies arise from different *in vitro* and *in vivo* experimental conditions used in different laboratories. Our studies show that in CAVD patients dysregulated Notch signaling is associated with pathological mineralization of the valve cells [54]. Specifically, we report that the profile of Notch-related gene expression is different in aortic valve interstitial cells of patients, compared to cells of healthy individuals. This difference is associated with dysregulated Notch-dependent events in the cells of the patients, such as NICD-dependent induction of EMT and calcification [54]. Consistent with the role of Notch in aortic valve calcification are genetic studies showing that the only proved candidate gene for BAV, a risk factor of CAVD development, is *NOTCH1* [55].

4.2. Aortic aneurysm

Thoracic aortic aneurysm (TAA) is a dangerous condition, which is manifested in patients by progressive growth of the thoracic aorta diameter due to destructive changes in the aortic wall. TAA could be a consequence of degenerative or hypertensive aortic enlargement or to less common genetic disorders, such as Marfan syndrome, Ehlers-Danlos, or other connective tissue diseases [56].

The aortic wall consists predominantly of endothelial cells and SMCs. Notch ligands expressed by endothelial cells activate Notch signaling in the underlying SMCs, which in turn ensures integrin adhesion to the endothelial basement membrane and induces maturation and differentiation of these cells [57]. There are several studies suggesting that lateral induction of Notch signaling within multiple SMC layers, ensures differentiation induced by an endothelium signal [58]. Mice with an endothelial deletion of the Jagged1 gene show poor SMCs differentiation and expression of SMCs markers [59]. Furthermore, activation of Notch signaling in SMCs by endothelial-expressed Jagged1 leads to increased expression of Jagged1 and Notch3 [60] which, together with Notch2, appears to be the most important Notch receptor for SMCs [61]. The critical role of Notch in blood vessels stabilization has been elegantly demonstrated by the group of Duarte that showed that upregulation of Jagged1 in the endothelium mediates the recruitment of pericytes needed for the maturation of the new vessels [62]. Lastly, we have recently shown that endothelial cells are capable of driving smooth muscle osteogenic gene expression via cell-cell contact and activation of Notch signaling [63].

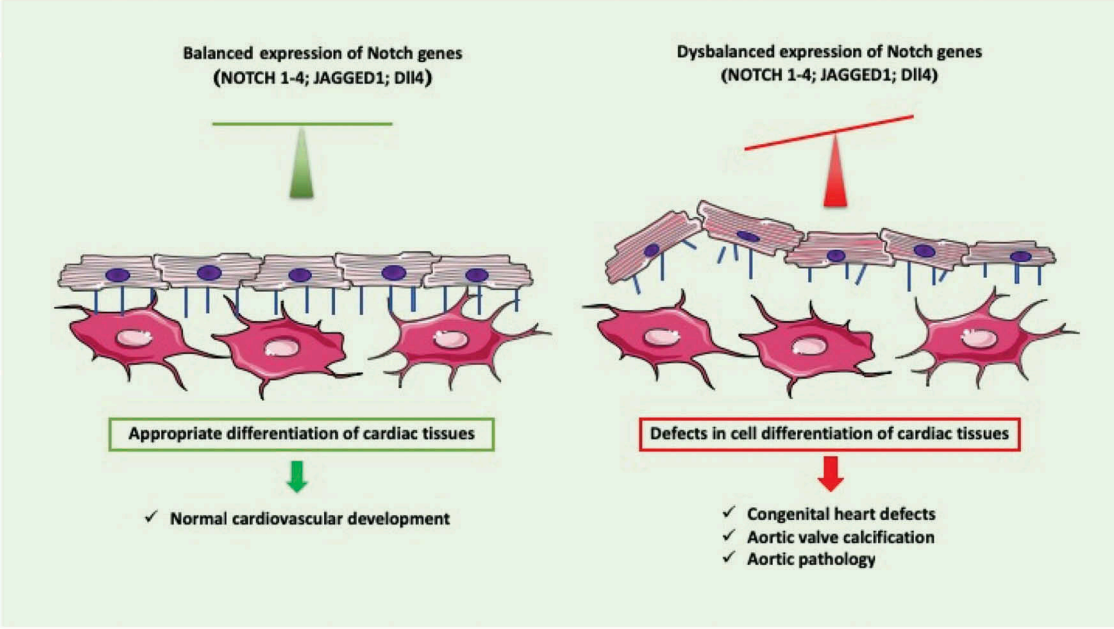

Figure 2. **Fine-tuned expression of Notch component genes is necessary for the proper maintenance of vessel walls and cardiac tissues**. Balanced expression of several Notch components is necessary for the proper development of cardiac tissues. Concerted actions of several Notch components are needed for the proper vessel function during development and lifetime. The data from various models suggest that dysregulation in expression levels of Notch components is associated with the pathological state of aorta and aortic valve and congenital heart defects.

Mutations in *NOTCH1* have been linked to BAV, which is associated with ascendic aorta aneurysm, but a clear involvement of *NOTCH1* mutations has not been described for aortic aneurysm in patients with the normal tricuspid aortic valve. In support for a role of Notch1 inactivating mutation in TAA, a recent study has shown that *Notch1*$^{+/-}$ mice develop aortic root dilation [64]. Consistent with the involvement of Notch in TAA, we have reported a dysregulation of Notch in aortic endothelial and SMCs cells from TAA patients, regardless the valve morphology and the presence of mutations in *NOTCH1* gene [65–68]. This was concomitant with decreased expression of contractile markers in aortic SMCs of TAA patients [69], in line with other observations suggesting that dysregulated Notch in endothelial and SMCs could be involved in changes in differentiation state of SMCs and subsequent disruption of aortic wall integrity. We have also shown that endothelial cells of TAA patients show dysregulated Notch, BMP and Wnt/β-catenin related signaling and impairment of Dll4-mediated Notch activation in response to flow [68]. Aortic wall is subjected to a constant mechanical stress and Notch, Wnt and BMP pathways are critical in maintaining endothelial integrity and proper differentiation state of endothelial cells [70,71]. Furthermore, activation of Notch in response to flow is an important differentiation and stress resistance mechanism [72]. Our data show that these Notch-regulated crucial protective mechanisms of the vascular wall are impaired in aneurysmal patients [68].

In conclusion, the Notch signaling is involved in the development and maintenance of the vessel wall (Figure 2). Concerted actions of several genes of the Notch family and Notch target genes are needed for proper vessel function, thus the dysregulation of the Notch pathway is associated with the pathological state of aorta and aortic valve.

4.3. Heart failure

Heart failure (HF), the last step of the so-called 'cardiovascular disease continuum', is caused by changes in size and shape of the ventricle (pathological remodeling) consequent to myocardial infarction (MI), pressure or volume overload, inflammation, or cardiomyopathy. All these conditions cause the dysregulation of common pathways, leading to altered gene expression, cellular metabolism, protein turnover and, eventually, to the impairment of the heart contractile function [73].

There is a limited number of studies in patients showing the involvement of Notch in HF (reviewed in [74]). This is due to limited access to myocardium biopsies and to the scarce availability of circulating markers to assess the status of activation of Notch in the failing heart. Increased levels of the Notch ligand Dll1 were found in the serum of patients with HF [75] and dilated cardiomyopathy [76]: in both studies, the levels of Dll1 correlated with the number of adverse cardiovascular events [75,76]. In patients with dilated cardiomyopathy, the levels of periostin, a non-canonical Notch ligand, were higher and correlated with the degree of diastolic dysfunction [76]. The biological role and source of these soluble mediators in the serum of HF patients need further investigation [77]. A role for Notch in the pathophysiology of HF has also emerged in the context of phase 1 clinical studies to assess safety of Dll4-blocking antibodies to prevent tumor angiogenesis, that reported HF in some of the patients undergoing this treatment [78,79].

In contrast with the limited knowledge in patients, there is a large number of *in vitro* and *in vivo* studies showing the critical role of Notch in the maintenance of the homeostasis of the heart and arteries and the dysregulation of this pathway in HF development. In the next paragraphs, we will focus on

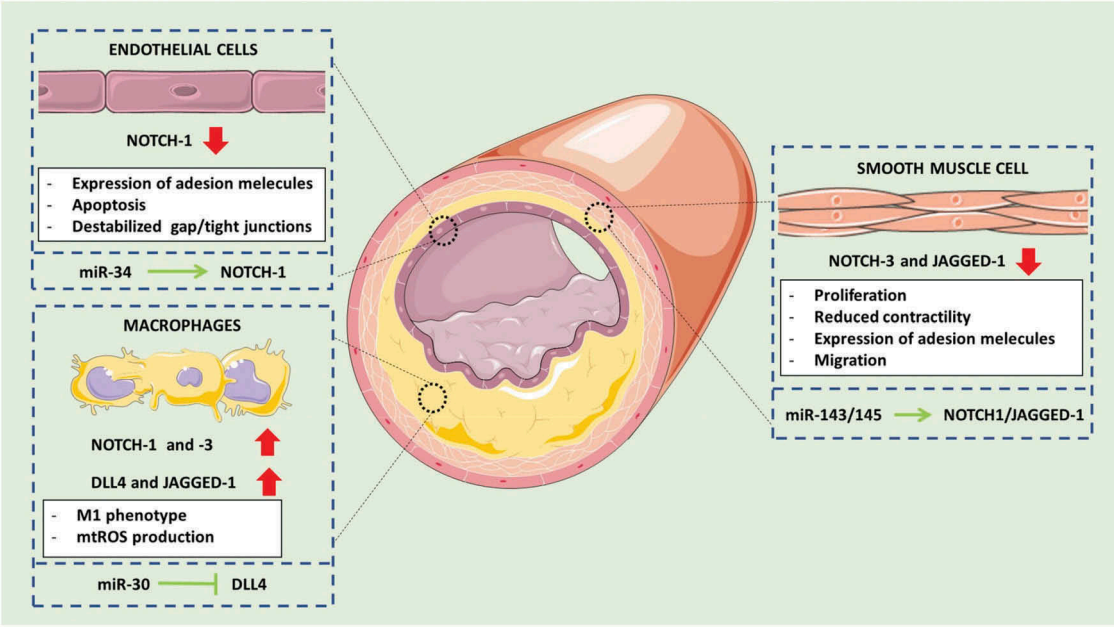

Figure 3. **The role of Notch pathway components in regulating the functions of the cells present in the vascular artery wall**. Dysregulated expression of Notch pathway components affects the onset and progression of atherosclerosis by causing endothelial cells dysfunctions, by favoring M1 phenotype in macrophages, and by enhancing the migration and proliferation of the vascular smooth muscle cells. miRNAs, by targeting specific components of the Notch pathway, may be used to repristinate the normal physiological functions of the different cells present in the vascular artery wall.

studies relative to the role of Notch in HF caused by atherosclerotic coronary artery disease.

