Novel Therapeutic Concepts

The Notch pathway: a novel target for myocardial remodelling therapy?

Roberto Ferrari1* and Paola Rizzo2

1Department of Cardiology and LTTA Centre, University Hospital of Ferrara, Ferrara, Italy; and 2GVM Care and Research, E.S. Health Science Foundation, Maria Cecilia Hospital, Cottignola, Italy

Received 23 December 2013; revised 24 April 2014; accepted 20 May 2014; online publish-ahead-of-print 26 June 2014

Pathological ventricle remodelling, which follows a cardiac insult, causes heart failure. Despite the existence of multiple pharmaceutical approaches, heart failure is one of the leading causes of death worldwide and there is an urgent need to explore new therapeutic avenues. The Notch pathway is an evolutionary conserved fundamental pathway that regulates cell fate during development as well as throughout postnatal life in self-renewing tissues. In the myocardium, Notch signalling is involved in the modulation of cardiomyocytes survival, cardiac stem cells differentiation, and angiogenesis which are factors known to determine the extent of pathological cardiac remodelling. Modulation of the Notch pathway could become a tool to limit ventricle remodelling and the associated inexorable deterioration of cardiac performance.

Keywords Cardiomyocytes • Apoptosis • Heart failure • Stem cells

Introduction

The heart is a dynamic organ, capable of cellular and chamber remodelling in response to pathological and physiological stimulation. Pathological ventricular remodelling may occur after myocardial infarction (MI), pressure overload (aortic stenosis, hypertension), inflammation (myocarditis), idiopathic dilated cardiomyopathy, or volume overload (valvular regurgitation). Although these diseases have different aetiologies, they all share similar pathways involving extrinsic and intrinsic signals that are transduced, through myocyte membrane-bound receptors, to the nuclei altering gene expression, metabolism, protein turnover, and, eventually, contractile function. Thus, remodelling is defined as genomic, molecular, cellular, and interstitial changes that manifest clinically as changes in size, shape, and function of the heart after injury or stress stimulation.1

After infarction, ventricular repair at the site of necrosis begins rapidly, within hours and lasts for almost a week, leading to scar formation (early remodelling). Extensive myocytes necrosis and degradation of collagen fibres begins immediately after the infarction and leads to infiltration of inflammatory cells for the re-absorption of necrotic tissue.2 While this inflammatory phase is needed to clear the infarct zone from dead cells and to activate reparative pathways, a timely suppression of the post-infarction inflammatory reaction may be crucial to protect the myocardium from dilative remodelling.3 By post-infarction Day 4, multiple mechanisms (cell stretching, reduction of intracellular space and mainly sliding or ‘slippage’ of cardiomyocytes consequent to the degradation of the collagen fibres) contribute to wall thinning of the infarcted tissue and regional dilatation of the left ventricle. During this phase, fibroblasts deposit collagen on the thinned tissue determining the formation of a scar that limits further expansion.4 After a MI, there is increased production and release of endothelial and mesenchymal stem cells (MSCs) from the bone marrow4–6 that may contribute to angiogenesis7,8 and to the reduction of myocyte loss.9,10 both processes potentially able to reduce pathological post-MI remodelling11,12.

The late phase of remodelling involves sites at a distance from the infarcted area. In response to the mechanical stretch (triggered by myocytes necrosis and the resultant increase in load), the still viable myocardium releases cytokines with autocrine and paracrine effects that activate pathways leading to cardiomyocytes survival and hypertrophy13–15 (Figure 1A). As the ventricle remodelling, its geometry changes: it becomes less elliptical and more spherical.1 At a cellular level, changes include myocyte hypertrophy, necrosis, apoptosis, fibroblast proliferation.1 Biopsies from HF patients show myocytes with a phenotype resembling foetal life with a pattern of embryonic myofilaments, down-regulation of sarcoplasmic reticulum calcium ATPase, increased expression of atrial natriuretic peptide and of ventricular myocytes expressing the i K current channels.16,17 Such a series of events is likely to exert a beneficial effect, and as a result, cardiac function can be maintained at least for a

---

*Corresponding author. Tel: +39 0532239882, Fax: +39 0532 237841, Email fier@unife.it
Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2014. For permissions please email: journals.permissions@oup.com.
limited period of time explaining why remodelling usually becomes evident only months or years after the infarction.\textsuperscript{18,19}

The molecular pathways involved in cardiac remodelling and the existing and novel therapeutic approaches to prevent it have been extensively reviewed.\textsuperscript{20 – 22} In this review, we focus on the role of the Notch signalling in the myocardium and discuss the possibility of targeting this pathway for the reduction of pathological cardiac remodelling. For simplicity, here we only addressed post-MI-remodelling which represents the most common form.

