International Journal of Cardiology 285 (2019) 72–79

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Remote ischemic perconditioning attenuates adverse cardiac remodeling and preserves left ventricular function in a rat model of reperfused myocardial infarction

Patrick M. Pilz [a], Ouafa Hamza [a], Olof Gidlöf [b], Ines F. Gonçalves [a], Eva Verena Tretter [c], Sandra Trojanek [d], Dietmar Abraham [d], Stefan Heber [e], Paul M. Haller [a], Bruno K. Podesser [a,*], Attila Kiss [a,*]

[a] Ludwig Boltzmann Cluster for Cardiovascular Research at the Center for Biomedical Research, Medical University of Vienna, Vienna, Austria
[b] Department of Cardiology, Clinical Sciences, Lund University, Skåne University Hospital, Lund, Sweden
[c] Department of Anesthesia, General Intensive Care and Pain Therapy, Medical University of Vienna, Austria
[d] Center for Anatomy and Cell Biology, Medical University of Vienna, Vienna, Austria
[e] Center for Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria

ARTICLE INFO

Article history:
Received 25 October 2018
Received in revised form 22 February 2019
Accepted 1 March 2019
Available online 6 March 2019

Keywords:
Myocardial infarction
Remote ischemic perconditioning
Neuregulin-1
Inflammation
Epigenetic
ErbB receptors

ABSTRACT

Aims: Remote ischemic conditioning (RIC) is considered a potential clinical approach to reduce myocardial infarct size and ameliorate adverse post-infarct left ventricular (LV) remodeling, however the mechanisms are unknown. The aim was to clarify the impact of RIC on Neuregulin-1 (NRG-1)/ErbBs expression, inflammation and LV hemodynamic function.

Methods and results: Male Sprague-Dawley rats were subjected to 30 min occlusion of the left coronary artery (LCA) followed by 2 weeks of reperfusion and separated into three groups: (1) sham operated (without LCA occlusion); (2) Myocardial ischemia/reperfusion (MIR) and (3) remote ischemic perconditioning group (MIR + RIPerc). Cardiac structural and functional changes were evaluated by echocardiography and on the isolated working heart system. The level of H3K4me3 at the NRG-1 promoter, and both plasma and LV tissue levels of NRG-1 were assessed. The expression of pro-inflammatory cytokines, ECM components and ErbB receptors were assessed by RT-qPCR. MIR resulted in a significant decrease in LV function and enlargement of LV chamber. This was accompanied with a decrease in the level of H3K4me3 at the NRG-1 promoter. Consequently NRG-1 protein levels were reduced in the infarcted myocardium. Subsequently, an upregulated influx of CD68+ macrophages, high expression of MMP-2 and -9 as well as an increase of IL-1β, TLR-4, TNF-α, TNC expression were observed. In contrast, RIPerc significantly decreased inflammation and improved LV function in association with the enhancement of NRG-1 levels and ErbB3 expression.

Conclusions: These findings may reveal a novel anti-remodeling and anti-inflammatory effect of RIPerc, involving activation of NRG-1/ErbB3 signaling.

1. Introduction

Despite the progressive and gradual decrease in incidence, acute myocardial infarction (MI) remains a significant public health problem, representing a major contributor to mortality and morbidity worldwide. Numbers of patients surviving the acute MI event exhibit adverse left ventricular (LV) remodeling, leading eventually to congestive heart failure (HF). The pathophysiology of post-infarct LV remodeling is associated with, and largely driven by, excessive inflammation [1–3]. In fact, the inflammatory response after MI plays a crucial role in cardiac repair, but it has been implicated in adverse post-infarct LV remodeling [2,4]. Upregulation of inflammatory cytokines and chemokine expression (IL-1β, TNF-α, IL-6 and MCP-1) plays a fundamental role in expansion of MI size, contractility impairment and progression of LV remodeling.

Abbreviations: AUC, area under the curve; Ang II, Angiotensin II; CO, cardiac output; ECM, extracellular matrix; EHW, external heart work; H&E, hematoxylin and eosin; HF, heart failure; LCA, left coronary artery; LV, left ventricular; LVSP, left ventricular systolic pressure; MI, myocardial infarction; MMPs, matrix metalloproteinases; NRG-1, Neuregulin-1; RIC, Remote ischemic conditioning; RIPerc, remote ischemic perconditioning; SV, stroke volume; TNC, Tenascin-C.

* Corresponding authors at: Ludwig Boltzmann Cluster for Cardiovascular Research at the Center for Biomedical Research, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria.
E-mail addresses: bruno.podesser@meduniwien.ac.at (B.K. Podesser), attila.kiss@meduniwien.ac.at (A. Kiss).

https://doi.org/10.1016/j.ijcard.2019.03.003
0167-5273/

Subsequently, the release of pro-inflammatory cytokines by macrophages largely contributes to the activation of matrix metalloproteinases (MMPs), ultimately leading to maladaptive LV remodeling and cardiac dysfunction [5]. With regard to extracellular matrix (ECM) responses in adverse LV remodeling, we demonstrated the pathophysiological importance of Tenascin-C (TNC) on MMP-2 regulation in a mouse model of chronic pressure overload induced LV hypertrophy [6]. Other investigators reported similar findings in post-infarct LV remodeling [7,8] via a signaling mechanism of TNC modulated M1/M2-macrophage polarization [9].

Remote ischemic conditioning (RIC) by brief non-lethal episodes of ischemia in the hind limb has recently emerged as a novel therapeutic method to achieve cardioprotection in patients with ST-elevation MI (STEMI) [10]. Despite numerous reports demonstrating that RIC reduces myocardial infarct size in acute MI [11], only a few studies attempted to clarify the role of RIC beyond MI size reduction [12]. Recently, Wei et al. [13] demonstrated that the anti-remodeling effects of RIC or repeated RIC were associated with reduced levels of inflammation in rat hearts. However, the mechanisms underlying the anti-remodeling effect of RIC after MI are largely unknown. More recently, experimental studies demonstrated that the acute infarct size limiting effect of ischemic local postconditioning was associated with the activation of Neuregulin-1 (NRG-1)/ErbB3 and ErbB4 signaling pathways [14,15]. In addition, a number of preclinical and clinical studies have shown the cytoprotective, anti-inflammatory and anti-remodeling effects of NRG-1-ErbBs signaling [16–18]. Nevertheless, the effect of RIC on the regulation of NRG-1/ErbB2/3/4 expressions and post-infarct LV remodeling has not been investigated.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (10–12 weeks old, 250–300 g, Department for Laboratory Animal Science and Genetics, Himberg, Austria) were used. The experimental protocol was approved by the regional Ethics Committee for Laboratory Animal Experiments at the Medical University of Vienna and the Austrian Ministry of Science Research and Economy (BMWFW-66.009/0023-WF/V/3b/2016), and conforms with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Experimental protocol

The experimental protocol is depicted in Supplemental Fig. 1. Male Sprague-Dawley rats were anaesthetized by intraperitoneal injection of a mixture of Xylazine (4 mg/kg; Bayer, Germany) and Ketamine (100 mg/kg; Dr. E. Gräub AG, Switzerland), intubated (14-gauge tube) and ventilated (9 ml/kg body weight, 75–85 stroke/min). Rats were allocated to MIR (n = 14) or RIPerc with three cycles of 5 min of IR of a hindlimb performed during myocardial ischemia (MIR + RIPerc, n = 15) and were subjected to 30 min of left coronary artery (LCA) occlusion followed by 14 days of reperfusion. To induce myocardial ischemia, the heart was exposed via a left thoracotomy and a ligature was placed around the left coronary artery 2–3 mm away from the origin. Sham operated animals (Sham, without LCA occlusion; n = 10) served as controls. Rectal temperature was measured and maintained at 37.5–38.5 °C by a heated operating table. Myocardial ischemia was associated with pallor of the myocardial area at risk and ST-elevation on ECG signal. Reperfusion was initiated after 30 min of LCA occlusion by removal of the snare and confirmed by the changes in color of the heart and signal on ECG. Analgesia was initiated by intraperitoneal injection of Piritramide (0.1 ml/kg body weight) preoperatively and Piritramide in drinking water was applied as a postoperative analgesic regimen (2 ampules of Piritramide with 30 ml of Glucose 5% in 250 ml water).