4.3.1. Role of Notch in plaque formation and progression

Coronary atherosclerosis, responsible in some patients for ischemic heart disease and its different clinical manifestations, is characterized by a long, often silent phase preceding the plaque formation. The process starts with endothelial dysfunction, defined as the disruption of endothelium integrity (caused by increased apoptosis and permeability), impairment of its function (reduced nitric oxide (NO) production) and increased expression of surface proteins involved in the recruitment of inflammatory cells [80,81]. During the last decade, the role of Notch in preventing endothelial dysfunctions caused by inflammation, turbulent shear stress, dyslipidemia, and low estrogen levels has been demonstrated by a plethora of *in vitro* and *in vivo* studies (as discussed in [23,80]) (Figure 3). The first detailed description of molecular mechanisms underlying the protective role of Notch in the endothelium came from the work in iPSC (inducible Pluripotent Stem Cell) by Theodoris et al. showing that Notch1 is required to transduce the positive effects of shear stress and for the normal function of the endothelium [53]. More evidence was provided by the study of Briot et al showing that the downregulation of endothelial *Notch1* is involved in dyslipidemia-induced endothelial dysfunction in regions at risk for plaque formation, such as the lower aortic arch exposed to pro-atherogenic, turbulent blood flow [82]. Conversely, ivabradine, a heart rate slowing drug, delays plaque formation in the endothelium of the lower aortic arch of dyslipidemic mice by inducing the expression of anti-atherogenic genes, including *Notch1* [83]. Consistent with these observations, Mack et al. reported that loss of endothelial *Notch1* results in increased inflammation and plaque burden in the aorta of adult mice unequivocally showing that Notch1 is required to maintain cell-cell junctions elongated cell morphology, and endothelial alignment with blood flow [84]. More details were provided by Polacheck et al. that showed that shear stress maintains endothelial barrier function by activating non-canonical Notch1 specifically required for intact adherens junctions [85]. Recent work by Jabs et al. has shown that lack of endothelial *RBP-Jκ* affects also the heart, by inhibiting the fatty acid transport to cardiomyocytes and causing a switch to glycolysis, increased expression of 'fetal genes' and, consequently, HF [86]. The studies discussed so far, suggestive of a protective role of Notch1 against inflammation- and/or dyslipidemia-induced endothelium damages, are in contrast with the results of a study showing a role for Notch1/Jagged1 in the interleukin (Il)-1β mediated induction of vascular cell adhesion protein 1 (VCAM-1) in endothelial cells [87]. In line with this *in vitro* study, a high cholesterol diet resulted in reduced atherosclerosis in the aortic arch in Apolipoprotein E (ApoE)-deficient mice carrying either endothelial-specific deletion of *Rbpj* or systemic deletion of *Notch1* [88]. Furthermore, mice with endothelial *Notch1* knockdown showed attenuated inflammation, due to reduced histone H3K27 acetylation at a subset of NF-κB-directed inflammatory enhancers [89]. The conflicting sets of data from the *in vivo* studies could be, at least in part, explained considering that RBP-Jκ deficiency does not impair the Notch non-canonical signal [90] and that systemic Notch1 deletion will affect the activity and differentiation of several components of the immune system and different aspects of inflammatory responses [91,92]. Overall these discrepancies indicate that the role of Notch in the endothelium requires more investigations [90].

Vascular SMCs are important for plaque stability [93] and the Notch signaling is required for their survival [94] and to maintain them in a non-proliferative, contractile phenotype [95,96] (Figure 3). Consistent with these studies, we found that cholesterol loading of rat aortic vascular SMCs leads to reduction and

induction of contractility and inflammatory markers, respectively, in association with reduced levels of Jagged1 and Hey2 and increased levels of Dll4 mRNAs [20]. Jagged1/Notch3 axis seems to be the main player in determining the quiescent phenotype of vascular SMCs [61] but recent work in endothelial *Notch1* KO mice has found that activation of Notch1 by Jagged1, expressed on the adjacent endothelial cells, is required for Akt-mediated survival of SMCs in the artery wall [94]. On the contrary, in the context of vascular wall remodeling following artery damage, Chen and collaborators found that miR-34a inhibited neointima formation, that is decreased vascular SMCs proliferation, by reducing Notch1 expression [97]. Similarly, neointimal formation and vascular SMCs proliferation, following carotid artery ligation, were inhibited by perivascular injection of Notch1 siRNA [98] and γ-secretase inhibitor DAPT prevented migration and proliferation of SMCs of ductus arteriosus induced by angiotensin I through the Notch3-Hes1/2/5 axis [99]. These results were confirmed by a study revealing that phospholipase C (PLC)γ 1, through Akt, specifically activates Notch1, necessary for intima formation after vessel injury [100]. These apparently opposite actions of the Notch1 and 3 receptors on vascular SMCs functions highlights once again the complexity of the Notch system. Based on the studies discussed so far they could be, at least partially, explained by i) the different roles played by Notch1 and Notch3 or by ii) their different expression levels in vascular SMCs and/or by iii) the different cellular context present in atherosclerotic plaques and in the damaged artery wall.

Macrophages play a major role in plaque formation and evolution. The role of Notch receptors, mainly Notch1, in promoting the proinflammatory, M1 phenotype, has been thoroughly investigated (as reviewed in [92,101]). Notch1, rather than Notch2 and Notch3, plays a crucial role in determining the M1 phenotype through the activation of the NF-κB pathway [102]. This observation has been confirmed and extended by Singla and collaborators that have reported the reduction of M1 macrophages and pro-inflammatory cytokines production in monocytes treated with Notch1 siRNA, followed by the enhancement of M2 macrophage differentiation, characterized by the production of anti-inflammatory cytokines [103]. Consistently, miR-148a-3p, a Notch1-induced miRNA, promotes the differentiation of monocytes into macrophages, inhibits M2 polarization and favors instead a M1 state [104]. In macrophages isolated from *Notch1* KO mice and in the macrophage cell line Raw 264.7 treated with Notch1 siRNA, lipopolysaccharides (LPS)-induced NF-κB and hypoxia-inducible factors (HIF)-1α activation were decreased, confirming that LPS requires Notch1 for the transcriptional upregulation of M1 genes (NO synthase-2, *Nos2*, Tumor necrosis factor-alpha, *Tnfa*, and Interleukin-1β, *Il-1β*) [105]. Of interest, in this study Notch1 activation was linked to metabolic upregulation of mitochondrial oxidative phosphorylation and reactive oxygen species (ROS) production [105]. In terms of ligands, both Dll4 and Jagged1 have been found involved in macrophages function. Dll4, induced by Il-1β and LPS, exacerbates the inflammatory response of macrophages [106] and interferes with Il-4-induced M2 phenotype, causing instead macrophages apoptosis [107]. Other groups have confirmed the role of Dll4, expressed on endothelial cells, in Notch-mediated promotion of a M1 pro-inflammatory fate [108] and shown that blockade of Dll4-mediated pro-inflammatory activation of macrophages interferes with atherosclerosis progression [109]. Foldi et al. have reported instead that LPS strongly induces Jagged1/Notch1 signaling, leading to the amplification of inflammatory response [110] (Figure 3). In addition to the studies described so far showing mainly a role for Notch1 in macrophages activation, other authors have reported the involvement of other isoforms of Notch receptors in the regulation of macrophages function. Specifically, Notch3-activated signaling has been shown to be crucial for inflammatory phenotype of macrophages [106] and canonical Notch2 signaling, together with Notch1, seems to promote macrophages function in wound repair [111]. Furthermore in mice has been shown that *Dll1* expressed in the endothelium activates Notch2 signaling in monocytes, promoting the conversion of Ly6Chi monocytes into Ly6Clo monocytes, involved in repair of ischemic tissues [112].

Macrophages are not the only cells of the immune system involved in atherosclerosis: CD4 and CD8 T-cells also contribute to plaques formation and the dysregulation of the functions of the regulatory T-cells (Tregs) is involved in the progression of this disease [92]. Trying to blunt this complex response, without compromising the immune defenses, represents a major translational goal of the field of immunology of atherosclerosis [113,114]. The role of Notch in the regulation of immunity has been thoroughly investigated in physiological conditions [92,115], and in cancer [116]. Little is known about Notch-mediated regulation of acquired immunity in the context of atherosclerosis [92]: this knowledge could provide extra tools to harness this host defense program in our favor.

