Cardioprotection by gene therapy: A review paper on behalf of the Working Group on Drug Cardiotoxicity and Cardioprotection of the Italian Society of Cardiology

Cardioprotection by gene therapy A review paper on behalf of the Working Group on Drug Cardiotoxicity and Cardioprotection of the Italian Society of Cardiology

Rosalinda Madonna a,k,l, Christian Cadeddu b, Martino Deidda b, Zoltán Giricz m,n, Clelia Madeddub, Donato Mele c, Ines Monte d, Giuseppina Novo e, Pasquale Pagliarof, Alessia Pepe g, Paolo Spallarossa h,Carlo Gabriele Tocchetti i, Zoltán V. Varga m,n, Concetta Zito j, Yong-Jian Geng k,l, Giuseppe Mercuro b, Peter Ferdinandy m,n

a Institute of Cardiology, Center of Excellence on Aging, "G. d'Annunzio" University, Chieti, Italy

b Department of Medical Sciences "Mario Aresu", University of Cagliari, Cagliari, Italy

c Cardiology Unit, University Hospital of Ferrara, Ferrara, Italy

d Department of General Surgery and Medical-Surgery Specialities, University of Catania, Catania, Italy

e Chair and Division of Cardiology, University of Palermo, Palermo, Italy

f Department of Clinical and Biological Sciences, University of Turin, Orbassano, Italy

g U.O.C. Magnetic Resonance Imaging, Fondazione G. Monasterio C.N.R., Pisa, Italy

h Clinic of Cardiovascular Diseases, IRCCS San Martino IST, Genoa, Italy

i Department of Translational Medical Sciences, Division of Internal Medicine, Federico II University, Naples, Italy

j U.O.C. Cardiology Intensive Unit, A.O.U. Policlinico "G. Martino" University of Messina, Messina, Italy

k Texas Heart Institute at St. Luke's Episcopal Hospital, Houston, TX, United States

l Department of Internal Medicine, Cardiology, The University of Texas Health Science Center at Houston, Houston, TX, United States

m Pharmahungary Group, Szeged, Hungary

n Cardiometabolic Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

Article history:

Received 8 February 2015

Received in revised form 28 April 2015

Accepted 30 April 2015

Available online 1 May 2015

Keywords: Genomics Gene therapy Cardioprotection Ischemic heart disease Preconditioning Postconditioning Remote conditioning

Abstract

Ischemic heart disease remains the leading cause of death worldwide. Ischemic pre-, post-, and remote conditionings trigger endogenous cardioprotection that renders the heart resistant to ischemic–reperfusion injury (IRI). Mimicking endogenous cardioprotection bymodulating genes involved in cardioprotective signal transduction provides an opportunity to reproduce endogenous cardioprotection with better possibilities of translation into the clinical setting. Genes and signaling pathways by which conditioning maneuvers exert their effects on the heart are partially understood. This is due to the targeted approach that allowed identifying one or a few genes associated with IRI and cardioprotection. Genes critical for signaling pathways in cardioprotection include protectomiRs (e.g., microRNA 125b*), ZAC1 transcription factor, pro-inflammatory genes such as cycloxygenase (COX)-2 and inducible nitric oxide synthase (iNOS), antioxidant enzymes such as hemoxygenase (HO)-1, extracellular and manganese superoxidase dismutases (ec-SOD and Mg-SOD), heat shock proteins (HSPs), growth factors such as insulin like growth factor (IGF)-1 and hepatocyte growth factor (HGF), antiapoptotic proteins such as Bcl-2 and Bcl-xL, pro-apoptotic proteins such as FasL, Bcl-2, Bax, caspase-3 and p53, and proangiogenic genes such as TGFbeta, sphingosine kinase 1 (SPK1), and PI3K–Akt. By identifying the gene expression profiles of IRI and ischemic conditioning, one may reveal potential gene targets responsible for cardioprotection. In this manuscript, we review the current state of the art of gene therapy in cardioprotection and propose that gene expression analysis facilitates the identification of individual genes associated with cardioprotection. We discuss signaling pathways associated with cardioprotection that can be targeted by gene therapy to achieve cardioprotection.