Two weeks after the reperfusion, hearts were either explanted or mounted on an isolated working heart system (Sham n = 5, MIR = 6 and MIR + RIPerc n = 7) or myocardial tissue samples were collected for further analysis (Sham n = 5, MIR = 7 and MIR + RIPerc n = 8).

2.3. Assessment of myocardial scar formation

Assessment of myocardial scar formation is described in detail in the Supplement. Two weeks after reperfusion hearts were explanted washed and perfused with saline before being transferred to a 4% paraformaldehyde solution for 24 h. After this fixation period, heart sections (5 μm) were stained with hematoxylin and eosin (H&E) and mounted on glass slides. In addition, heart sections were stained with Masson-Goldner to measure collagen contents. Images were acquired by microscopy (Olympus VS120 Virtual Slide Microscope System, Olympus, Japan) and captured by digital camera (AVT PIKE F-505C VC 50, Allied Vision Technologies, Germany). To assess scar formation at 14 days after MI, the LV area was estimated using a slice obtained from the central part of the myocardium at mid-papillary muscle level. The percentage of fibrosis was acquired with Adobe Photoshop Element (Adobe Photoshop, Version 14.1) based on the equation: % fibrosis (scar formation) = fibrotic area / (fibrotic area + non-fibrotic area of LV).

2.4. Transthoracic echocardiography assessment

Transthoracic echocardiography assessment is described in detail in the Supplement.

2.5. Assessment of left ventricular hemodynamic function in isolated working heart system

Assessment of left ventricular hemodynamic function is described in detail in the Supplement.

2.6. Assessment of NRG-1 and TNC levels

Plasma levels of TNC (CSB-E13377r, Maryland, USA) and plasma as well as LV tissue levels of NRG-1 (E-EL-R0790 Elabscience, Texas, USA) were determined by ELISA. ELISA kits were used according to the manufacturer's protocol.

2.7. Chromatin immunoprecipitation (ChIP)

Assessment of ChIP-qPCR is described in detail in the Supplement.

2.8. Quantitative polymerase chain reaction (RT-qPCR)

Assessment of RT-qPCR is described in detail in the Supplement.

2.9. Immunohistochemistry

Streptavidin-biotin immunostaining for CD68, MMP-2, and MMP-9 of paraffin-embedded tissue sections was performed in three slices per heart as described previously [19]. Sections were incubated with antibodies against CD68 (1:100; mouse monoclonal, ED1, ab31630, Abcam, Cambridge, MA, USA) to evaluate the density of tissue macrophages, MMP-2 (1:500; rabbit polyclonal, NB200-193, Novus Biologicals, CO), and MMP-9 (1:100; rabbit monoclonal, EP1254; ab76003, Abcam). Primary antibodies were detected with biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) and peroxidase conjugated streptavidin (Dako, Glostrup, Denmark), developed with 3,3′-diaminobenzidine (Vector Laboratories), counterstained with hematoxylin, dehydrated and mounted in DPX (Merck, Darmstadt, Germany). Digitalized images were generated with a Nikon Eclipse 80i (Tokyo, Japan) microscope.

2.10. Statistical analysis

Data are presented as mean ± SEM. One-way ANOVA followed by Tukey post hoc tests were used for comparisons of hemodynamic parameters in vivo and ex vivo, extent of myocardial scar formation and expression of cytokines, MMPs as well as NRG-1 and TNC protein levels. Analysis was performed using Prism™ 6 software (GraphPad Inc., San Diego, CA, USA). Paired *t*-test was used for comparison of two groups. In addition, to compare external heart work and cardiac output, the area under the curve (AUC) was estimated for each dependent variable for each experimental condition separately as described previously [20]. Resulting estimates with standard errors were compared between experimental conditions using one-way ANOVA followed by post-hoc tests corrected by Sidak's method. Statistical analysis was performed with GraphPad Prism 7. Only two-sided p-values were used, $p < 0.05$ was considered significant.

3. Results

3.1. Animal characteristics

There were no differences in body weight between the groups (data not shown). However, myocardial infarction resulted in increased heart to body weight ratio (Sham: 4.01 ± 0–01 mg/g vs MIR: 4.98 ± 0.06 mg/g; $p < 0.001$), which was significantly reduced by RIPerc (MIR: 4.98 ± 0.06 mg/g vs MIR + RIPerc: 4.35 ± 0.01; $p < 0.01$ vs MIR). This result indicates the potential positive effect on post MI by RIPerc.

3.2. RIPerc reduces scar formation and improves left ventricular function after myocardial infarction

The extent of MI (scar formation) was significantly reduced by RIPerc as compared to MIR (Fig. 1A and B, $p < 0.01$). Transthoracic echocardiography was used in order to assess cardiac structure and function. The summarized results with a representative image (Fig. 1C) of

echocardiography are presented in Fig. 1. Myocardial infarction resulted in a significant reduction in LVEF (Fig. 1D, $p < 0.001$ vs Sham) and LV chamber enlargement (LVESD and LVEDD) (Fig. 1E–F; $p < 0.001$ vs Sham; respectively). The improvement of LV dilatation (Fig. 1E and F), $p < 0.05$) by RIPerc was associated with a marked increase in LVEF (Fig. 1D, $p < 0.05$ vs MIR).

3.3. RIPerc improves left ventricular dysfunction ex vivo

To further evaluate LV function, an erythrocyte-perfused isolated working heart model was used to assess cardiac output (CO), stroke volume, LV systolic pressure (LVSP), heart rate (HR) and coronary flow. No differences in HR and basal CF between groups were observed (data are not shown). However, LVSP, CO and SV were reduced in the MIR group as compared to Sham (Fig. 2A–C, respectively). In contrast, rats with RIPerc showed a tendency to improve LVSP ($p = 0.06$, Fig. 2A) and significantly increased CO and SV as compared to the MIR group (Fig. 2B–C, $p < 0.05$ and $p < 0.01$, respectively). In addition, measured at a physiologically relevant afterload, the recovery of CO and EHW were significantly reduced in both MIR groups compared to Sham (Fig. 2D–E). Of note, the RIPerc group showed a significantly higher recovery of both parameters (Fig. 2F and G, $p < 0.05$, respectively) within the physiologic range (50 mmHg–170 mmHg) compared to the MIR group.