4.3.2. Notch in the ischemic heart and post-infarction remodeling

The role of Notch1 in stimulating the proliferation of immature cardiomyocytes and of their precursors has been shown by a large number of studies (reviewed in [74]). Recent work addressing heart regeneration in neonatal mice has shed more light on the mechanism by which Notch 1 sustains cardiomyocyte proliferation by showing that acetylation extends the half-life of the Notch1 intracellular domain and enhances its transcriptional activity [117]. It is widely accepted that Notch1 is turned off in mature cardiomyocytes [74] because of epigenetic modifications at Notch-responsive promoters that appear to be irreversible [118]. There are, nevertheless, numerous studies showing that the reactivation of Notch1 in the border zone of the infarct or in overloaded heart prevents cardiomyocytes apoptosis, reduces the extent of fibrosis and limits the hypertrophic response (reviewed in [74]). Both in the developing and the stressed heart, Notch1 signaling is mainly activated via expression of Jagged1 on the surface of cardiomyocytes. After activating Notch1, Jagged1 is endocytosed by the signaling cells and processed to a Jagged1 intracellular domain which binds to N1ICD and blunts its activity in the nucleus, thus contributing to the regulation of Notch signaling [119].

The Notch1 signaling pathway has been widely investigated in animal models of ischemia/reperfusion (I/R) injury. In the heart subjected to I/R injury, Notch1 has a protective function by counteracting oxidative/nitrate stress, decreasing

expression of inducible NOS (iNOS), increasing endothelial NOS (eNOS) phosphorylation and increasing the phosphorylation of Akt [120,121]. In addition to Notch1, Notch3 overexpression is involved in cardioprotection during I/R injury, by activating Akt signaling and by maintaining mitochondrial function [122]. Consistently with the protective role of Notch during I/R injury, diabetes seems to augment the severity of MI by downregulating Notch1 and Jagged1 [123]. Furthermore, inhibition of miR-363, upregulated during *in vitro* ischemia, protects cardiomyocytes through the activation of Notch1 and the induction of *Hes1* and *Hey1* [124]. Several compounds able to limit the I/R damage have been shown to activate Notch1 signaling, such as G1, an agonist of the G protein-coupled estrogen receptor (GPER30) [125], 2,3,5,4′-Tetrahydroxystilbene-2-O-β-D-glucoside, a compound extracted from *Polygonum multiflorum Thunb* [126], and the alkaloid berberine [127]. For both G1 and berberine, the cardioprotective action has been linked to Notch1-mediated activation of Akt [125,127]. Lastly, the Notch1 signaling is activated and contributes to the reduction of cardiomyocyte apoptosis associated with ischemic pre- and post-conditioning, both approaches being used to limit heart damages caused by an MI [128,129].

An important question to be answered about the role played by Notch1 in heart repair is: in which cell types of the damaged heart Notch1 is reactivated, and according to which molecular mechanisms? Felician et al. have shown that, due to the methylation of its promoter [118], it is unlikely that Notch1 is reactivated in mature cardiomyocytes. On the contrary, work by Boni et al. found that activation of Notch1 in c-kit-positive-cardiac precursor cells favors their myocytes rather than fibroblast lineage, still maintaining them in a highly proliferative state [130]. This observation is consistent with the study by Nemir et al. in a mouse model of overloaded myocardium in which Jagged1 overexpressed in cardiomyocytes stimulates the expansion of Nkx2.5-positive cardiac precursor cells [131].

The extent of early ventricular remodeling following an MI is determined by a delicate balance between timing and intensity of the inflammatory response, first required to remove the dead cells debris, and the fibrosis, which will replenish the space left by the dead cardiomyocytes [74,132]. In adult heart subjected to pressure overload, Notch1 reduces fibrotic response by a Jagged1-mediated differentiation of cardiac precursor cells into cardiomyocytes rather than fibroblasts [131]. Additionally, Notch1 activation ameliorates cardiac fibrosis by inhibiting transforming growth factor-β (TGF-β) signaling- induced fibroblast-myofibroblast transition [133,134]. Noteworthy, relaxin, a natural hormone with antifibrotic capacity, reduces aberrant TGF-β-mediated collagen deposition and fibrosis by activating Notch1 signaling [133]. In agreement with these data showing the involvement of Jagged1/Notch1- mediated signaling in the damaged heart, intra-myocardial delivery of a peptide mimic of Jagged1 improves cardiac function in rats subjected to MI by decreasing myocardial fibrosis [135].

MI elicited-inflammation leads to activation of interferon regulatory factor 3 (IRF3) and type I interferons (IFNs) in cardiac macrophages which, in turn, induces further damages to the heart. Interruption of IRF3-dependent signaling decreases cardiac expression of inflammatory cytokines and attenuates ventricular dilation, thus improving cardiac function [136]. Based on this evidence, IRF3 and the type I IFN response could be a potential therapeutic target for cardioprotection. Given the role played by Notch in the stimulation of inflammatory response in macrophages and in the production of type I IFN by plasmacytoid dendritic cells [137], it would be interesting to determine if Notch inhibition could reduce heart damages associated with IRF3/IFN and, in general, with inflammatory response during I/R. In agreement with this hypothesis, in an animal model of stroke, treatment with a Notch inhibitor reduced the size of cerebral damage by interfering with the recruitment of neutrophils leukocytes [138].

A Notch involvement may be relevant also for arrhythmias. Inflammation-dominated sympathetic sprouting adjacent to the necrotic area has been implicated in the etiology of arrhythmias resulting in sudden cardiac death. At 3 days post-MI, high NICD levels were observed in the macrophages infiltrating the infarct area. The administration of DAPT (30 min before MI and then daily) decreased the number of macrophages and attenuated the expression of nerve growth factor, thus preventing the process of sympathetic hyperinnervation and arrhythmias [139].

4.3.3. Notch and post-infarction angiogenesis

Targeting angiogenesis could restore the microcirculation in the reperfused MI, reducing adverse clinical outcome [140]. The role of Dll4/Notch1 in regulating angiogenesis during development and after birth, both under physiological conditions or tumor angiogenesis, has been thoroughly investigated [80,141]. In the heart, Dll4 is expressed mainly in the endothelium, which also expresses Notch1, nevertheless little is known about the role of Dll4/Notch1 in the ischemic heart and in the progression to HF. Kratsios et al. were the first to show that overexpression of Notch1 in the infarcted myocardium leads to increased angiogenesis and improved cardiac function [142]. Consistent with an active role of Dll4/Notch1 in regulating heart angiogenesis, Jabs et al. reported reduced fractional shortening and ejection fraction following injection of an anti-Dll4 antibody in wild type C57BL/6 mice and increased blood vessels density, without defects in blood perfusion, in the heart of mice lacking endothelial *RBP-Jκ* [86]. It should be noted that in the context of myocardial angiogenesis, RBP-Jκ may also act, independently of Notch1, by binding and inhibiting HIF-1α and HIF-2α [143]. In agreement with these observations suggesting a role for Notch in angiogenesis in the context of HF, we showed that treatment with serum of HF patients promotes sprouting angiogenesis and regulates Notch signaling in human umbilical vein endothelial cells [144].

4.3.4. Notch in stem cells for heart repair

The role of Notch in the regulation of stemness is well defined, both in embryonic and adult organs. In some tissues, as in the myocardium, Notch1 maintains cells in an undifferentiated, high proliferative state and its downregulation is required for the acquisition of the differentiated state [130]. In other tissues, such as the skin, Notch1 is turned off in the stem cells and its expression and activation is required for cells differentiation [145]. Depending on its role as an inhibitor or

promoter of stemness, Notch1 will behave as an oncogene or tumor suppressor gene, respectively, within a specific cell type [146].

In the last 20 years, a large body of data has been accumulated on the existence of cancer stem cells (CSCs), derived from the transformation of tissue stem cells or from the acquisition of progressive mutations of cancer cells [147]. These cells, characterized by tissue-specific surface markers [148], grow very slowly, compared to the bulk tumor cells, and therefore do not respond to classical chemotherapy, thus causing tumor recurrence [149]. Of interest, CSCs in tumors rely on Notch signaling for their survival, thus inhibition of Notch in these cells seems to be a promising attempt to prevent cancer growth and recurrence [150].

After an MI there is increased recruitment from the bone marrow of endothelial progenitor and mesenchymal stem cells (EPCs and MSCs, respectively) that may contribute to the repair of the damaged myocardium [74]. This observation provided the basis for the use of intracoronary injection of patients stem cells for heart regeneration following an MI. However and unfortunately, so far there has been no clinical benefit from these treatments [151]. It should be noted, however, that this approach is relatively new, thus, more knowledge of the molecular mechanisms regulating the function of these stem cells, together with more studies to identify the best cell types to be used and/or new modalities to enhance engraftment and cells function, should help to achieve heart regeneration by using patients stem cells [151]. Of relevance in this context, Notch1 modulates the recruitment and functions of EPCs and MSCs (as reviewed in [74,80]). Noteworthy, hypoxic preconditioning enhances proliferation, migration of EPCs and the secretion of NO and VEGF by activating the Notch-Jagged1 axis [152]. Targeting Notch could, therefore, help to obtain more 'performing' stem cells to be used for myocardium repair.

One of the mechanisms by which stem cell injection may favor heart repair is the formation of new cardiomyocytes from resident cardiac progenitor cells (CPCs) [153]. According to several studies the myocardium has the potential to heal itself: cardiomyocytes renewal in human has been documented, with a gradual decrease from 1% turning over annually at the age of 20% to 0.3% at the age of 75 [154] and CPCs have been identified in human explanted hearts [155]. Targeting Notch1 could enhance this intrinsic ability of the heart to repair itself since, as already discussed, Notch1 maintains CPCs in a high proliferative state [130]. Consistent with this observation, Adeno-associated virus- based gene transfer of activated Notch1-intracellular or soluble-Jagged1 increases number and size of cardiospheres originated from a heterogeneous population of poorly differentiated cells outgrowing from *in vitro* cultured cardiac explants [156]. Interestingly, CPCs isolated from human heart differentiate predominantly in smooth muscle cells but can be redirected to the cardiomyocyte fate by transient activation, followed by inhibition, of Notch1 signaling [157]. However, future studies aimed to promote myocardium repair by targeting CPCs should consider a recent work using single cell mRNA sequencing that found no evidence for the existence of a quiescent cardiac stem cell population, further quenching the enthusiasm toward the promotion of heart repair by activation of resident cardiac stem cells [158].