Introduction

Ischemia/reperfusion injury (IRI) including myocardial infarction and its long termconsequences aremajor causes of morbidity andmortality worldwide. Although early mechanical reperfusion therapy dramatically increases survival of acute infarction patients, cardioprotective drug therapy is still an unmet clinical need. Ischemic pre-, post-, and remote conditioning, are potential therapeutic strategies for protecting organs against the detrimental effects of acute IRI. Mimicking ischemic cardiac conditioning through prophylactic transfection of cardioprotective genes into the myocardium would represent a therapeutic tool to protect the heart throughout an ischemia/reperfusion episode. A thorough knowledge of the genes and signaling pathways

associatedwith IRI and ischemic conditioning are a prerequisite for the development of cardioprotective gene therapy [1]. (See Fig. 1.)

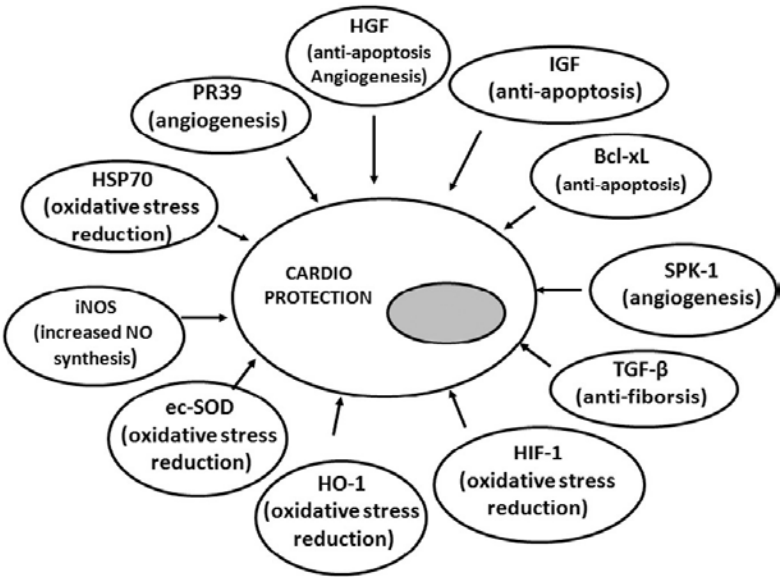

Fig. 1. Target genes and mechanisms of cardioprotection. Abbreviations: HGF, hepatocyte growth factor; IGF, insulin like growth factor; PR39, proline 39; HSP70, heat shock protein 70; iNOS inducible nitric oxide synthase; ec-SOD, superoxide dismutase; HO-1, hemoxygenase 1; HIF-1, hypoxia inducible factor 1; TGFbeta, tumor growth factor beta; SPK1, sphingosine kinase 1.

1.1. Cardioprotection by ischemic conditioning of the heart

During ischemic pre-, post- and remote conditioning, cytoprotective cellular mechanisms are activated by brief episodes of ischemia/ reperfusion that render the heart more resistant to a major ischemic/ reperfusion event causing IRI. Preconditioning consists of two distinct phases: the early phase, which develops within a few minutes from the exposure to brief cycles of ischemia/reperfusion and lasts for 1–2 h; and the late phase, which develops within 6–12 h, and lasts for 3–4 days [2–6]. The early phase is capable of limiting myocardial infarction, while the late phase protects against both myocardial infarction and reversible myocardial dysfunction. In ischemic postconditioning, protective endogenous mechanisms are activated by very brief episodes of ischemia/ reperfusion applied immediately after a major ischemic/reperfusion event to render the heartmore resistant to reperfusion injury. In remote ischemic conditioning the application of one or more brief cycles of non-lethal ischemia/reperfusion to an organ or tissue protects a remote organ or tissue from a major episode of lethal IRI [7,8]. In remote ischemic conditioning the conditioning stimulus can be applied before (remote preconditioning), during (remote preconditioning), or after (remote postconditioning) a major ischemic event. Since the remote conditioning stimuli could be applied non-invasively by simply inflating and deflating a blood pressure cuff placed on a limb, this simplicity facilitates the translation of remote conditioning into the clinical arena as shown by several ongoing clinical studies.