3.4. Effect of RIPerc on NRG-1 promoter activity and protein levels in plasma and LV tissue

The level of H3K4me3, a chromatin modification indicative of an activated promoter, at the NRG-1 promoter was measured by ChIP-qPCR. ChIP was performed using a H3K4me3 antibody on chromatin from cardiac tissue (border zone and infarcted) from Sham, MIR, and MIR + RIPerc groups and analyzed by qPCR with primers specific for the core NRG-1 promoter. MIR resulted in significantly reduced levels of H3K4me3 at the NRG-1 promoter in the infarcted tissue as compared to the border zone (Fig. 3A, $p < 0.05$). This was accompanied by a significant reduction of NRG-1 protein levels in both the infarcted myocardium and plasma (Fig. 3B and C; $p < 0.01$ vs Sham). These effects were reversed by RIPerc (Fig. 3A–C, $p < 0.01$, respectively). Of note, mRNA expression of ErbB2 and −4 tended to be downregulated in both MIR groups (Fig. 3D and E). However, RIPerc exclusively affected ErbB3 expression in the reperfused myocardium (Fig. 3D, $p < 0.01$).

3.5. RIPerc reduces the influx of macrophages, expression of MMP-2 and MMP-9 and inflammatory cytokines, TLR-4 and TNC

Immunohistochemistry was performed to evaluate the influx of CD68+ macrophages and the expression of MMP-9 and MMP-2 (Fig. 4A–C). No CD68+ cells were detected in the Sham group

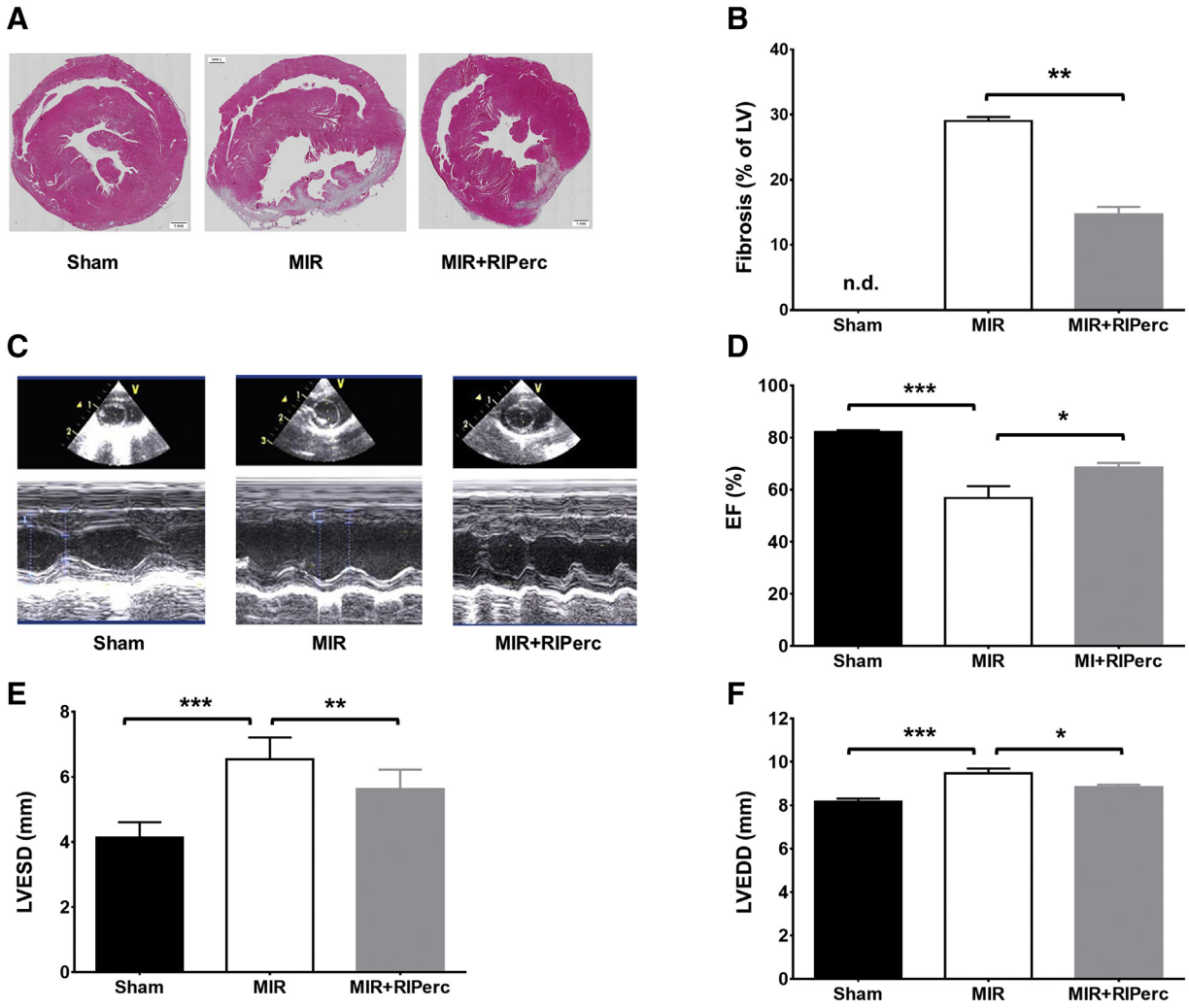

Fig. 1. Effect of remote ischemic conditioning on scar formation, left ventricular function and remodeling. (A) Representative Masson-Goldner staining on LV slices was obtained on day 14 post myocardial reperfusion and (B) quantified results of fibrosis in bar graphs. (C) Representative M-mode echocardiograms for each group, with (D) EF; (E) LVESD and (F) LVEDD quantified in bar graphs. Data are expressed as mean ± SEM. IR: ischemia-reperfusion, RIPerc: remote ischemic perconditioning, EF: ejection fraction, LVESD: left ventricular end-systolic diameter, LVEDD: left ventricular end-diastolic diameter, LV: left ventricular. Data are expressed as mean ± SEM. *$p < 0.05$; **$p < 0.01$ and ***$p < 0.001$.

(Fig. 4A). In contrast, high numbers of CD68+ macrophages were present in the infarcted myocardial tissue of the MIR group. Furthermore, high expression of MMP-9 and MMP-2 was found in the infarcted area (Fig. 4B and C), indicating a pivotal role for these MMPs in the remodeling of the reperfused myocardium. More importantly, improvement of LV remodeling by RIPerc was associated with reduced influx of CD68 + macrophages (Fig. 4A) and reduced expression of both MMP-9 and MMP-2 (Fig. 4B and C).

In addition, mRNA expression of inflammatory cytokines, chemokines, ECM components and TNC expression were assessed in LV tissue samples. Myocardial ischemia and long-term reperfusion resulted in a significant upregulation of the expression of pro-inflammatory cytokines such as TNF-α (Fig. 4D) and IL-1β (Fig. 4E, $p < 0.05$ and $p < 0.001$ vs Sham, respectively). Similar to these findings, TLR-4 was upregulated in the reperfused myocardium (Fig. 4F, $p < 0.05$, respectively). In contrast, RIPerc markedly reversed this upregulation of cytokines (Fig. 4D–E, $p < 0.05$ and $p < 0.01$, respectively) and TLR-4 (Fig. 4F, $p < 0.05$). Furthermore, both tissue and plasma levels of TNC were significantly upregulated in the MIR group as compared to Sham (Fig. 4G and H, $p < 0.05$ and $p < 0.01$, respectively), an effect that was reversed by RIPerc (Fig. 4G and H, $p < 0.05$, respectively).

4. Discussion

To the best of our knowledge, this is the first study to reveal the impact of RIPerc on epigenetic changes of the NRG-1 promoter. This may ultimately lead to an increase of NRG-1 levels associated with increased ErbB3 expression, thereby affecting the NRG-1/ErbB3 signaling capacity, and revealing a potential mechanism to improve post-infarct LV remodeling and cardiac dysfunction. In addition, reversal of MIR by RIPerc was associated with a marked decline in influx of CD68+ macrophages, and the expression of inflammatory cytokines, TLR-4, TNC and MMPs.