5. Conclusions

In summary, the Notch pathway plays a crucial role during the development and postnatally, for the maintenance of the homeostasis of many tissues and organs, including the heart, arteries, and immune cells. Today, a large body of evidence shows that the dysregulation of Notch, as already shown in cancer, is involved in congenital and not congenital cardiovascular diseases (Table 1).

6. Expert opinion: Notch a novel therapeutic target for cardiovascular disease?

Accumulating evidence shows that cancer and cardiovascular disease share risk factors and numerous underlying molecular pathways. One of the most recent examples of the overlapping between these deadly diseases is the discovery that mutations in hematopoietic blood cells, known to increase the risk of hematological malignancies, are also associated with nearly a doubling in the risk of coronary heart disease in humans and with accelerated atherosclerosis in mice [159]. Additionally, the work by Meijers et al. has shown that a heart damaged by MI releases soluble factors that promote cancer progression [160] shedding light on the possible mechanisms underlying the increased risk of cancer observed in HF patients [161]. This connection between HF and cancer has also emerged from a study showing a 14% increase of risk of lung cancer in patients under angiotensin converting enzyme (ACE) inhibitors [162]. Statins, which are widely used as 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors for LDL lowering therapies, have been recently under investigation for possible effects on cancer incidence and progression (as reviewed in [163]).

Based on all these links, it is not surprising that drugs that are successfully used for cancer therapy are now being used to treat cardiovascular disease, and *vice versa*. One of these drugs is teniposide, a topo II (DNA topoisomerase II) inhibitor used for cancer treatment which recently has been shown to reduce vascular calcification [134]. Additionally, inhibitors of the EGFR (Epidermal Growth Factor Receptor), such as erlotinib, used for targeted therapy of lung cancer, also reduces atherosclerosis by inducing anergy in CD4 T cells [113,164]. Exploratory analyses have found that anti-inflammatory therapy with canakinumab, a monoclonal antibody that neutralizes Il-1β, not only reduces cardiovascular events in patients with cardiovascular disease [165] and the development of chronic viral myocarditis [166] but also the incidence of mortality attributable to lung cancer [114].

The Notch pathway represents another link between cancer and cardiovascular diseases. Since Notch activation promotes the survival of cancer cells in solid tumors and leukemias, large effort is being put into the inhibition of Notch to render cancer more responsive to existing chemotherapeutic agents [150,167]. Clinical trials are currently investigating small molecules inhibitors of γ-secretase, but, in parallel, antibodies against the different isoforms of Notch receptors and/or ligands together with decoy ligands, are also being tested in preclinical [150,167] and pilot clinical [168] studies. The clinical trials conducted so far, targeting Notch alone or in combination with

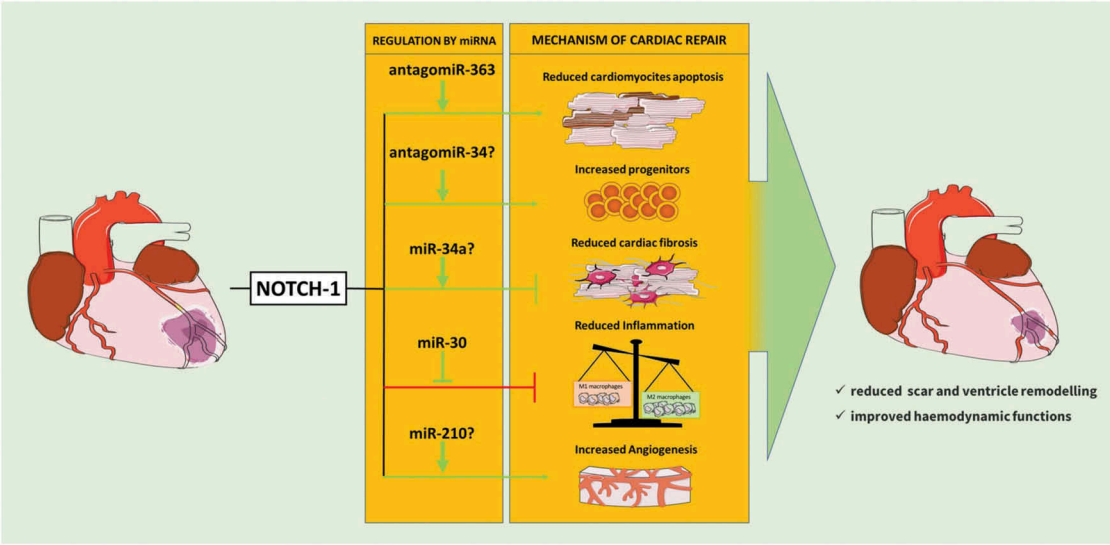

Figure 4. Notch in cardiac remodeling following myocardial infarction. Myocardial Notch1 modulation, by miRNAs or antagomiRNAs, in the infarcted heart may limit pathological remodeling by reducing cardiomyocytes apoptosis and cardiac fibrosis, by enhancing the proliferation of cardiac stem cells, by promoting angiogenesis and by reducing the ratio M1/M2 macrophages. Fine-tuned Notch1 activity in the damaged myocardium may thus lead to improved hemodynamic functions by reducing scar formation and ventricle remodeling.

other agents, have shown little, not extraordinary response [169]. A recent case study has reported total remission in a patient with early T-cell progenitor acute lymphoblastic leukemia treated with γ-secretase inhibitor BMS-906,024. Next Generation Sequencing analyses have shown the presence of Notch mutations in leukemic blasts possibly driving this tumor [170] suggesting the need for genomic analyses to identify super-responders to anti-Notch treatment. When taking under consideration the challenges of targeting Notch in cancer, it should be also considered that Notch is a suppressor gene in some tissues, such as the skin [171] and not surprising skin tumors have been observed following treatment with Notch inhibitors [167]. Additionally, since Notch is a major player in the modulation of the immune system [92,172] long-term studies should evaluate the effects on the immunity of Notch inhibitors. Also, based on the discussed crucial role of Notch in the cardiovascular system, the potential cardiotoxicity of these Notch-targeting cancer drugs should be evaluated in long-term studies [173].

Conversely, Notch1 could be targeted to reduce the cardiotoxicity caused by some anti-cancer agents. It is widely recognized that lack of estrogens causes endothelial dysfunction [23] and our data show that estrogens protects the endothelium by activating Notch1 [174]. Hence, the cardiotoxicity observed in association with anti-estrogen treatment for breast cancer could be due to endothelial Notch1 inhibition, and thus, it could be reduced by restablishing Notch1 signaling in this tissue [23]. Similarly, given the pivotal role of Notch1 in the stressed heart, studies aimed to investigate the involvement of this receptor in cardiomyocytes death, caused by doxorubicin, trastuzumab, and lapatinib treatments, could lead to novel, Notch-based, therapeutic approaches to prevent cardiotoxicity linked to the treatment with these drugs [74,173].

Due to the complexity of the Notch signaling, thoroughly discussed in the previous paragraphs, the dissection of the mechanism of actions of the different Notch receptors and their ligands, similarly to cancer, should be a crucial step for the targeting of this pathway for cardiovascular diseases. Specifically, studies aimed to identify upstream or downstream regulators and effectors of Notch signaling could facilitate the targeting of Notch in this context. For example, it is known that Notch1 inhibition could prevent intima thickening, but given the divergent effects of Notch receptors on vascular remodeling and in the endothelium, the use of a γ-secretase inhibitor (GSI) to inhibit Notch1 may give undesired effects to other Notch receptors. To overcome this problem Jiang et al, based on data showing that PLCγ1-Akt–mediated Notch1 signaling is crucial for intima formation, found that specific inhibition of the PLCγ1 and Akt interaction could be a promising therapeutic strategy for preventing vascular remodeling [100]. Another issue, given the multiple and opposite roles played by Notch in multiple tissues, is the modality of administration of novel pharmacological agents. Activation of Notch1 to reduce ischemia-caused damages in cardiomyocytes and to prevent endothelial dysfunction, should be accomplished locally to avoid general activation of inflammatory pathways. Nanoparticles, successfully utilized to reduce the MI damage by delivering drugs targeting infiltrating cells [175], could be used to deliver miRNAs that regulate Notch1 [124,176,177]. These miRNAs could be also administrated systemically [178] or locally, by intracardiac injection [179] or by hydrogels-functionalized with a peptide mimic of Jagged1 [135] (Figures 3 and 4). At this purpose hydrogels-functionalized with miRNAs, similarly to those with a peptide mimic of Jagged1 [135] could be used. Similar approaches could be exploited to inhibit Notch1 in leucocytes, to diminish inflammatory response, or to reduce Dll4-mediated Notch signaling in plaques. In the context of atherosclerosis, GSI-eluting stent could also be developed to prevent vascular SMCs proliferation and intima thickening.

To the best of our knowledge, probably due to the many challenges to be overcome discussed in this review, no clinical trials targeting Notch for cardiovascular disease have been

conducted so far. Based on our discussion, it emerges that effective strategies for the targeting of Notch in cardiovascular diseases should benefit from the interaction between basic and clinical researchers involved in cancer or cardiovascular diseases. This collaboration, aimed to further understand the pathological role of Notch pathway and to identify novel therapeutic interventions targeting both cancer and heart and vessels disease, will expand the established field of the cardio-oncology, which today aims mainly to reduce the negative effect of cancer drugs on the cardiovascular system.