**Notch signalling pathway**

The Notch system controls proliferation, differentiation, and apoptosis in multiple tissues by regulating the communication between adjacent cells.\textsuperscript{23} Humans, and mammals in general, have four Notch receptors (Notch1–4) and five ligands (Delta-like1, 3, 4 and Jagged1 and 2). They are all located on the cell surface. Binding of ligand to receptor triggers two proteolytic cleavages and leads to the release of the active form of Notch (NIC). NIC translocates into the nucleus where it binds to the transcription factor RBP-jk (recombination signal binding protein for the immunoglobulin kappa j region) and activates transcription of target genes.\textsuperscript{2141}

**Figure 1** (A) Ischaemia causes cardiomyocytes apoptosis leading to stretching of the viable cardiomyocytes and secretion of cytokines acting in an autocrine and paracrine manner. (B) In the stressed myocardium, HER2/HER4 and HER2/gp130 pathways could reduce cardiomyocytes apoptosis by activating Notch1. (C) Trastuzumab, by binding HER2, could inhibit both HER2/HER4 and HER2/gp130 pathways and block activation of Notch1 leading to increased cardiomyocytes apoptosis. (HER2, human epidermal growth factor receptor 2; HER4, human epidermal growth factor receptor 4, IL-6, interleukin-6, TRZ, trastuzumab, NRG, neuregulin).

**Figure 2** The interaction between Notch receptors (Notch 1–4) with their ligands (Jagged1, 2 and Dll1, 3, 4) induces cleavages of the receptor by two proteases (red arrows) which release the Notch intracellular domain (NIC). In the nucleus NIC associates with the transcription factor RBP-jk and activates transcription of target genes. (Dll, delta-like ligand; RBP-jk, recombination signal binding protein for the immunoglobulin kappa j region).
is tightly regulated by post-translational modifications as well as by cross-talks with inflammatory cytokines, NF-kB, oestrogen receptor α, erbB-2, and VEGF (vascular endothelium growth factor) receptors (for more details on these cross-talks, the reader is referred to Espinoza and Miele and Rizzo et al.23,24). As a result of these modifications and complex interactions, Notch activity is extremely cell-context dependent and the output is affected by timing, duration and dose of activation. The complexity of this pathway has been recently confirmed by genome-scale studies revealing a wide network of genes which either affect or are affected by Notch.23

What is the role of Notch in the myocardium?

The Notch pathway plays a major role in the heart during development.22 Mutations in the Notch pathway have been identified in human congenital heart defects and, more recently, in individuals affected by left ventricular non-compaction cardiomyopathy.28

Notch1 and Jagged1 are the pre-dominant forms of Notch receptors and ligands expressed in adult myocytes.29 In hearts of adult rats, Notch signalling is absent under normal physiological conditions, but it becomes activated in the border zone of MI30 or in stressed hearts.29,31 Expression of Notch signalling has also been observed in myocardial biopsies from heart failure (HF) patients undergoing heart transplant.32 Inhibition of Notch1 worsened remodelling and hypertrophic response in transgenic mice with cardiomyopathy due to angiotensin II overproduction.39 On the contrary, Notch1 activation by myocardial injection of viral vector expressing Notch130 or Notch1 activating monoclonal antibody33 reduced myocardium damage and remodelling due to ligation of left coronary artery in mice. Overexpression of Jagged1 in cardiomyocytes reduced remodelling induced by transaortic constriction in mice.31 These observations suggest a role for Notch signalling in the repair of damaged, infarcted, or overloaded myocardium. A review of the current literature shows that, even though the exact role played by Notch in myocardial repair is still not completely understood, the Notch signalling seems to be involved in many crucial steps determining the extent of post-MI remodelling (cardiomyocytes survival and regeneration, fibrotic response, angiogenesis) and, therefore, it could represent a novel pathway to be harnessed to enhance myocardial repair.

Does Notch modulate cardiomyocytes survival and proliferation?