Post-infarct LV remodeling is a predictor of congestive HF, and for this reason it assumes a negative prognostic value [21]. Although β-receptor blockers and inhibitors of the renin-angiotensin system, or angiotensin II (Ang II) receptor blockers, are commonly used to prevent progression of LV remodeling and hence development of HF, the number of patients suffering from HF is still increasing. Adverse, post-infarct, LV remodeling is closely intertwined with an excessive inflammatory response that ultimately results in fibrous tissue deposition and cardiac dysfunction [22]. Accordingly, we demonstrated a significant upregulation of pro-inflammatory cytokines such as IL-1β and TNF-α as well as an elevated influx of CD68+ macrophages in the reperfused myocardium. This was accompanied by a significant enlargement of the LV chamber and cardiac dysfunction. Despite a growing understanding of the molecular signals regulating the post-infarct inflammatory response, therapeutic targeting of inflammatory mediators in patients with MI has proved challenging. A recent preclinical study by Wei et al. [13] suggested an additional benefit of RIC besides the observed initial reduction in infarct size, indicating a novel benefit of RIC acting directly on post-infarct LV remodeling. They found that RIC markedly reduced the infiltration of macrophages and monocytes, and

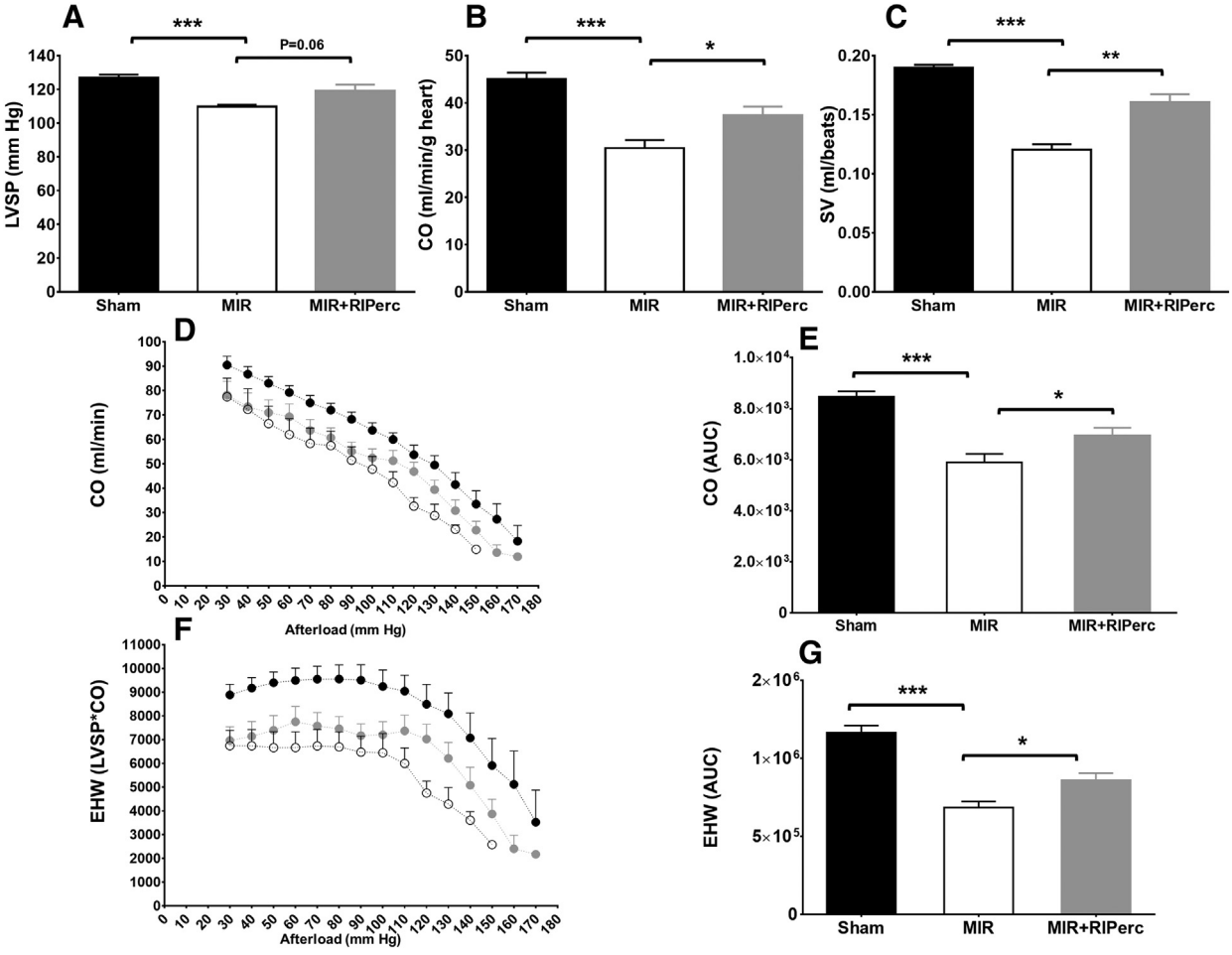

Fig. 2. Effect of remote ischemic conditioning on left ventricular hemodynamic function. (A) Left ventricular systolic pressure (B) cardiac output, (C) stroke volume results were obtained from isolated working heart on day 14 post myocardial reperfusion (D) cardiac output is depicted as a function of afterload; (F) external heart work as function of afterload and quantified results in bar graph (E and G). Data are expressed as mean ± SEM and n = 4–7/group. *$p < 0.05$; **$p < 0.01$ and ***$p < 0.001$. MIR: myocardial ischemia/reperfusion and RIPerc: remote ischemic perconditioning, CO: cardiac output; EHW: external heart work; SV: stroke volume; LVSP: left ventricular systolic pressure; AUC: area under the curve.