Funding

This study was funded by Fondazione Annamaria Sechi per il Cuore to L Marracino, Fondo Ateneo per la Ricerca 2017 to P Rizzo, and the Russian Science Foundation 18-14-00152 to A Malaschicheva.

Declaration of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial relationships or otherwise to disclose.

ORCID

Anna Malaschicheva http://orcid.org/0000-0002-0820-2913

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Miele L. Notch signaling. Clin Cancer Res. 2006;12(4):1074–1079.
2. D'Souza B, Meloty-Kapella L, Weinmaster G. Canonical and non-canonical Notch ligands. Curr Top Dev Biol. 2010;92:73–129.
3. Kovall RA, Gebelein B, Sprinzak D, *et al*. The Canonical Notch Signaling Pathway: structural and Biochemical Insights into Shape, Sugar, and Force. Dev Cell. 2017;41(3):228–241.
4. Brou C, Logeat F, Gupta N, *et al*. A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. Mol Cell. 2000;5(2):207–216.
5. Struhl G, Greenwald I. Presenilin-mediated transmembrane cleavage is required for Notch signal transduction in Drosophila. Proc Natl Acad Sci U S A. 2001;98(1):229–234.
6. Selkoe DJ, Wolfe MS. Presenilin: running with scissors in the membrane. Cell. 2007;131(2):215–221.
7. Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism. Cell. 2009;137(2):216–233.
8. Morel V, Lecourtois M, Massiani O, *et al*. Transcriptional repression by suppressor of hairless involves the binding of a hairless-dCtBP complex in Drosophila. Curr Biol. 2001;11(10):789–792.
9. Wallberg AE, Pedersen K, Lendahl U, *et al*. p300 and PCAF act cooperatively to mediate transcriptional activation from chromatin templates by notch intracellular domains in vitro. Mol Cell Biol. 2002;22(22):7812–7819.
10. Bray SJ. Notch signalling in context. Nat Rev Mol Cell Biol. 2016;17(11):722–735.
11. Skalska L, Stojnic R, Li J, *et al*. Chromatin signatures at Notch-regulated enhancers reveal large-scale changes in H3K56ac upon activation. Embo J. 2015;34(14):1889–1904.
12. Ayaz F, Osborne BA. Non-canonical notch signaling in cancer and immunity. Front Oncol. 2014;4:345.
13. Siebel C, Lendahl U. Notch signaling in development, tissue homeostasis, and disease. Physiol Rev. 2017;97(4):1235–1294.
• **This review provides an overview of the role of Notch signaling in regulating tissue homeostasis, with examples from skin, liver, lung, intestine, and the vasculature.**
14. Palomero T, Ferrando A. Therapeutic targeting of NOTCH1 signaling in T-cell acute lymphoblastic leukemia. Clin Lymphoma Myeloma. 2009;9(Suppl 3):S205–S210.
15. Marcel N, Sarin A. Notch1 regulated autophagy controls survival and suppressor activity of activated murine T-regulatory cells. Elife. 2016 5.
16. Yamamoto S, Schulze KL, Bellen HJ. Introduction to Notch signaling. Methods Mol Biol. 2014;1187:1–14.
17. Borggrefe T, Lauth M, Zwijsen A, *et al*. The Notch intracellular domain integrates signals from Wnt, Hedgehog, TGFbeta/BMP and hypoxia pathways. Biochim Biophys Acta. 2016;1863(2):303–313.
18. Andersson ER, Sandberg R, Lendahl U. Notch signaling: simplicity in design, versatility in function. Development. 2011;138(17):3593–3612.
19. Kakuda S, Haltiwanger RS. Deciphering the Fringe-Mediated Notch Code: identification of Activating and Inhibiting Sites Allowing Discrimination between Ligands. Dev Cell. 2017;40(2):193–201.
20. Aquila G, Fortini C, Pannuti A, *et al*. Distinct gene expression profiles associated with Notch ligands Delta-like 4 and Jagged1 in plaque material from peripheral artery disease patients: a pilot study. J Transl Med. 2017;15(1):98.
• **This study reveals a possible role of Notch signaling in the pathophysiology of peripheral artery disease and suggests that ligand-specific activation of this pathway could determine the plaque characteristics and therefore progression of the disease.**
21. Sprinzak D, Lakhanpal A, Lebon L, *et al*. Cis-interactions between Notch and Delta generate mutually exclusive signalling states. Nature. 2010;465(7294):86–90.
22. Osipo C, Golde TE, Osborne BA, *et al*. Off the beaten pathway: the complex cross talk between Notch and NF-kappaB. Lab Invest. 2008;88(1):11–17.
23. Fortini F, Vieceli Dalla Sega F, Caliceti C, *et al*. Estrogen-mediated protection against coronary heart disease: the role of the Notch pathway. J Steroid Biochem Mol Biol. 2019;189:87–100.
24. Aster JC, Pear WS, Blacklow SC. The varied roles of notch in cancer. Annu Rev Pathol. 2017;12:245–275.
25. Luxán G, D'Amato G, MacGrogan D, *et al*. Endocardial Notch signaling in cardiac development and disease. Circ Res. 2016;118(1):e1–e18.
26. Papoutsi T, Luna-Zurita L, Prados B, *et al*. Bmp2 and Notch cooperate to pattern the embryonic endocardium. Development. 2018.
27. de la Pompa JL, Epstein JA. Coordinating tissue interactions: notch signaling in cardiac development and disease. Dev Cell. 2012;22(2):244–254.
28. D'Amato G, Luxan G, de la Pompa JL. Notch signalling in ventricular chamber development and cardiomyopathy. Febs J. 2016;283(23):4223–4237.
29. Del Monte G, Grego-Bessa J, Gonzalez-Rajal A, *et al*. Monitoring Notch1 activity in development: evidence for a feedback regulatory loop. Dev Dyn. 2007;236(9):2594–2614.
30. Fischer A, Steidl C, Wagner TU, *et al*. Combined loss of Hey1 and HeyL causes congenital heart defects because of impaired epithelial to mesenchymal transition. Circ Res. 2007;100(6):856–863.
31. Krebs LT, Shutter JR, Tanigaki K, *et al*. Haploinsufficient lethality and formation of arteriovenous malformations in Notch pathway mutants. Genes Dev. 2004;18(20):2469–2473.
32. Duarte A, Hirashima M, Benedito R, *et al*. Dosage-sensitive requirement for mouse Dll4 in artery development. Genes Dev. 2004;18(20):2474–2478.
33. Krebs LT, Starling C, Chervonsky AV, *et al*. Notch1 activation in mice causes arteriovenous malformations phenocopied by ephrinB2 and EphB4 mutants. Genesis. 2010;48(3):146–150.