1.2. Cellular mechanisms of cardioprotection: importance of gene expression

In the early phase after conditioning protocols, several signaling cascades are recruited including phosphorylative/dephosphorylative processes and redox signaling such as S-nitrosation/-nitrosylation, S-

sulfhydration, and O-linked glycosylation [6–9]. These processes converge on mitochondria improving their function and lead to modulation of gene expression profile after the conditioning stimuli. In fact, a transient (a few days) new synthesis of proteins due to upregulation of several cardioprotective genes, is mainly responsible for the protection afforded by late ischemic preconditioning. Mimicking the effect of ischemic conditioning through expression of cardioprotective genes would represent a therapeutic tool to limit infarct size of the heart and is an attractive research hypothesis worthy of consideration [9]. This type of gene expression would make the heart resistant to IRI for a long period. As of today, genes and signaling pathways by which conditioning exerts its effects on the heart are partially understood, due at least in part to the arbitrarily selected gene targets pursued so far that allowed identifying one or a few genes associated with IRI and with cardioprotection by conditioning. An alternative way to approach this fundamental issue is to use functional genomic approaches to identify genes that are differentially expressed in conditioning [10–12]. Genomics refers to the study of multiple genetic variants and/or gene expression profiles simultaneously [13, 14]. Genomics utilizes technologies designed to elucidate hundreds to thousands of DNA variants (e.g., single-nucleotide polymorphism [SNP] arrays/chips) as well as alterations in RNA expression levels (e.g., microarrays). Functional genomic analysis followed by analysis of alterations in protein levels (i.e., proteomics) and even final metabolic products (e.g., metabolomics) [14] could provide a global understanding of the cardioprotective genomic and proteomic program providing cardioprotection. The use of genome-based technologies allows for the interactive analysis of a collateral, cognate gene, thus leading to the identification of relevant processes and pathways involved in IRI and cardioprotection. Because thousands of gene products are analyzed simultaneously, intense mathematical analysis is required to interpret the final results. In this review, we describe how functional genomics may facilitate the identification of genes or gene sets/pathways that are associated with cardioprotection by conditioning. Then,we discuss someof the potential cardioprotective genes that could be or has been already targeted by gene therapy to mimic the cardioprotection afforded by ischemic conditioning (Table 1).

Table 1
Summary of preclinical studies that successfully applied gene therapy for cardioprotection.

Target gene	Therapeutic vector	Experimental model of ischemia–reperfusion	Outcome summary	Ref.
iNOS	Adenovirus	Ligation and release of left anterior descending coronary artery in mouse	Reduction in myocardial infarct size	[27,35]
ec-SOD	Adenovirus	Ligation and release of left anterior descending coronary artery in rabbit	Reduction in myocardial infarct size	[29]
HO-1	Adenovirus	Ligation and release of left anterior descending coronary artery in rat	Prevention of myocardial infarction	[41]
HO-1	Adeno-associated virus	Ligation and release of left anterior descending coronary artery in rat	Reduction in myocardial infarct size	[42–44]
HIF-1α	Adeno-associated virus	Rat neonatal cardiomyocytes in hypoxia for 3 h, followed by normoxia for 24 h followed by lethal ischemia (depletion of serum and glucose and hypoxia) for 2 h	Overexpression of VEGF and HSP70	[46]
HIF-1α	Exosomal microRNA + cardiac progenitor cells	Permanent ligation of left anterior descending coronary artery in mouse	Enhanced recovery of cardiac function	[47]
HSP70	Adenovirus	Ligation and release of left anterior descending coronary artery in mouse	Higher ventricle developed pressure	[48]
HSP70	Adenovirus	Ligation and release of left anterior descending coronary artery in rabbit	Reduction in myocardial infarct size	[50]
HSP70	HVJ-liposome	Ligation and release of left anterior descending coronary artery in rat	Enhanced recovery of cardiac function	[51]
PR39	Adenovirus	Ligation and release of left anterior descending coronary artery in mouse	Reduction in myocardial infarct size	[56]
TGFβ3	Adeno-associated virus	Ligation and release of left anterior descending coronary artery in rat	Reduction in myocardial infarct size	[34]
SPK1	Adenovirus	Ligation and release of left anterior descending coronary artery in rat	Reduction in myocardial infarct size	[59]
Bcl-xL	Adenovirus	Ligation and release of left anterior descending coronary artery in rat	Reduction in myocardial infarct size	[33]
IGF	Adenovirus	Ligation and release of left anterior descending coronary artery in rat	Reduction in myocardial infarct size	[69]
HGF	Adenovirus	Ligation and release of left anterior descending coronary artery in rabbit	Reduction in myocardial infarct size	[76]