Progenitor cells of mature cardiomyocytes express high levels of active Notch1, which is required for their proliferation.34 Primary cultures of rat neonatal cardiomyocytes, isolated at birth, express high levels of active Notch1 and are actively proliferating but after several passages in culture, Notch1 expression becomes undetectable and these cells lose their proliferative ability.35 These data indicate that activation of Notch1 signalling is required for the expansion of cardiac immature cells, but it has to be down-regulated to achieve terminal differentiation. Reactivation of Notch in mature cardiomyocytes has been linked to apoptosis protection.30,33 Following activation of a c-Met receptor by the hepatocyte growth factor, which is elevated in infarcted heart, Notch1 becomes re-activated in cardiomyocytes near the border with the infarct zone leading to increased phosphorylation of the pro-survival protein Akt and to reduction in cardiomyocytes apoptosis.30 Similarly, in mice genetically modified to express the active form of Notch1 in cardiomyocytes, a decreased number of apoptotic cells following a myocardium infarction was observed.33 Consistently, cardiomyocytes apoptosis caused by increased haemodynamic load in hypertensive mice was higher in the absence of cardiac Notch1 signalling.29 Furthermore, in cardiomyocytes grown under hypoxia, Notch1 activation induced the expression of anti-apoptotic genes36 and inhibition of Notch signalling caused increased apoptosis.31 In comparison to wild-type, in mice overexpressing active Notch1 in cardiomyocytes, MI led also to increased number of ki67-positive cardiomyocytes (a protein linked to cell proliferation), suggesting their re-entry in cell cycle and proliferation. Nevertheless, no differences were found in this context in the number of phospho-Hist3-positive cardiomyocytes, suggesting that Notch activation induces incomplete cell cycle progression in adult cardiomyocytes.31 In agreement with these data, forced activation of Notch in mature cardiomyocytes led to cell cycle progression followed by G2/M interphase arrest block and apoptosis.37 These results suggest that following a myocardial damage, a temporary activation of Notch1 would increase cardiomyocytes survival. It remains to be established whether under these conditions, a prolonged Notch activation would also be able to induce their proliferation.

Does Notch control cardiovascular stem cells?

Notch is a fundamental pathway for proliferation and differentiation of resident cardiac precursor cells (CPCs). These are myocardial niche of c-kit, stem cell antigen 1 (Sca1) and multi-drug resistant gene product 1 (MDR1) positive, self-renewing, and multi-potent stem cells that could play a role in cardiac repair.39,38,40 The ability of cardiac stem cells present in adult myocardium to differentiate into cardiomyocytes in a post-infarction environment has been questioned by studies that have shown c-kit+ precursors support post-infarction myogenesis in the neonatal, but not in adult heart.41

Cardiac precursor cells express mainly the Notch1 receptor.34 Its activation by Jagged1 on the surface of adjacent cardiomyocytes, induces the expression of Nkx2.5, a transcription factor which promotes proliferation and expression of cardiomyogenic transcripts and it inhibits the expression of markers of vascular cells.42 Thus Notch1 favours myocyte lineage specification of CPCs and maintains them in a high-proliferative state. By doing so, Notch1 exerts control not only of heart homeostasis but also of adaptation to pathological states. Consistently, Notch1 inhibition in newborn healthy mice causes a 56% reduction in cardiomyocytes and induces dilated cardiomyopathy.34 Additionally, Notch1 inhibition causes a decrease in Nkx2.5-positive cells and a reduction in the generation of new myocytes in a mouse model of myocardium infarction.42 In transgenic mice overexpressing Jagged1 on cardiomyocytes, remodelling caused by transaortic constriction is attenuated and there is improved cardiac function due to Notch1 activation in CPCs which...
promotes their differentiation into Nkx2.5-positive CPCs rather than into fibrosis-causing myofibroblasts.

Notch signalling is also involved in the regulation of bone marrow-derived endothelial precursor cells (EPCs) and MSCs. Co-culture of EPCs with Jagged1-expressing cardiomyocytes shows that activation of Notch1 is necessary for the expression of cardiomyocytes markers in EPCs. Deletion of Notch1 in bone marrow-derived MSCs impairs their recruitment, proliferation and survival leading to a decreased ability to repair myocardium damage compared with MSCs with a functional Notch1. Conversely, activation of Notch1 signalling in bone marrow MSCs by soluble Jagged1 increases their differentiation into cardiomyocytes.

These findings suggest that it could be important to evaluate the status of Notch signalling in stem cells obtained from patients for therapeutic purposes. Mesenchymal stem cells isolated from adipose tissue of HF patients express low levels of Notch1 and Jagged1 in comparison with cells obtained from healthy subjects. This reduced Notch activity could hamper their therapeutic use.

**Does Notch modulate myocardial angiogenesis?**

The growth of new capillaries and arterioles is often inadequate in the post-MI heart and this lack of adequate blood perfusion contributes to infarct expansion and transition to HF. Since exercise training is able to promote only limited sprouting of new blood vessels, stem cells, and pharmacological approaches have been utilized in the pre-clinical setting to enhance post-MI angiogenesis.