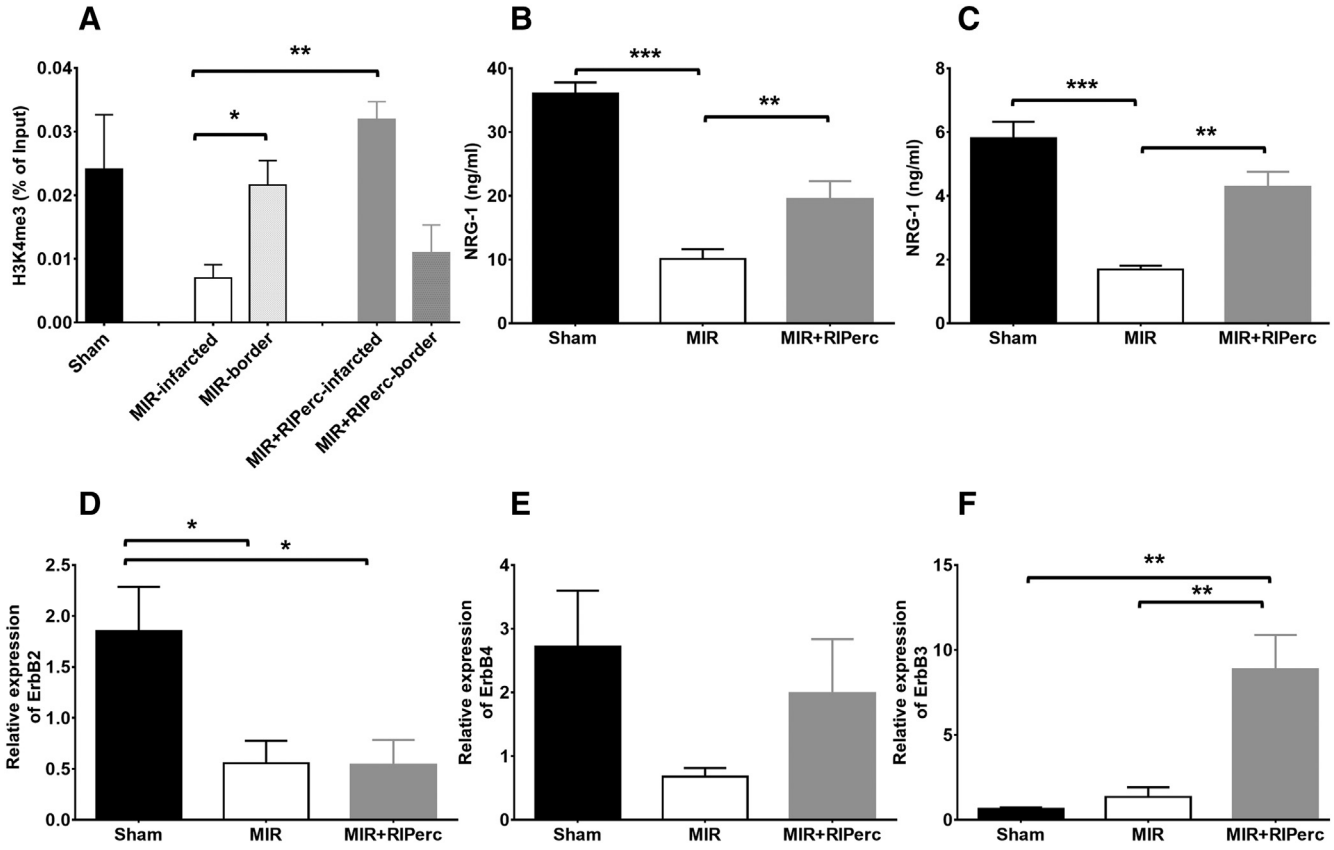

Fig. 3. Effect of remote ischemic perconditioning on NRG-1 promoter activity, expression of NRG-1 and ErbBs. The level of H3K4me3, a chromatin modification indicative of an activated promoter, at the NRG-1 promoter was measured by ChIP-qPCR. ChIP was performed using a H3K4me3 antibody on chromatin from (A) infarcted and border zone from each group. (B) Infarcted myocardial tissue and (C) plasma protein levels of NRG-1. mRNA expression of (D) ErbB2; (E) ErbB4 and (F) ErbB3 in reperfused LV tissue. Data are expressed as mean ± SEM and n = 4–7/group. *p < 0.05; **p < 0.01 and ***p < 0.001. MIR: myocardial ischemia/reperfusion, NRG-1: Neuregulin-1 and RIPerc: remote ischemic perconditioning.

subsequently the expression of IL-1β in the infarcted myocardium on day 4 after MI [13]. However, the mechanisms underlying the anti-remodeling effect of RIC after MI are largely unknown. Therefore, our study aimed to clarify the role of RIPerc on the regulation of NRG-1/ErbB expression. Recently, Wang et al. [15] demonstrated that the MI size limiting effect of local ischemic postconditioning was associated with an upregulation of NRG-1/ErbB4 expression. Neuregulin-1 is critical for cardiac development and repair, and recombinant forms are currently being assessed as possible therapeutics for systolic HF [23]. In addition, numerous preclinical studies confirmed the anti-remodeling and anti-inflammatory effects of NRG-1 [24,25]. Subsequently, we demonstrated for the first time that both promoter activity of NRG-1 and NRG-1 levels were declined in the infarcted myocardium. These findings may indicate a process for excessive inflammation, fibrosis and subsequently LV dysfunction. In line with this, recombinant of NRG-1 protein attenuated Ang II-induced myocardial inflammation, reducing influx of macrophages and fibrosis [26], suggesting the putative role of NRG-1 on inflammation. Accordingly, we found that enhancement of NRG-1 by RIPerc was associated with a decrease in influx of CD68+ macrophages and subsequently the expression of IL-1β and TNF-α. In addition, it is also worth pointing out that myocardial IR and local preconditioning causes epigenetic effects at specific loci throughout the genome [27]. Next, we characterized the epigenetic state of the NRG-1 promoter with particular focus on H3K4me3, a histone mark characteristic of activated promoters. We demonstrated for the first time that remote ischemic perconditioning markedly enhanced the activity of NRG-1 promoter in comparison with controls after 2 weeks of reperfusion. Upregulation of NRG-1 levels by RIPerc also may contribute to the improvement of cardiac contractile dysfunction after MI [28,29]. Accordingly, the recovery of cardiac output and external heart work were remarkable by RIPerc. However, these results did not prove that the cardioprotective effect of RIPerC is driven by NRG-1 solely, but these findings elucidate that the NRG-1 signaling pathways should be essential components in the anti-remodeling effect of RIPerc. Of importance, we need to emphasize that our study was not aimed to investigate the causative role of a loss of NRG-1 signaling in adverse post-MI LV remodeling and cardioprotection from RIPerc, further studies are needed to clarify that.

Therefore, another interesting finding of our study was a sole upregulation of ErbB3 expression by RIPerc. NRG-1 binds to the ErbB family of tyrosine kinase receptors, i.e. ErbB2, ErbB3 and ErbB4, and subsequently activates downstream cardioprotective and anti-inflammatory signaling [16,23,30]. More recently, NRG-1 has been shown to inhibit the transition of cardiac fibroblasts to myofibroblasts, cells primarily responsible for the massive collagen deposition that characterizes the process of fibrosis after MI [31]. This mechanism is more likely mediated through the activation of the ErbB3 receptor, which is highly expressed by cardiac fibroblasts [32]. Furthermore, the activation of ErbB3 by NRG-1 on monocytes leads to a significant reduction in the release of inflammatory cytokines, indicating the anti-inflammatory effect of ErbB3 activation [33]. Based on these findings, we strongly believe that ErbB3 receptor upregulation by RIPerc largely contributes to the marked reduction in fibrosis and inflammation. In addition, we demonstrated that both ErbB2 and ErbB4 receptor mRNA expression levels show a tendency to decrease, presumably because of irreversible loss of cardiomyocytes within the infarcted area.

Finally, we investigated the effect of RIPerc on TNC expression. There is substantial evidence that IL-1β and TNF-α are secreted by activated macrophages and subsequently regulate turnover of ECM components, ultimately contributing to an increase of MMPs expression