iNOS, inducible nitric oxide synthase; ec-SOD, extracellular superoxide synthase; HO-1, hemoxygenase isoform 1; HIF-1α, hypoxia inducible factor isoform 1α; HSP, heat shock protein; PR, proline; TGF-β, tumor growth factor isoform β; SPK1, sphingosine kinase isoform 1; Bcl-xL, an antiapoptotic protein; IGF, insulin growth factor; HGF, hepatocyte growth factor.

2. Identification of potential cardioprotective target genes by analysis of cardiac gene expression profile

In spite of 3 decades of research on the mechanism of cardioprotection, we still do not have cardioprotective drugs on the market. This is due to several reasons including the complexity of the cellular mechanism of cardioprotection which is far from being understood [7, 8,15]. Therefore, it is somehow surprising that a very

small number of studies (compared to the thousands of studies on cardioprotection by conditioning) applied the functional genomics approach to reveal the complex mechanism of cardioprotection and to identify novel target genes to treat ischemic heart disease. Nevertheless, some studies have shown that both pre- and postconditioning significantly alter the gene expression profile of the ischemic heart at the transcript level. The first studies that described the effect of preconditioning on cardiac gene expression profile have been published a decade ago in rabbits [16] and rats [17]. These pioneer studies receive supportive information from several additional investigations showing that brief episodes of conditioning ischemia trigger a cardioprotective genomic response in the heart [18]. Moreover, recent studies have shown that ischemic postconditioning is able to modify the gene expression profile of mice [19] and rat hearts [20]. Expression profile of microRNAs, has been recently shown to bemarkedly changed by both pre- and postconditioning [21].

So far no genomics approach has been applied to identify gene targets for remote conditioning, although extracellular vesicles, inter-organ carriers of microRNAs, have been recently identified as a potential mediator of remote conditioning [22]. The use of genomics-based technologies allows for the interactive analysis of collateral, cognate gene, thus leading to the identification of relevant processes and pathways involved in IRI and in cardioprotection by conditioning. Accordingly, Vincent et al., by using the DNA microarray technology, identified that the transcription factor ZAC1 gene is downregulated by both pre- and postconditioning [19]. Therefore, ZAC1 could be an interesting target for gene silencing. Varga et al. [21] made a systematic comparison in the direction of microRNA expression changes due to IRI between preconditioning and postconditioning, and they were able to identify protectomiRs, microRNA targets and specific mimics or antagomiRs of these targets that may have pre- and postconditioning-like cardioprotective effects. They termed these cardioprotective microRNA mimics or angatomirs as “protectomiRs”. By using this approach, the mimic microRNA 125b* has been identified and validated as a powerful cardio-cytoprotective protectomir. Based on the functional genomic approach, thousands of potential target genes can be identified by intense bioinformatic analysis of the results including several mathematical approaches such as e.g., network analysis [23]. It is encouraging to have more genomic studies to determine the complex cardioprotective genomic pattern and screen the key players to be targeted by gene therapy approach. Since the vast majority of the studies so far arbitrarily selected some potential gene targets to study in cardioprotection, the next chapters will review the most important cardioprotective genes identified so far by this traditional way.