34. Mack JJ, Iruela-Arispe ML. NOTCH regulation of the endothelial cell phenotype. Curr Opin Hematol. 2018;25(3):212–218.
•• **This study reveals that NOTCH1 is required to maintain junctional integrity and to prevent atherosclerosis. NOTCH1 signaling is also necessary in adult arteries to interpret hemodynamic forces and initiate appropriate biological responses required for vascular homeostasis and atheroprotection.**
35. Garg V, Muth AN, Ransom JF, *et al*. Mutations in NOTCH1 cause aortic valve disease. Nature. 2005;437(7056):270–274.
36. Preuss C, Capredon M, Wünnemann F, *et al*. Family based whole exome sequencing reveals the multifaceted role of Notch signaling in congenital heart disease. PLoS Genet. 2016;12(10):e1006335.
37. Ducharme V, Guauque-Olarte S, Gaudreault N, *et al*. NOTCH1 genetic variants in patients with tricuspid calcific aortic valve stenosis. J Heart Valve Dis. 2013;22(2):142–149.
38. Freylikhman O, Tatarinova T, Smolina N, *et al*. Variants in the NOTCH1 gene in patients with aortic coarctation. Congenit Heart Dis. 2014;9(5):391–396.
39. Irtyuga O, Malashicheva A, Zhiduleva E, *et al*. NOTCH1 Mutations in Aortic Stenosis: association with Osteoprotegerin/RANK/RANKL. Biomed Res Int. 2017;2017:6917907.
40. Guida V, Chiappe F, Ferese R, *et al*. Novel and recurrent JAG1 mutations in patients with tetralogy of Fallot. Clin Genet. 2011;80 (6):591–594.
41. Page DJ, Miossec MJ, Williams SG, *et al*. Whole exome sequencing reveals the major genetic contributors to nonsyndromic tetralogy of fallot. Circ Res. 2019;124(4):553–563.
42. Iascone M, Ciccone R, Galletti L, *et al*. Identification of de novo mutations and rare variants in hypoplastic left heart syndrome. Clin Genet. 2012;81(6):542–554.
43. Southgate L, Sukalo M, Karountzos AS, *et al*. Haploinsufficiency of the NOTCH1 receptor as a cause of Adams–Oliver syndrome with variable cardiac anomalies. Circ Genomic Precis Med. 2015;8 (4):572–581.
44. Shaheen R, Aglan M, Keppler-Noreuil K, *et al*. Mutations in EOGT confirm the genetic heterogeneity of autosomal-recessive Adams-Oliver syndrome. Am J Hum Genet. 2013;92(4):598–604.
45. Kerstjens-Frederikse WS, van de Laar IM, Vos YJ, *et al*. Cardiovascular malformations caused by NOTCH1 mutations do not keep left: data on 428 probands with left-sided CHD and their families. Genet Med. 2016;18(9):914–923.
46. Spinner NB, Leonard LD, Krantz ID. Alagille syndrome. 2013. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2019. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1273/.
47. Joutel A, Corpechot C, Ducros A, *et al*. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. Nature. 1996;383(6602):707.
48. Rutkovskiy A, Malashicheva A, Sullivan G, *et al*. Valve interstitial cells: the key to understanding the pathophysiology of heart valve calcification. J Am Heart Assoc. 2017;6(9).
49. Acharya A, Hans CP, Koenig SN, *et al*. Inhibitory role of Notch1 in calcific aortic valve disease. PloS One. 2011;6(11):e27743.
50. Nigam V, Srivastava D. Notch1 represses osteogenic pathways in aortic valve cells. J Mol Cell Cardiol. 2009;47(6):828–834.
51. Hadji F, Boulanger MC, Guay SP, *et al*. Altered DNA methylation of long noncoding RNA H19 in calcific aortic valve disease promotes mineralization by silencing NOTCH1. Circulation. 2016;134 (23):1848–1862.
52. Zeng Q, Song R, Ao L, *et al*. Notch1 promotes the pro-osteogenic response of human aortic valve interstitial cells via modulation of ERK1/2 and nuclear factor-κB activation. Arterioscler Thromb Vasc Biol. 2013;33(7):1580–1590.
53. Theodoris CV, Li M, White MP, *et al*. Human disease modeling reveals integrated transcriptional and epigenetic mechanisms of NOTCH1 haploinsufficiency. Cell. 2015;160(6):1072–1086.
54. Kostina A, Shishkova A, Ignatieva E, *et al*. Different Notch signaling in cells from calcified bicuspid and tricuspid aortic valves. J Mol Cell Cardiol. 2018;114:211–219.
55. Michelena HI, Khanna AD, Mahoney D, *et al*. Incidence of aortic complications in patients with bicuspid aortic valves. Jama. 2011;306(10):1104–1112.
56. Davis FM, Rateri DL, Daugherty A. Mechanisms of aortic aneurysm formation: translating preclinical studies into clinical therapies. Heart. 2014;100(19):1498–1505.
57. Scheppke L, Murphy EA, Zarpellon A, *et al*. Notch promotes vascular maturation by inducing integrin-mediated smooth muscle cell adhesion to the endothelial basement membrane. Blood. 2012;119 (9):2149–2158.
58. Manderfield LJ, High FA, Engleka KA, *et al*. Notch activation of Jagged1 contributes to the assembly of the arterial wall. Circulation. 2012;125(2):314–323.
59. High FA, Lu MM, Pear WS, *et al*. Endothelial expression of the Notch ligand Jagged1 is required for vascular smooth muscle development. Proc Natl Acad Sci U S A. 2008;105(6):1955–1959.
60. Baeten JT, Lilly B. Notch signaling in vascular smooth muscle cells. Adv Pharmacol. 2017;78:351–382.
61. Baeten JT, Lilly B. Differential regulation of NOTCH2 and NOTCH3 contribute to their unique functions in vascular smooth muscle cells. J Biol Chem. 2015;290(26):16226–16237.
62. Pedrosa A-R, Trindade A, Fernandes A-C, *et al*. Endothelial Jagged1 antagonizes Dll4 regulation of endothelial branching and promotes vascular maturation downstream of Dll4/Notch1. Arterioscler Thromb Vasc Biol. 2015;35(5):1134–1146.
63. Kostina A, Semenova D, Kostina D, *et al*. Human aortic endothelial cells have osteogenic Notch-dependent properties in co-culture with aortic smooth muscle cells. Biochem Biophys Res Commun. 2019;514(2):462–468.
64. Koenig SN, LaHaye S, Feller JD, *et al*. Notch1 haploinsufficiency causes ascending aortic aneurysms in mice. JCI Insight. 2017;2 (21):e91353.
65. Kostina AS, Uspensky VE, Irtyuga OB, *et al*. Notch-dependent EMT is attenuated in patients with aortic aneurysm and bicuspid aortic valve. Biochim Biophys Acta (BBA) - Mol Basis Dis. 2016;1862 (4):733–740.
66. Ignatieva E, Kostina D, Irtyuga O, *et al*. Mechanisms of smooth muscle cell differentiationare distinctly altered in thoracic aortic aneurysms associated with Bicuspid or Tricuspid Aortic Valves. Front Physiol. 2017;8.
67. Maleki S, Kjellqvist S, Paloschi V, *et al*. Mesenchymal state of intimal cells may explain higher propensity to ascending aortic aneurysm in bicuspid aortic valves. Sci Rep. 2016;6:35712.
68. Kostina A, Bjork H, Ignatieva E, *et al*. Notch, BMP and WNT/beta-catenin network is impaired in endothelial cells of the patients with thoracic aortic aneurysm. Atheroscler Suppl. 201835:e6–e13.
69. Malashicheva A, Kostina D, Kostina A, *et al*. (2016) Phenotypic and functional changes of endothelial and smooth muscle cells in thoracic aortic aneurysms. Int J Vasc Med. 2016;2016:3107879
70. Rostama B, Turner JE, Seavey GT, *et al*. DLL4/Notch1 and BMP9 interdependent signaling induces human endothelial cell quiescence via P27KIP1 and thrombospondin-1. Arterioscler Thromb Vasc Biol. 2015;35(12):2626–2637.
71. Morini MF, Dejana E. Transcriptional regulation of arterial differentiation via Wnt, Sox and Notch. Curr Opin Hematol. 2014;21 (3):229–234.
72. Jahnsen ED, Trindade A, Zaun HC, *et al*. Notch1 is pan-endothelial at the onset of flow and regulated by flow. PloS One. 2015;10(4): e0122622.
73. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling–concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. J Am Coll Cardiol. 2000;35(3):569–582.
74. Ferrari R, Rizzo P. The Notch pathway: a novel target for myocardial remodelling therapy?. Eur Heart J. 2014;35(32):2140–2145.
75. Norum HM, Gullestad L, Abraityte A, *et al*. Increased serum levels of the Notch ligand DLL1 are associated with diastolic dysfunction, reduced exercise capacity, and adverse outcome in chronic heart failure. J Card Fail. 2016;22(3):218–223.

•• **This study provides evidence that serum levels of the Notch ligand Delta-like-1 (DLL1) is associated with clinical and hemodynamic variables in patients with heart failure, suggesting that the Notch/DLL1 signaling pathway is involved in the pathophysiology of heart failure, potentially affecting diastolic function.**

76. Norum HM, Broch K, Michelsen AE, *et al*. The Notch Ligands DLL1 and Periostin Are Associated with Symptom Severity and Diastolic Function in Dilated Cardiomyopathy. J Cardiovasc Transl Res. 2017.
77. Chiplunkar A, Rentschler S. Notch Activation Associated with Poor Outcomes in Heart Failure: is it Harmful, or not Enough of a Good Thing?. J Card Fail. 2016;22(3):224–225.
78. Chiorean EG, LoRusso P, Strother RM, et al. A Phase I First-in-Human Study of Enoticumab (REGN421), a Fully Human Delta-like Ligand 4 (Dll4) Monoclonal Antibody in Patients with Advanced Solid Tumors. Clin Cancer Res. 2015;21(12):2695–2703.
79. Smith DC, Eisenberg PD, Manikhas G, *et al*. A Phase I Dose Escalation and Expansion Study of the Anticancer Stem Cell Agent Demcizumab (Anti-DLL4) in Patients with Previously Treated Solid Tumors. Clin Cancer Res. 2014;20:6295–6303.
80. Rizzo P, Miele L, Ferrari R. The Notch pathway: a crossroad between the life and death of the endothelium. Eur Heart J. 2013;34 (32):2504–2509.
81. Vieceli Dalla Sega F, Aquila G, Fortini F, *et al*. Context-dependent function of ROS in the vascular endothelium: the role of the Notch pathway and shear stress. Biofactors. 2017;43(4):475–485.
82. Briot A, Civelek M, Seki A, *et al*. Endothelial NOTCH1 is suppressed by circulating lipids and antagonizes inflammation during atherosclerosis. J Exp Med. 2015;212(12):2147–2163.

• **This study provides in vitro evidence that reduction of endothelial NOTCH1 is a predisposing factor in the onset of vascular inflammation and initiation of atherosclerosis.**

83. Aquila G, Morelli MB, Vieceli Dalla Sega F, *et al*. Heart rate reduction with ivabradine in the early phase of atherosclerosis is protective in the endothelium of ApoE-deficient mice. J Physiol Pharmacol. 2018;69(1):35–52.
84. Mack JJ, Mosqueiro TS, Archer BJ, *et al*. NOTCH1 is a mechanosensor in adult arteries. Nat Commun. 2017;8(1):1620.
85. Polacheck WJ, Kutys ML, Yang J, *et al*. A non-canonical Notch complex regulates adherens junctions and vascular barrier function. Nature. 2017;552(7684):258–262.
86. Jabs M, Rose AJ, Lehmann LH, *et al*. Inhibition of Endothelial Notch Signaling Impairs Fatty Acid Transport and Leads to Metabolic and Vascular Remodeling of the Adult Heart. Circulation. 2018;137:2592–2608.