Notch receptors (1, 2, and 4), Delta-like ligands (DLL) 1, 4, and Jagged1, 2 are all expressed in the endothelium. Notch signalling determines arterial-venous specification during the development and, after birth, it maintains vascular homeostasis by controlling endothelial cells survival and proliferation and by regulating sprouting angiogenesis (for a review on Notch regulation of angiogenesis the reader is referred to Rizzo et al.52). Notch signalling is involved also in vascular smooth muscle cell growth, apoptosis, and migration.

Notch1 is active in endothelial cells and vascular muscle cells of cardiac vessels. In mouse heart, Notch1 activation by intramyocardial delivery of a monoclonal antibody (pseudo-ligand), 4 weeks after infarction, led to higher levels of angiogenesis markers which were associated with reduced scar and improved cardiac functions. On the contrary, inhibition of Notch signalling by low doses of a soluble DLL4 extracellular domain fused to human IgG1-promoted reperfusion of acutely ischaemic limbs by inducing functional neo-angiogenesis. These contrasting results, probably due to the context-dependent dependency of Notch signalling, show that more studies are required to fully understand how Notch signalling could be manipulated to promote angiogenesis in ischaemic tissues.

**Targeting Notch in oncology: side effects on the heart?**

Notch signalling is activated in the majority of solid tumours and leukemias and it is a promising therapeutic target in oncology. Notch inhibition not only sensitizes cancer cells to apoptosis-inducing drugs, but it affects also tumour angiogenesis and the growth of cancer stem cells involved in tumour recurrence and metastasis. There are ~30 clinical studies ongoing to investigate efficacy and/or safety of a combined treatment of Notch inhibitors with existing oncology drugs (source: www.clinicaltrial.gov). The majority of these studies employs small molecules inhibitors of the gamma secretase (GSI), the enzymatic complex involved in Notch activation. Even though short-term treatment with GSIs causes only minor gastrointestinal toxicity (resulting from inhibition of Notch1 and Notch2 in the intestinal progenitor cells) which can be overcome by intermittent treatment, alternative strategies to target Notch pathway with higher tumour-selectivity and minimum toxicity are being developed. These include monoclonal antibodies directed against individual Notch receptors. Four Phase I/II studies are ongoing to investigate the efficacy of Notch inhibiting—monoclonal antibodies in cancer patients (source: www.clinicaltrial.gov).

Since Notch inhibitors have the potential to target not only the tumour, but also the heart and blood vessels, the possible cardiotoxicity associated with long-term Notch inhibition will have to be investigated.

The Notch signalling could be playing a role also in the context of cardiotoxicity associated with drugs that are commonly used in oncology. Cardiotoxicity has been observed following treatment with trastuzumab, an antibody against Human Epidermal growth factor Receptor 2 (HER2, also known as gp185), used as an additional therapy in patients with HER2-overexpressing breast cancer. Similar issues are being faced today with other clinically approved kinase-targeted oncology agents.

Trastuzumab-induced cardiotoxicity is still poorly understood, but it is thought to be due to the blocking of HER2 which causes inhibition of survival signalling pathways in cardiomyocytes. In agreement with this hypothesis, cardiotoxicity occurs more often when trastuzumab is administered together or immediately after anthracycline treatment, which acts as a stressor to the heart, as does an acute MI.

HER2 is a highly characterized oncoprotein and cross-talks between HER2 and Notch have been identified in breast cancer in which Notch signalling is also activated. Treatment of MCF10A cells (immortalized breast epithelial cell line) with heregulin, to induce activation of HER2, leads to cyclin D1-mediated activation of Notch1. Furthermore, in a HER2-transgenic mouse model of breast cancer, Notch signalling plays a critical role in tumour maintenance since its inhibition leads to reduced cancer cells proliferation.

These data suggest that HER2 activation in breast cancer leads to Notch1 activation even though it has been shown that trastuzumab treatment of HER2-overexpressing SKBR3 breast cancer cell lines activates Notch signalling. In prostate carcinoma and breast cancer, HER2 activation is required for activation of the pro-survival gp130-mediated pathway. Of interest, in neural stem cells and breast cancer, gp130 activates Notch signalling.