3. Gene therapy targeting nitric oxide synthase and cycloxygenase

Up to now, the vast majority of the studies on cardioprotection has been based on arbitrary selection of potential target genes. The first gene identified as a mediator of late ischemic PC was the inducible isoform of nitric oxide synthase (iNOS) [24–26], which has been shown to increase modestly in cardiomyocytes within the ischemic–reperfused region after ischemic preconditioning. Subsequently, other genes involved in cardioprotection have been discovered, including cycloxygenase (COX)-2 [27], heme oxygenase (HO)-1 [28], antioxidant enzymes such as extracellular and manganese superoxidase dismutases (ec-SOD and Mg-SOD) [29], heat shock proteins (HSPs) [30,31], insulin like growth factor (IGF)-1 [32], antiapoptotic proteins such as Bcl-xL [33] and tumor growth factor (TGF)-alpha [34]. Li and colleagues [35] investigated short-term effects of iNOS gene transfer on myocardial IRI. The authors used adenovirus 5 (Ad5) vector with deletions of the E1, E2a and E3 regions, carrying the human iNOS gene (Ad5/iNOS) or control LacZ gene (Ad5/LacZ). Li and colleagues [35] compared the effects of iNOS gene transferwith that of late preconditioning by using a murine model of myocardial infarction, which was produced by a 30 min coronary occlusion followed by 4 h reperfusion. Gene

transfer was produced profilactically 3 days before infarction, by intramyocardial injection in the anterior left ventricular wall of an adenoviral vehicle (PBS/1% sucrose) or Ad5/LacZ or Ad5/iNOS. Late preconditioning was produced by a sequence of six 4-min occlusion/4 min reperfusion cycles 24 h before the 30-min occlusion. In this model of gene therapy-mediated cardioprotection, it was demonstrated that intramyocardial injection of Ad5/iNOS resulted in a significant increase in iNOS protein expression, iNOS activity and nitric oxide (NO) levels, indicating effective transduction of myocytes with functionally competent iNOS. These effects were associated with a 67% reduction in infarct size compared with the control virus group (Ad5/LacZ). The role of cyclooxygenase (COX)-2 in the cardioprotection afforded by iNOS [27] was also investigated. Mice received an intramyocardial injection of Ad5/LacZ or Ad5/iNOS with additional administration of the selective COX-2 inhibitor NS-398, 30 min before the coronary occlusion. Those mice had increased iNOS expression accompanied by increased expression and activity of COX-2. These effects were abrogated by coadministration of COX-2 selective inhibitor, thus demonstrating that COX-2 is an obligatory downstream effector of iNOS-dependent cardioprotection. In a following work, Li and colleagues [36] investigated the long-term effects of iNOS gene therapy on cardiac function after myocardial injury. Gene transfer of iNOS was produced one or two months before infarction (30-min coronary occlusion followed by 4 h of reperfusion), by using the same recombinant Ad5 vector employed in the previous study. In mice transduced with Ad5/iNOS, infarct size was smaller than in the control virus group (Ad5/lacZ) at either 1 month and 2 months after gene transfer, demonstrating that the expression of iNOS was associated with long-term cardioprotection.

4. Gene therapy targeting oxidative stress

Considerable evidence indicates that reactive oxygen species (ROS), such as superoxide anion (O2•−), hydroxyl radical (HO•) and hydrogen peroxide (H2O2), contribute importantly to myocardial ischemia–reperfusion injury [37]. Li and colleagues [29] investigated the role of ec-SOD in myocardial protection, by using a conscious rabbit model of myocardial infarction [38,39]. However, ROS have been identified as essential cardioprotective signaling molecules either in pre or post-conditioning processes. The reasons of the switch from protective oxidative signaling to deleterious oxidative stress are not fully understood. The complex regulation of this switch is, at least in part, responsible for the diminished or absent cardioprotection by conditioning protocols observed in aging and in the presence of some comorbidities [8,40]. Since exogenous antioxidants in human trials failed to provide a beneficial result or even resulted in toxicity under certain conditions, it is likely that the endogenous antioxidant subcellular localization, the amount of ROS produced and the time course of their synthesis during ischemia/reperfusion could all be of primary importance for explaining the dual role of ROS and antioxidants. Authors postulated that gene therapy capable of creating an endogenous source of antioxidant enzymes, could limit lethal myocellular injury during ischemia–reperfusion in vivo. The infarction protocol in this study consisted of a 30 min coronary artery occlusion by placing a balloon occluder around a major branch of the left coronary artery, followed by 3 days of reperfusion. The effects of ec-SODgene transfer were compared with that of late preconditioning, which was realized with a sequence of six 4-min coronary occlusions interspersed with 4 min of reperfusion performed 4 h before the 30-min occlusion. It was shown that in the gene-therapy group, the average infarct size was 57% smaller than in the control-treated group, indicating the expression of ec-SOD as responsible for the marked cardioprotective effect, quite similar to that induced by the late phase of ischemic preconditioning. The protective role of antioxidant gene transfer in defense against ischemia–reperfusion injury, has been also investigated by Francis et al. [41] and Melo et al. [42]. Francis and colleagues proved that intramyocardial delivery of recombinant adenoviral vectors encoding