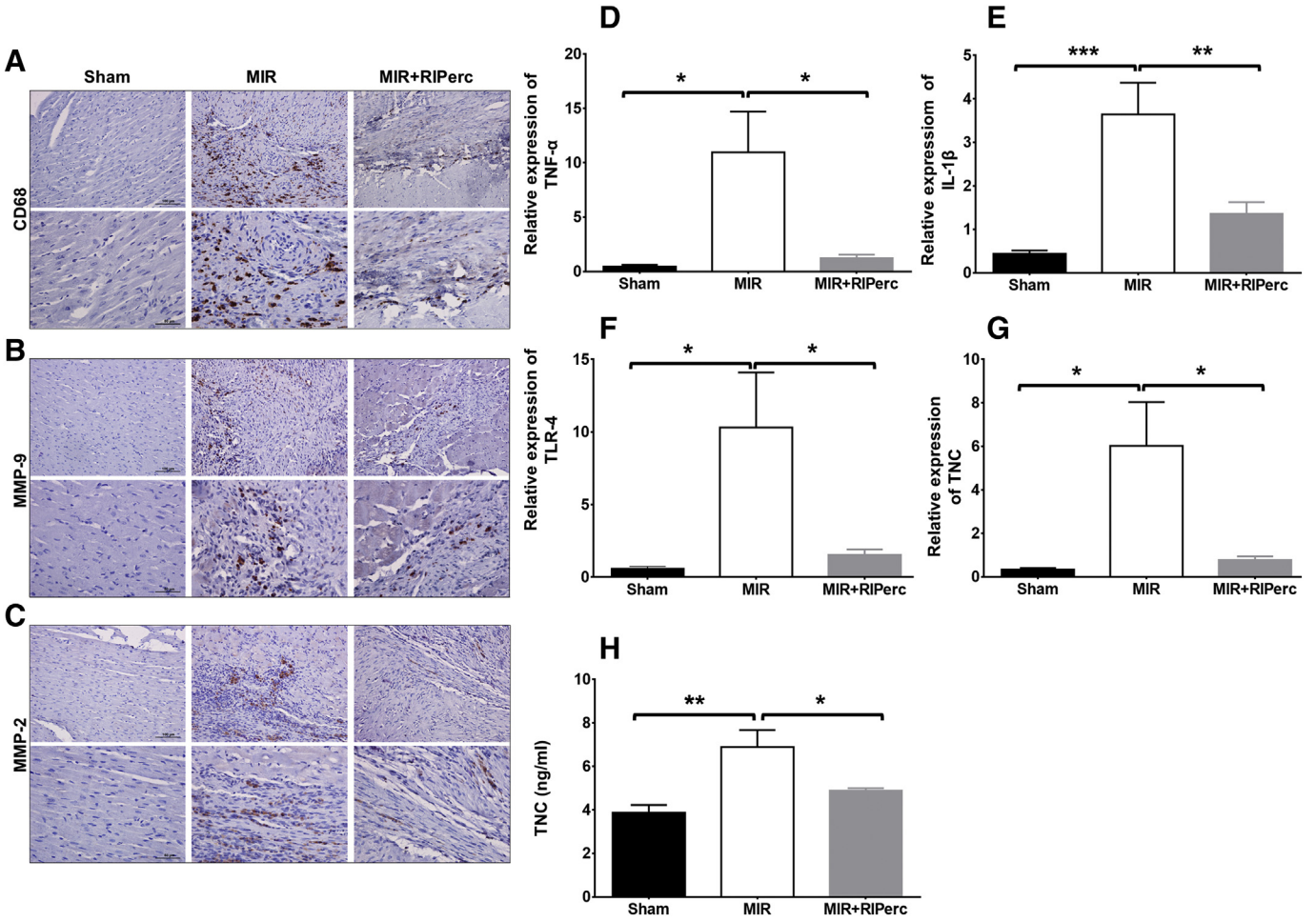

Fig. 4. Effect of remote ischemic perconditioning on influx of CD68+ cells, expression of MMP-2 and MMP-9 and mRNA expression of inflammatory cytokines, TLR-4 and TNC. Representative images from each group of rats, showing (A) CD68+ macrophages, (B) MMP-2 and (C) MMP-9 in the reperfused myocardium (magnification 200× and 400×, scale bar: 100 and 50 μm, respectively). mRNA expression of (D) TNF-α; (E) IL-1β, (F) TLR-4, and (G) TNC in reperfused LV tissue as well as (H) plasma levels of TNC. Data are expressed as mean ± SEM and n = 4–7/group. *p < 0.05; **p < 0.01 and ***p < 0.001. MIR: myocardial ischemia/reperfusion; MMP-2: matrix metalloproteinase 2; MMP-9: matrix metalloproteinase; TLR-4: toll-like receptor 4; TNC: Tenascin-C and RIPerc: remote ischemic perconditioning.

and activity [34]. In line with this, we demonstrated a significant upregulation of CD68+ macrophages and MMPs expression in the infarcted myocardium. Of importance, RIPerc markedly reduced the influx of CD68+ macrophages and MMPs expression in association with upregulation of NRG-1 levels. In addition, besides playing a role in fibrosis, degradation of cardiac myosin light chain kinase by MMP-2 contributes to LV contractile dysfunction [35]. Our study demonstrated that cardiac dysfunction was reversed by RIPerc in association with a significant reduction in MMP-2 expression. Mechanistically, H9c2 cells exposed to human purified TNC protein lead to a significant upregulation of MMP-2 [6]. Re-expression of TNC in an early stage of post-MI remodeling is culminating in a worse clinical outcome, pointing at the pathophysiological importance of TNC [36] in modulating M1/M2-macrophage polarization [9]. Accordingly, RIPerc reduced both CD68+ macrophage infiltration and M1 macrophage markers such as IL-1β and TNF-α expression. In addition, the activation of TLR-4 receptor plays a critical role in the enhancement of the inflammatory response in adverse LV remodeling [37,38]. Furthermore, TLR-4 receptor upregulation on cardiomyocytes leads to a marked inflammatory response in HF [38] and contributes to a process associated with an upregulation of MMP-2 in post-infarct LV remodeling [39] via a signaling mechanism that may be driven by TNC [40]. Of importance, RIPerc markedly reduced both TNC and TLR-4 upregulation in the infarcted myocardium.

4.1. Limitations

Certain limitations of the study need to be acknowledged. First, only mRNA expression of TNF-α, IL-1β, TNC, TLR-4 and not their protein levels are presented. However, previous studies demonstrated the upregulation of pro-inflammatory cytokines, TNC and TLR-4 at protein levels in rodent models of MI [5]. Second, we demonstrated RIPerc improves cardiac dysfunction and post-infarct LV remodeling in association with activation of NRG-1 and ErbB3 expression, but our data do not provide evidence regarding a causative role. Nevertheless, our results strongly suggest the importance of NRG-1/ErbB3 signaling in cardioprotection from RIPerc. Third, we did not aim to clarify the profound underling signaling mechanisms by NRG-1 affecting LV remodeling and cardiac dysfunction. However, a number of previous studies showed that recombinant human NRG-1 markedly improved cardiac remodeling after MI [28] via a signaling mechanisms modulating inflammatory processes or acting on myosin chain kinase/myosin light chain 2 pathways, respectively. Nevertheless, further studies are warranted to clarify whether these mechanisms are involved in the cardioprotective effects of RIPerc. Fourth, we used only young animals. However, worse post-infarct adverse LV remodeling, cardiac dysfunction, and the apparent limited therapies existing to improve myocardial function in elderly patients with MI ultimately require better understanding of LV remodeling [41]. In addition, reduced ischemic tolerances

and increased susceptibility of the heart to IR injury is a hallmark of adaptation of both aged human and rodent hearts. Furthermore, the aged heart is also refractory to endogenous protection from interventions like ischemic remote preconditioning [42].

5. Conclusions

In conclusion, our data reveal novel anti-remodeling and anti-inflammatory effects of RIPerc, hence activation of NRG-1/ErbB3 signaling. RIPerc markedly improved cardiac function and LV remodeling in the rat model of reperfused MI in association with an enhancement of NRG-1 promoter and NRG-1 protein levels as well as ErbB3 expression. Of importance, RIPerc significantly reduced the expression of inflammatory cytokines, MMP-2, MMP-9, TNC and TLR-4 in the infarcted LV tissue. Collectively, our data suggest that RIPerc has beneficial effects on post-MI remodeling by positively influencing inflammation and cardiac function.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.03.003.