It is well established that, after an infarction, the IL-6 (interleukin-6)-type cytokines-dependent gp130 receptor pathway enhances cardiomyocytes survival by activating the signal transducer and activator of the transcription (STAT)-1/3 pathway and the SH2 domain-containing cytoplasmic protein tyrosine phosphatase (SHP2)/MEK/ extracellular signal-regulated kinase pathway. Furthermore, neuregulin, another important stress-mediated paracrine growth factor, promotes survival and hypertrophy in the heart by activating...
signalling mediated by HER2/HER4. Based on these observations, it could be of interest to determine whether in the infarcted myocardium HER2 activation, directly and/or through interaction with gp130, activates Notch1, which would be needed for cardiomyocytes survival. If that were the case, these studies could have translational implications since Notch1 activation in the heart could counteract trastuzumab-induced cardiotoxicity (Figure 1B and C).

**Notch: a potential target for cardiovascular therapy?**

The evidences presented so far show that some pathways utilized by Notch to promote survival and proliferation are in common between cancer cells and cardiomyocytes. The hypoxic environment in tumour leads to Notch1 activation which in turn activates the pro-survival protein AKT. In cardiomyocytes, as in cancer cells, Notch1 controls cyclin D1 expression and nuclear localization and similarly to CPCs, Notch is needed for proliferation of cancer stem cells.

It follows that Notch activation could be a target for the treatment of remodelling and HF. This possibility is particularly relevant after acute MI, a context where myocytes death prevails while regeneration from exogenous or endogenous stem cells is, at best, insufficient to compensate for the myocytes loss.

Compared with the oncology, translational research in the cardiovascular field is still in its infancy. Considered the complex series of events characterizing cardiac remodelling and the multifaceted role of Notch in the infarcted myocardium, the targeting of Notch could prove to be more difficult in this setting. Inflammation plays a major role in the pathogenesis of post-infarction HF since, immediately after the infarction, it worsens the damage (ischaemia/reperfusion injury) and then it is required to re-adsorb the necrotic tissue. In a mouse model of ischaemic stroke, Notch inhibition led to reduced brain damage by decreasing the inflammatory response which follows ischaemia and reperfusion. On the contrary, in mouse ischaemic myocardium, Notch1 signalling protected against reperfusion injury. More studies are needed to clarify the role of Notch in the inflammatory response within the infarction. During the repair phase, activation of Notch signalling could have a beneficial effect on cardiac recovery (Figure 3). More pre-clinical studies are needed to validate the results obtained so far by a still limited number of laboratories. Additionally, timing, safety, feasibility and endpoints of these putative treatments will have to be investigated.

**Ex vivo** Notch activation of stem cells for clinical use has been successfully attempted to expand stem cells isolated from bone marrow of leukaemia patients. It remains to be established whether the activation of Notch signalling in EPCs or MSCs would render them more suitable to repair myocardial damage.

**Conclusions**

Recognition of the most emerging concepts in cellular and ventricular remodelling is needed for future cardiovascular drug discovery,
which, today, is viewed as an extremely risky enterprise, particularly in the area of remodeling and HF. After identifying that a neuroendocrine response in HF is deleterious, no other fundamental steps have been taken to understand the biology of the disease. Not surprisingly, after recognition of the usefulness of angiotensin-converting enzyme inhibitors (ACEI), β-blockers and mineral corticoid antagonists for slowing remodeling progression, no real innovations have been made other than identifying hypothetically ‘dangerous’ hormones or cytokines to be blocked. As a consequence, cardiology has experienced several clinical trials with anti-angiotensin I receptors, anti-renin, anti-endothelins, anti-tumour necrosis factor α. None of these, however, has provided exciting results, suggesting that a different approach is needed.72

There is little doubt that cardiac remodeling is multi-mechanistic and complex. By no means this brief review has examined the large number of pathways that contribute to ventricular remodeling. Our intention was to highlight the possible role of the Notch pathway in this context. Notch has important functions in the development of the heart and its activation following a myocardial injury suggests a role for this pathway in cardiac repair. The oncologists have been actively pursuing the targeting of Notch for cancer therapy, thus providing the stimulus to investigate Notch in the cardiovascular field. However, a lot of work is needed to confirm the role of Notch in this context and whether it can develop into a therapeutic target to improve heart function. The collaboration between cardiologists and cancer researchers will be crucial for investigational Notch inhibitors and activators. Cardiovascular researchers can rely on a large body of experimental data accumulated during the last 20 years with the intent to target Notch for the induction of apoptosis in cancer cells and for the inhibition of tumour angiogenesis. On the other hand, the oncologists will also benefit from this collaboration, which may lead to target Notch for cancer therapy with minimal cardiovascular negative effects.

**Funding**

This work was supported by a grant from Fondazione Anna Maria Sechi per il Cuore (FASC), Italy.

**Conflict of interests** none declared.

**References**