•• **This study shows that Notch signaling in the endothelium controls blood vessel formation and fatty acid transport and that its inhibition could lead to heart failure due to vascular and metabolic remodelling of the heart**

87. Verginelli F, Adesso L, Limon I, *et al*. Activation of an endothelial Notch1-Jagged1 circuit induces VCAM1 expression, an effect amplified by interleukin-1beta. Oncotarget. 2015;6(41):43216–43229.
88. Nus M, Martinez-Poveda B, MacGrogan D, *et al*. Endothelial Jag1-RBPJ signalling promotes inflammatory leucocyte recruitment and atherosclerosis. Cardiovasc Res. 2016;112:568–580.
89. Poulsen LC, Edelmann RJ, Kruger S, *et al*. Inhibition of Endothelial NOTCH1 Signaling Attenuates Inflammation by Reducing Cytokine-Mediated Histone Acetylation at Inflammatory Enhancers. Arterioscler Thromb Vasc Biol. 2018;38(4):854–869.
90. Guruharsha KG, Kankel MW, Artavanis-Tsakonas S. The Notch signalling system: recent insights into the complexity of a conserved pathway. Nat Rev Genet. 2012;13(9):654–666.
91. Shang Y, Smith S, Hu X. Role of Notch signaling in regulating innate immunity and inflammation in health and disease. Protein Cell. 2016;7(3):159–174.
92. Vieceli Dalla Sega F, Fortini F, Aquila G, *et al*. Notch signaling regulates immune response in atherosclerosis. Front Immunol. 2019.
93. Rizzo P, Ferrari R. The Notch pathway: a new therapeutic target in atherosclerosis?. Eur Heart J Suppl. 2015;17(suppl_A):A74–A76.
94. Kerr BA, West XZ, Kim YW, *et al*. Stability and function of adult vasculature is sustained by Akt/Jagged1 signalling axis in endothelium. Nat Commun. 2016;7:10960.
95. Clement N, Gueguen M, Glorian M, *et al*. Notch3 and IL-1beta exert opposing effects on a vascular smooth muscle cell inflammatory pathway in which NF-kappaB drives crosstalk. J Cell Sci. 2007;120 (Pt 19):3352–3361.
96. Boucher JM, Peterson SM, Urs S, *et al*. The miR-143/145 cluster is a novel transcriptional target of Jagged-1/Notch signaling in vascular smooth muscle cells. J Biol Chem. 2011;286(32):28312–28321.
97. Chen Q, Yang F, Guo M, *et al*. miRNA-34a reduces neointima formation through inhibiting smooth muscle cell proliferation and migration. J Mol Cell Cardiol. 2015;89(Pt A):75–86.
98. Redmond EM, Liu W, Hamm K, *et al*. Perivascular delivery of Notch 1 siRNA inhibits injury-induced arterial remodeling. PLoS One. 2014;9(1):e84122.
99. Wu JR, Yeh JL, Liou SF, *et al*. Gamma-secretase Inhibitor Prevents Proliferation and Migration of Ductus Arteriosus Smooth Muscle Cells through the Notch3-HES1/2/5 Pathway. Int J Biol Sci. 2016;12 (9):1063–1073.
100. Jiang D, Zhuang J, Peng W, *et al*. Phospholipase Cgamma1 Mediates Intima Formation Through Akt-Notch1 Signaling Independent of the Phospholipase Activity. J Am Heart Assoc. 2017;6(7).
101. Aquila G, Marracino L, Martino V, *et al*. The Use of Nutraceuticals to Counteract Atherosclerosis: the Role of the Notch Pathway. Oxid Med Cell Longev. 2019;(2019):30.
102. Ruan ZB, Fu XL, Li W, *et al*. Effect of notch1,2,3 genes silicing on NF-kappaB signaling pathway of macrophages in patients with atherosclerosis. Biomed Pharmacother. 2016;84:666–673.
103. Singla DK, Wang J, Singla R. Primary human monocytes differentiate into M2 macrophages and involve Notch-1 pathway. Can J Physiol Pharmacol. 2017;95(3):288–294.
104. Huang F, Zhao JL, Wang L, *et al*. miR-148a-3p Mediates Notch Signaling to Promote the Differentiation and M1 Activation of Macrophages. Front Immunol. 2017;8:1327.
105. Xu J, Chi F, Guo T, *et al*. NOTCH reprograms mitochondrial metabolism for proinflammatory macrophage activation. J Clin Invest. 2015;125(4):1579–1590.
106. Fung E, Tang SM, Canner JP, *et al*. Delta-like 4 induces notch signaling in macrophages: implications for inflammation. Circulation. 2007;115(23):2948–2956.
107. Pagie S, Gerard N, Charreau B. Notch signaling triggered via the ligand DLL4 impedes M2 macrophage differentiation and promotes their apoptosis. Cell Commun Signal. 2018;16(1):4.
108. Pabois A, Pagie S, Gerard N, *et al*. Notch signaling mediates crosstalk between endothelial cells and macrophages via Dll4 and IL6 in cardiac microvascular inflammation. Biochem Pharmacol. 2016;104:95–107.
109. Nakano T, Fukuda D, Koga J, *et al*. Delta-Like Ligand 4-Notch Signaling in Macrophage Activation. Arterioscler Thromb Vasc Biol. 2016;36(10):2038–2047.
110. Foldi J, Chung AY, Xu H, *et al*. Autoamplification of Notch signaling in macrophages by TLR-induced and RBP-J-dependent induction of Jagged1. J Immunol. 2010;185(9):5023–5031.
111. Kimball AS, Joshi AD, Boniakowski AE, *et al*. Notch Regulates Macrophage-Mediated Inflammation in Diabetic Wound Healing. Front Immunol. 2017;8:635.
112. Gamrekelashvili J, Giagnorio R, Jussofie J, *et al*. Regulation of monocyte cell fate by blood vessels mediated by Notch signalling. Nat Commun. 2016;7:12597.
113. Libby P, Hansson GK. Taming Immune and Inflammatory Responses to Treat Atherosclerosis. J Am Coll Cardiol. 2018;71(2):173–176.
114. Libby P, Ebert BL. CHIP (Clonal Hematopoiesis of Indeterminate Potential). Circulation. 2018;138(7):666–668.
115. Radtke F, MacDonald HR, Tacchini-Cottier F. Regulation of innate and adaptive immunity by Notch. Nat Rev Immunol. 2013;13 (6):427–437.
116. Hossain F, Majumder S, Ucar DA, *et al*. Notch Signaling in Myeloid Cells as a Regulator of Tumor Immune Responses. Front Immunol. 2018;9:1288.