Acknowledgement

This article is based upon work from COST Action EU-CARDIOPROTECTION CA16225 supported by COST (European Cooperation in Science and Technology). The authors would like to thank Milat Inci, Eylem Acar, Miriam Lang, Petra Lujza Szabo and Simon Watzinger (Center for Biomedical Research, Medical University of Vienna, Austria) for technical assistance.

Source of funding

This study was supported by an unrestricted research grant from Ludwig Boltzmann Cluster for Cardiovascular Research, Vienna, Austria (REM, 2017–2020 project).

Conflict of interest

None declared.

References

[1] S.B. Ong, S. Hernández-Reséndiz, G.E. Crespo-Avilan, R.T. Mukhametshina, X.Y. Kwek, H.A. Cabrera-Fuentes, D.J. Hausenloy, Inflammation following acute myocardial infarction: multiple players, dynamic roles, and novel therapeutic opportunities, Pharmacol. Ther. 186 (2018) 73–87.
[2] I.M. Seropian, S. Toldo, B.W. Van Tassell, A. Abbate, Anti-inflammatory strategies for ventricular remodeling following St-segment elevation acute myocardial infarction, J. Am. Coll. Cardiol. 63 (2014) 1593–1603.
[3] P.C. Westman, M.J. Lipinski, D. Luger, R. Waksman, R.O. Bonow, E. Wu, S.E. Epstein, Inflammation as a driver of adverse left ventricular remodeling after acute myocardial infarction, J. Am. Coll. Cardiol. 67 (2016) 2050–2060.
[4] K. Trescher, O. Bernecker, B. Fellner, M. Gyöngyösi, R. Schäfer, S. Aharinejad, R. DeMartin, E. Wolner, B.K. Podesser, Inflammation and postinfarct remodeling: overexpression of IκB prevents ventricular dilation via increasing TIMP levels, Cardiovasc. Res. 69 (2006) 746–754.
[5] D. Vanhoutte, M. Schellings, Y. Pinto, S. Heymans, Relevance of matrix metalloproteinases and their inhibitors after myocardial infarction: a temporal and spatial window, Cardiovasc. Res. 69 (2006) 604–613.
[6] B.K. Podesser, M. Kreibich, E. Dzilic, D. Santer, L. Förster, S. Trojanek, D. Abraham, M. Krššák, K.U. Klein, E.V. Tretter, C. Kaun, J. Wojta, B. Kapeller, I.F. Gonçalves, K. Trescher, A. Kiss, Tenascin-C promotes chronic pressure overload-induced cardiac dysfunction, hypertrophy and myocardial fibrosis, J. Hypertens. 36 (4) (2018) 847–856.
[7] A. Sato, K. Aonuma, K. Imanaka-Yoshida, T. Yoshida, M. Isobe, D. Kawase, N. Kinoshita, Y. Yazaki, M. Hiroe, Serum tenascin-C might be a novel predictor of left ventricular remodeling and prognosis after acute myocardial infarction, J. Am. Coll. Cardiol. 47 (2006) 2319–2325.
[8] T. Nishioka, K. Onishi, N. Shimojo, Y. Nagano, H. Matsusaka, M. Ikeuchi, T. Ide, H. Tsutsui, M. Hiroe, T. Yoshida, K. Imanaka-Yoshida, Tenascin-C may aggravate left ventricular remodeling and function after myocardial infarction in mice, Am. J. Physiol. Heart Circ. Physiol. 298 (2010) H1072–H1078.
[9] T. Kimura, K. Tajiri, A. Sato, S. Sakai, Z. Wang, T. Yoshida, T. Uede, M. Hiroe, K. Aonuma, M. Ieda, K. Imanaka-Yoshida, Tenascin-C accelerates adverse ventricular remodelling after myocardial infarction by modulating macrophage polarization, Basic Res. Cardiol. 113 (3) (2018 Mar 7) 14, https://doi.org/10.1007/s00395-018-0672-3.
[10] A. Gaspar, A. Lourenco, P. Azevedo, M. Pereira, J. Marques, A. Leite-Moreira, Randomized controlled trial of remote ischaemic conditioning in ST-elevation myocardial infarction as adjuvant to primary angioplasty (RIC-STEMI). Basic Res. Cardiol. 113(3): 14.
[11] P.M. Haller, K.G. Vargas, M.C. Haller, E. Piackova, J. Wojta, M. Gyöngyösi, B.J. Gersh, A. Kiss, B.K. Podesser, K. Huber, Remote ischaemic conditioning for myocardial infarction or elective PCI: systematic review and meta-analyses of randomised trials, Eur. Hear. J. Acute Cardiovasc. Care. (2018 Jun 1), 2048872618784150. https://doi.org/10.1177/2048872618784150 (Epub ahead of print).
[12] A.P. Vanezis, G.C. Rodrigo, I.B. Squire, N.J. Samani, Remote ischaemic conditioning and remodelling following myocardial infarction: current evidence and future perspectives, Heart Fail. Rev. 21 (2016) 635–643.
[13] M. Wei, P. Xin, S. Li, J. Tao, Y. Li, J. Li, M. Liu, J. Li, W. Zhu, A.N. Redington, Repeated remote ischemic postconditioning protects against adverse left ventricular remodeling and improves survival in a rat model of myocardial infarction, Circ. Res. 108 (2011) 1220–1225.
[14] M. Morano, C. Angotti, F. Tullio, G. Gambarotta, C. Penna, P. Pagliaro, S. Geuna, Myocardial ischemia/reperfusion upregulates the transcription of the Neuregulin1 receptor ErbB3, but only postconditioning preserves protein translation: role in oxidative stress, Int. J. Cardiol. 233 (2017) 73–79.
[15] F. Wang, H. Wang, X. Liu, H. Yu, B. Zuo, Z. Song, N. Wang, W. Huang, G. Wang, Pharmacological postconditioning with Neuregulin-1 mimics the cardioprotective effects of ischaemic postconditioning via ErbB4-dependent activation of reperfusion injury salvage kinase pathway, Mol. Med. 24 (1) (2018) 39.
[16] O. Odiete, M.F. Hill, D.B. Sawyer, Neuregulin in cardiovascular development and disease, Circ. Res. 111 (2012) 1376–1385.
[17] J.D. Gardner, Neuregulin-1β as a potential therapeutic for targeting fibroblasts in heart disease, J. Mol. Cell. Cardiol. 112 (2017) 132–133.
[18] X. Liu, X. Gu, Z. Li, X. Li, H. Li, J. Chang, P. Chen, J. Jin, B. Xi, D. Chen, D. Lai, R.M. Graham, M. Zhou, Neuregulin-1/erbB-activation improves cardiac function and survival in models of ischemic, dilated, and viral cardiomyopathy, J. Am. Coll. Cardiol. 48 (2006) 1438–1447.
[19] K. Zins, R. Schäfer, P. Paulus, S. Dobler, N. Fakhari, M. Sioud, S. Aharinejad, D. Abraham, Frizzled2 signaling regulates growth of high-risk neuroblastomas by interfering with β-catenin-dependent and β-catenin- independent signaling pathways, Oncotarget. 7 (29) (2016) 46187–46202.
[20] R.C. Gagnon, J.J. Peterson, Estimation of confidence intervals for area under the curve from destructively obtained pharmacokinetic data, J. Pharmacokinet. Biopharm. 26 (1998) 87–102.
[21] M.A. Pfeffer, E. Braunwald, Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications, Circulation. 81 (1990) 1161–1172.
[22] B. Chen, N.G. Frangogiannis, Immune cells in repair of the infarcted myocardium, Microcirculation. 24 (2017) 1–10.
[23] P. Mendes-Ferreira, G.W. De Keulenaer, A.F. Leite-Moreira, C. Brás-Silva, Therapeutic potential of neuregulin-1 in cardiovascular disease, Drug Discov. Today 18 (2013) 836–842.
[24] C.L. Galindo, E. Kasasbeh, A. Murphy, S. Ryzhov, S. Lenihan, F.A. Ahmad, P. Williams, A. Nunnally, J. Adcock, Y. Song, F.E. Harrell, T.-L. Tran, T.J. Parry, J. Iaci, A. Ganguly, I. Feoktistov, M.K. Stephenson, A.O. Caggiano, D.B. Sawyer, J.H. Cleator, Anti-remodeling and anti-fibrotic effects of the neuregulin-1 glial growth factor 2 in a large animal model of heart failure, J. Am. Heart Assoc. 3 (2014), e000773–e000773. .
[25] F.R. Formiga, B. Pelacho, E. Garbayo, I. Imbuluzqueta, P. Díaz-Herráez, G. Abizanda, J.J. Gavira, T. Simón-Yarza, E. Albiasu, E. Tamayo, F. Prósper, M.J. Blanco-Prieto, Controlled delivery of fibroblast growth factor-1 and neuregulin-1 from biodegradable microparticles promotes cardiac repair in a rat myocardial infarction model through activation of endogenous regeneration, J. Control. Release 173 (2014) 132–139.
[26] Z. Vermeulen, A.-S. Hervent, L. Dugaucquier, L. Vandekerckhove, M. Rombouts, M. Beyens, D.M. Schrijvers, G.R.Y. De Meyer, S. Maudsley, G.W. De Keulenaer, V.F. Segers, Inhibitory actions of the NRG-1/ErbB4 pathway in macrophages during tissue fibrosis in heart, skin and lung, Am. J. Physiol. - Hear. Circ. Physiol. (2017) ajpheart. (2017) 00206.
[27] O. Gidlöf, A.L. Johnstone, K. Bader, B.B. Khomtchouk, J.J. O'Reilly, S. Celik, D.J. Van Booven, C. Wahlestedt, B. Metzler, D. Erlinge, Ischemic preconditioning confers epigenetic repression of Mtor and induction of autophagy through G9a-dependent H3K9 dimethylation, J. Am. Heart Assoc. 5 (2016).
[28] X. Gu, X. Liu, D. Xu, X. Li, M. Yan, Y. Qi, W. Yan, W. Wang, J. Pan, Y. Xu, B. Xi, L. Cheng, J. Jia, K. Wang, J. Ge, M. Zhou, Cardiac functional improvement in rats with myocardial infarction by up-regulating cardiac myosin light chain kinase with neuregulin, Cardiovasc. Res. 88 (2010) 334–343.
[29] A. Brero, R. Ramella, A. Fitou, C. Dati, G. Alloatti, M.P. Gallo, R. Levi, Neuregulin-1β1 rapidly modulates nitric oxide synthesis and calcium handling in rat cardiomyocytes, Cardiovasc. Res. 88 (2010) 443–452.
[30] N. Hedhli, Q. Huang, A. Kalinowski, M. Palmeri, X. Hu, R.R. Russell, K.S. Russell, Endothelium-derived neuregulin protects the heart against ischemic injury, Circulation. 123 (2011) 2254–2262.
[31] C.L. Galindo, S. Ryzhov, D.B. Sawyer, Neuregulin as a heart failure therapy and mediator of reverse remodeling, Curr. Heart Fail. Rep. 11 (2014) 40–49.
[32] A. Kirabo, S. Ryzhov, M. Gupte, S. Sengsayadeth, R.J. Gumina, D.B. Sawyer, C.L. Galindo, Journal of Molecular and Cellular Cardiology Neuregulin-1 β induces