for hemoxygenase 1 (HO-1) in a rat model of myocardial IRI, induced by ligation and release of the left anterior descending coronary artery, results in the prevention of myocardial infarct [41]. Melo and colleagues showed that intramyocardial delivery of recombinant adeno-associated virus encodingforHO-1 (AAV-HO-1)8weeks before coronary artery ligation and release, led to significant reduction of myocardial infarct size [42]. These effects were accompanied by a decrease of proapoptotic and proinflammatory proteins such as Bax and interleukin-1beta, respectively [42]. By using a similar model of intramyocardial gene delivery of a recombinant adeno-associated virus encoding for HO-1, followed by ischemia and reperfusion, Liu and colleagues analyzed the chronic effects of HO-1 gene delivery on myocardial fibrosis and remodeling after acute myocardial infarction [43]. They showed an amelioration of ejection fraction and left ventricular volumes at 1.5 months after HO-1 gene delivery, and significant reduction of myocardial scarring and fibrosis at three months [43]. The existence of positive effects of intramyocardial HO-1 gene deliverywas also confirmed in a different model of chronic recurring myocardial ischemia and reperfusion [44]. In this study, Pachori and coworkers delivered AAV-HO-1 five weeks in advance of the injury, followed by left coronary artery occlusion for 15 min and release performed daily for 5 days. The authors showed the prevention ofmyocardial wall thinning, inflammation, fibrosis and deterioration of cardiac function in the rats transduced with AAV-HO-1 [44]. By using a similar model of chronic recurring myocardial ischemia Liu and colleagues analyzed the chronic effects of HO-1 gene delivery on long-term survival, myocardial function and left ventricular remodeling 1 year after myocardial infarction [45]. The authors showed a significant reduction of mortality and greater recovery of left ventricular developed pressure and end-diastolic volumes in the HO-1-treated animals compared with LacZ-treated animals. Furthermore, morphometric analysis revealed extensive reduction of myocardial scarring and fibrosis in the infarcted area after HO-1 gene delivery [45]. Elevated levels of hypoxia-inducible factor (HIF)-1, a heterodimeric transcription factor composed by HIF-alpha and HIF-beta subunits, have been shown in rat brain and retina after a brief exposition to sublethal hypoxia. Date and colleagues [46] investigated the effects of adenovirus-mediated expression of HIF-1alpha in an in vitro model of preconditioning and IRI. Preconditioning was induced by placing cultured neonatal rat ventricular cardiomyocytes in a hypoxic chamber (1% O2, 5% CO2; 37 °C) for 3 h and then returning to control conditions (21% O2, 5% CO2, 37 °C) for 24 h, followed by simulated lethal ischemia (depletion of serum and glucose and hypoxia) for 2 h. Exposure to an episode of lethal hypoxia decreased cell death and increased mRNA levels of protective HIF-1 target genes such as Vascular Endothelial Growth Factor (VEGF) and HSP70. These effects of preconditioning were mimicked by preinfection of cultured cardiomyocytes with recombinant adenoviral vectors encoding for HIF-1alpha (Ad2/HIF-1alpha) 48 h before simulated ischemia–reperfusion. Cardioprotective effects of HIF-1alpha have been recently demonstrated by the codelivery of cardiac progenitor cells (CPC) with a nonviral minicircle plasmid carrying HIF-1alpha (MC-HIF-1alpha) in a murine model of myocardial infarction [47]. This has resulted in better recovery of ejection fraction 6 weeks after myocardial infarction in the hearts injected with CPC + MC-HIF-1alpha. Targets for HIF are cardioprotective genes such as VEGF, iNOS, and erythropoietin, survivin and insulin growth factor binding protein [48]. Belke and colleagues [31] investigated the role of heat shock protein (HSP)-mediated adaptive response in limiting IRI. HSPs are a family of inducible and constitutive intracellular proteins, specifically expressed after exposure to heat and a wide spectrumof stressful and potentially deleterious stimuli such as brief ischemia (see for an extensive review: [49]). In the study by Belke and colleagues the role of viral-mediated gene therapy delivering HSP70 in the cardioprotection after ischemia–reperfusion was investigated. By the direct injection of replication-deficient adenovirus type 5 vectors expressing HSP70 in the left ventricular free wall of a mouse heart, the authors were able to moderate functional loss and cellular damage (measured via creatine kinase release) following ischemia–reperfusion.