117. Collesi C, Felician G, Secco I, *et al*. Reversible Notch1 acetylation tunes proliferative signalling in cardiomyocytes. Cardiovasc Res. 2018;114(1):103–122.
118. Felician G, Collesi C, Lusic M, *et al*. Epigenetic modification at Notch responsive promoters blunts efficacy of inducing notch pathway reactivation after myocardial infarction. Circ Res. 2014;115 (7):636–649.
119. Metrich M, Bezdek PA, Berthonneche C, *et al*. Jagged1 intracellular domain-mediated inhibition of Notch1 signalling regulates cardiac homeostasis in the postnatal heart. Cardiovasc Res. 2015;108 (1):74–86.
120. Pei H, Yu Q, Xue Q, *et al*. Notch1 cardioprotection in myocardial ischemia/reperfusion involves reduction of oxidative/nitrative stress. Basic Res Cardiol. 2013;108(5):373.
•• **This study shows that cardiac-specific Notch1 knockdown results in significantly aggravated I/R injury. Conversely, activation of Notch1 by Jagged1 protects against myocardial I/R injury partly though PTEN/Akt mediated anti-oxidative and anti-nitrative effects.**
121. Pei H, Song X, Peng C, *et al*. TNF-alpha inhibitor protects against myocardial ischemia/reperfusion injury via Notch1-mediated suppression of oxidative/nitrative stress. Free Radic Biol Med. 2015;82:114–121.
122. Zhang M, Wang C, Hu J, *et al*. Notch3/Akt signaling contributes to OSM-induced protection against cardiac ischemia/reperfusion injury. Apoptosis. 2015;20(9):1150–1163.
123. Wu F, Yu B, Zhang X, *et al*. Cardioprotective effect of Notch signaling on the development of myocardial infarction complicated by diabetes mellitus. Exp Ther Med. 2017;14(4):3447–3454.
124. Meng X, Ji Y, Wan Z, *et al*. Inhibition of miR-363 protects cardiomyocytes against hypoxia-induced apoptosis through regulation of Notch signaling. Biomed Pharmacother. 2017;90:509–516.
125. Rocca C, Femmino S, Aquila G, *et al*. Notch1 Mediates Preconditioning Protection Induced by GPER in Normotensive and Hypertensive Female Rat Hearts. Front Physiol. 2018;9:521.
126. Zhang M, Yu LM, Zhao H, *et al*. 2,3,5,4′-Tetrahydroxystilbene -2-O-beta-D-glucoside protects murine hearts against ischemia/reperfusion injury by activating Notch1/Hes1 signaling and attenuating endoplasmic reticulum stress. Acta Pharmacol Sin. 2017;38 (3):317–330.
127. Yu L, Li F, Zhao G, *et al*. Protective effect of berberine against myocardial ischemia reperfusion injury: role of Notch1/Hes1-PTEN/Akt signaling. Apoptosis. 2015;20(6):796–810.
128. Yu B, Song B. Notch 1 signalling inhibits cardiomyocyte apoptosis in ischaemic postconditioning. Heart Lung Circ. 2014;23 (2):152–158.
129. Zhou XL, Wan L, Xu QR, *et al*. Notch signaling activation contributes to cardioprotection provided by ischemic preconditioning and postconditioning. J Transl Med. 2013;11:251.
130. Boni A, Urbanek K, Nascimbene A, *et al*. Notch1 regulates the fate of cardiac progenitor cells. Proc Natl Acad Sci U S A. 2008;105 (40):15529–15534.
131. Nemir M, Metrich M, Plaisance I, *et al*. The Notch pathway controls fibrotic and regenerative repair in the adult heart. Eur Heart J. 2014;35(32):2174–2185.
132. Frangogiannis NG. Targeting the transforming growth factor (TGF)-beta cascade in the remodeling heart: benefits and perils. J Mol Cell Cardiol. 2014;76:169–171.
133. Sassoli C, Chellini F, Pini A, *et al*. Relaxin prevents cardiac fibroblast-myofibroblast transition via notch-1-mediated inhibition of TGF-beta/Smad3 signaling. PLoS One. 2013;8(5):e63896.
134. Zhou XL, Fang YH, Wan L, *et al*. Notch signaling inhibits cardiac fibroblast to myofibroblast transformation by antagonizing TGF-beta1/Smad3 signaling. J Cell Physiol. 2018;234(6):8834–8845.
135. Boopathy AV, Martinez MD, Smith AW, *et al*. Intramyocardial Delivery of Notch Ligand-Containing Hydrogels Improves Cardiac Function and Angiogenesis Following Infarction. Tissue Eng Part A. 2015;21(17–18):2315–2322.
136. King KR, Aguirre AD, Ye YX, *et al*. IRF3 and type I interferons fuel a fatal response to myocardial infarction. Nat Med. 2017;23 (12):1481–1487.
137. Svensson A, Jakara E, Shestakov A, *et al*. Inhibition of gamma-secretase cleavage in the notch signaling pathway blocks HSV-2-induced type I and type II interferon production. Viral Immunol. 2010;23(6):647–651.
138. Arumugam TV, Chan SL, Jo DG, *et al*. Gamma secretase-mediated Notch signaling worsens brain damage and functional outcome in ischemic stroke. Nat Med. 2006;12(6):621–623.
139. Yin J, Hu H, Li X, *et al*. Inhibition of Notch signaling pathway attenuates sympathetic hyperinnervation together with the augmentation of M2 macrophages in rats post-myocardial infarction. Am J Physiol Cell Physiol. 2016;310(1):C41–C53.
140. van der Laan AM, Piek JJ, Van Royen N. Targeting angiogenesis to restore the microcirculation after reperfused MI. Nat Rev Cardiol. 2009;6(8):515–523.
141. Gridley T. Notch signaling in vascular development and physiology. Development. 2007;134(15):2709–2718.
142. Kratsios P, Catela C, Salimova E, *et al*. Distinct roles for cell-autonomous Notch signaling in cardiomyocytes of the embryonic and adult heart. Circ Res. 2010;106(3):559–572.
143. Diaz-Trelles R, Scimia MC, Bushway P, *et al*. Notch-independent RBPJ controls angiogenesis in the adult heart. Nat Commun. 2016;7:12088.
144. Pannella M, Caliceti C, Fortini F, *et al*. Serum From Advanced Heart Failure Patients Promotes Angiogenic Sprouting and Affects the Notch Pathway in Human Endothelial Cells. J Cell Physiol. 2016;231 (12):2700–2710.
145. Restivo G, Nguyen BC, Dziunycz P, *et al*. IRF6 is a mediator of Notch pro-differentiation and tumour suppressive function in keratinocytes. Embo J. 2011;30(22):4571–4585.
146. South AP, Cho RJ, Aster JC. The double-edged sword of Notch signaling in cancer. Semin Cell Dev Biol. 2012;23(4):458–464.
147. Visvader JE. Cells of origin in cancer. Nature. 2011;469 (7330):314–322.
148. Plaks V, Kong N, Werb Z. The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells?. Cell Stem Cell. 2015;16(3):225–238.
149. Pannuti A, Foreman K, Rizzo P, *et al*. Targeting Notch to target cancer stem cells. Clin Cancer Res. 2010;16(12):3141–3152.
150. Takebe N, Miele L, Harris PJ, *et al*. Targeting Notch, Hedgehog, and Wnt pathways in cancer stem cells: clinical update. Nat Rev Clin Oncol. 2015;12(8):445–464.
151. Banerjee MN, Bolli R, Hare JM. Clinical Studies of Cell Therapy in Cardiovascular Medicine: recent Developments and Future Directions. Circ Res. 2018;123(2):266–287.
152. Wang Q, Liu L, Li Y, *et al*. Hypoxic Preconditioning Enhances Biological Function of Endothelial Progenitor Cells via Notch-Jagged1 Signaling Pathway. Med Sci Monit. 2017;23:4665–4667.
153. Bollini S, Smits AM, Balbi C, *et al*. Triggering Endogenous Cardiac Repair and Regeneration via Extracellular Vesicle-Mediated Communication. Front Physiol. 2018;9:1497.
154. Bergmann O, Bhardwaj RD, Bernard S, *et al*. Evidence for cardiomyocyte renewal in humans. Science. 2009;324(5923):98–102.
155. Cesselli D, Beltrami AP, D'Aurizio F, *et al*. Effects of age and heart failure on human cardiac stem cell function. Am J Pathol. 2011;179 (1):349–366.
156. Secco I, Barile L, Torrini C, *et al*. Notch pathway activation enhances cardiosphere in vitro expansion. J Cell Mol Med. 2018;22 (11):5583–5595.
157. Plaisance I, Perruchoud S, Fernandez-Tenorio M, *et al*. Cardiomyocyte Lineage Specification in Adult Human Cardiac Precursor Cells Via Modulation of Enhancer-Associated Long Noncoding RNA Expression. JACC Basic Transl Sci. 2016;1(6):472–493.
158. Kretzschmar K, Post Y, Bannier-Helaouet M, *et al*. Profiling proliferative cells and their progeny in damaged murine hearts. Proc Natl Acad Sci U S A. 2018;115(52):E12245–E12254.

159. Jaiswal S, Natarajan P, Silver AJ, *et al*. Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. N Engl J Med. 2017;377(2):111–121.
160. Meijers WC, Maglione M, Bakker SJL, *et al*. The Failing Heart Stimulates Tumor Growth by Circulating Factors. Circulation. 2018.
161. Bertero E, Canepa M, Maack C, *et al*. Linking Heart Failure to Cancer. Circulation. 2018;138(7):735–742.
162. Hicks BM, Filion KB, Yin H, *et al*. Angiotensin converting enzyme inhibitors and risk of lung cancer: population based cohort study. BMJ. 2018;363:k4209.
163. Van Wyhe RD, Rahal OM, Woodward WA. Effect of statins on breast cancer recurrence and mortality: a review. Breast Cancer (Dove Med Press). 2017;9:559–565.
164. Zeboudj L, Maitre M, Guyonnet L, *et al*. Selective EGF-Receptor Inhibition in CD4(+) T Cells Induces Anergy and Limits Atherosclerosis. J Am Coll Cardiol. 2018;71(2):160–172.
165. Ridker PM, Everett BM, Thuren T, *et al*. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N Engl J Med. 2017;377(12):1119–1131.
•• **This study shows that anti-inflammatory therapy with canakinumab, a monoclonal antibody that neutralizes Il-1β, at a dose of 150 mg every 3 months laeds to a significantly lower rate of recurrent cardiovascular events in patients with previous myocardial infarction**
166. Kraft L, Erdenesukh T, Sauter M, *et al*. Blocking the IL-1β signalling pathway prevents chronic viral myocarditis and cardiac remodeling. Basic Res Cardiol. 2019;114(2):11.
167. Tamagnone L, Zacchigna S, Rehman M. Taming the Notch Transcriptional Regulator for Cancer Therapy. Molecules. 2018;23 (2):431.
168. Jimeno A, Moore KN, Gordon M, *et al*. A first-in-human phase 1a study of the bispecific anti-DLL4/anti-VEGF antibody navicixizumab (OMP-305B83) in patients with previously treated solid tumors. Invest New Drugs. 2018;37(3):461–472.
169. Pant S, Jones SF, Kurkjian CD, *et al*. A first-in-human phase I study of the oral Notch inhibitor, LY900009, in patients with advanced cancer. Eur J Cancer. 2016;56:1–9.
170. Knoechel B, Bhatt A, Pan L, *et al*. Complete hematologic response of early T-cell progenitor acute lymphoblastic leukemia to the gamma-secretase inhibitor BMS-906024: genetic and epigenetic findings in an outlier case. Cold Spring Harb Mol Case Stud. 2015;1(1):a000539.
171. Dotto GP. Notch tumor suppressor function. Oncogene. 2008;27 (38):5115–5123.
172. Osborne B, Miele L. Notch and the immune system. Immunity. 1999;11(6):653–663.
173. Rizzo P, Mele D, Caliceti C, *et al*. The role of notch in the cardiovascular system: potential adverse effects of investigational notch inhibitors. Front Oncol. 2014;4:384.
174. Fortini F, Vieceli Dalla Sega F, Caliceti C, *et al*. Estrogen receptor beta-dependent Notch1 activation protects vascular endothelium against tumor necrosis factor alpha (TNFalpha)-induced apoptosis. J Biol Chem. 2017;292(44):18178–18191.
175. Katsuki S, Matoba T, Koga JI, *et al*. Anti-inflammatory Nanomedicine for Cardiovascular Disease. Front Cardiovasc Med. 2017;4:87.
176. Huang Y, Qi Y, Du JQ, *et al*. MicroRNA-34a regulates cardiac fibrosis after myocardial infarction by targeting Smad4. Expert Opin Ther Targets. 2014;18(12):1355–1365.
177. Iannolo G, Sciuto MR, Raffa GM, *et al*. MiR34 inhibition induces human heart progenitor proliferation. Cell Death Dis. 2018;9 (3):368.
178. Schober A, Nazari-Jahantigh M, Wei Y, *et al*. MicroRNA-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. Nat Med. 2014;20(4):368–376.
179. Sundararaman S, Miller TJ, Pastore JM, *et al*. Plasmid-based transient human stromal cell-derived factor-1 gene transfer improves cardiac function in chronic heart failure. Gene Ther. 2011;18 (9):867–873.