proliferation, survival and paracrine signaling in normal human cardiac ventricular fi broblasts, J. Mol. Cell. Cardiol. 105 (2017) 59–69.
[33] S. Ryzhov, A. Matafonov, C.L. Galindo, Q. Zhang, T.-L. Tran, D.J. Lenihan, C.G. Lenneman, I. Feoktistov, D.B. Sawyer, ERBB signaling attenuates proinflammatory activation of nonclassical monocytes, Am. J. Physiol. Heart Circ. Physiol. 312 (2017) H907–H918.
[34] D.A. Siwik, D.L.-F. Chang, W.S. Colucci, Interleukin-1 and tumor necrosis factor- decrease collagen synthesis and increase matrix metalloproteinase activity in cardiac fibroblasts in vitro, Circ. Res. 86 (2000) 1259–1265.
[35] A.D. Kandasamy, A.K. Chow, M.A.M. Ali, R. Schulz, Matrix metalloproteinase-2 and myocardial oxidative stress injury: beyond the matrix, Cardiovasc. Res. 85 (2010) 413–423.
[36] T. Yokokawa, Y. Sugano, T. Nakayama, T. Nagai, T.A. Matsuyama, K. Ohta-Ogo, Y. Ikeda, H. Ishibashi-Ueda, T. Nakatani, S. Yasuda, Y. Takeishi, H. Ogawa, T. Anzai, Significance of myocardial tenascin-C expression in left ventricular remodelling and long-term outcome in patients with dilated cardiomyopathy, Eur. J. Heart Fail. 18 (2016) 375–385.
[37] T. Eckle, H.K. Eltzschig, Toll-like receptor signaling during myocardial ischemia, Anesthesiology. 114 (2011) 490–492.
[38] L. Liu, Y. Wang, Z.Y. Cao, M.M. Wang, X.M. Liu, T. Gao, Q.K. Hu, W.J. Yuan, L. Lin, Up-regulated TLR4 in cardiomyocytes exacerbates heart failure after long-term myocardial infarction, J. Cell. Mol. Med. 19 (2015) 2728–2740.
[39] L. Timmers, J.P.G. Sluijter, J.K. Van Keulen, I.E. Hoefer, M.G.J. Nederhoff, M.J. Goumans, P.A. Doevendans, C.J.A. Van Echteld, J.A. Joles, P.H. Quax, J.J. Piek, G. Pasterkamp, D.P.V. De Kleijn, Toll-like receptor 4 mediates maladaptive left ventricular remodeling and impairs cardiac function after myocardial infarction, Circ. Res. 102 (2008) 257–264.
[40] T. Machino-Ohtsuka, K. Tajiri, T. Kimura, S. Sakai, A. Sato, T. Yoshida, M. Hiroe, Y. Yasutomi, K. Aonuma, K. Imanaka-Yoshida, Tenascin-C aggravates autoimmune myocarditis via dendritic cell activation and Th17 cell differentiation, J. Am. Heart Assoc. 3 (2014) 1–15.
[41] H. Shih H, B. Lee B, RJ Lee, AJ Boyle. The aging heart and post-infarction left ventricular remodeling, J. Am. Coll. Cardiol. (2011) 57(1):9–17.
[42] A. Heinen, F. Behmenburg, A. Aytulun, M. Dierkes, L. Zerbin, W. Kaisers, M. Schaefer, T. Meyer-Treschan, S. Feit, I. Bauer, M.W. Hollmann, R. Huhn, The release of cardioprotective humoral factors after remote ischemic preconditioning in humans is age- and sex-dependent, J. Transl. Med. 16 (2018) 1–11.