Contractile function was measured in isolated Langendorff perfused hearts 5 days and 8 months following in vivo gene therapy. In preischemic aerobically perfuse hearts, developed pressure as well as the rate of contraction (+dP/dt) and relaxation (−dP/dt) did not differ between mice injected with adenovirus expressing HSP70 or those expressing empty vector. Following 20 min of ischemia and 120 min reperfusion, analysis of pressure tracings generated by the heart indicated a higher developed pressure in the HSP70 group (46% increase). Analysis of the rates of contraction and relaxation also showed a significant increase in the HSP70-treated group. Analysis of creatine kinase release during reperfusion indicated a greater release of enzyme in the control group than in the HSP70 group, suggesting greater cellular damage in the control group. Belke and colleagues observed the protective effects of HSP70 also up to 8 months after gene therapy, demonstrating a potential therapeutic effect for chronic conditions. Previous work by Okubo and colleagues showed the cardioprotective effects of HSP70 in ischemic hearts [50]. Recombinant adenovirus encoding for the inducible form of human HSP 70 (Ad.HSP70), was injected in the ventricles, and such procedure was followed by 30 min ligation of the left coronary artery and 3 h reperfusion. The authors showed a significant reduction of infarct size in Ad.HSP70-injected hearts compared with the saline- and Ad.LacZ-treated hearts [50]. The cardioprotective effects of HSP70 in ischemic injury have also been found by Suzuki and colleagues, by using a different method of cardiac gene transfer [51]. The HSP70 gene was transfected by intracoronary infusion of the hemagglutinating virus of the Japan (HVJ)-liposome containing human HSP70 gene, and Langendorff perfusion was performed to evaluate the effect of HSP70 on myocardial protection. Hearts overexpressing HSP70 showed enhanced recovery of both systolic and diastolic cardiac function, as well as less damage after ischemia–reperfusion as compared with control. The study showed that gene therapy by HSP70 has a potential to enhance myocardial tolerance to ischemia better than the heat stress by itself [3]. In a similar model of HSP70 gene therapy by intracoronary infusion of the HVJ-liposome containing human HSP70 gene, Suzuki and colleagues [51] showed that the enhanced activity of mitochondrial manganese SOD (Mn-SOD) during ischemia–reperfusion injury, is one of the possible mechanisms explaining HSP70-induced myocardial protection, likely by mitochondrial protection and apoptosis reduction [52]. After ischemia, Mn-SOD content and activity in the HSP70- transfected hearts enhanced compared to the controls, and this was further associated with improved mitochondrial respiratory function [51]. However, there is conflicting evidence about HSP70 expression within the atherosclerotic plaque. It has been reported that HSP70 expression is increased in necrotic plaques [53]. A more recent study reported no difference in HSP70 staining between fibrotic plaques and inflammatory plaques, whereas HSP70 expression was increased in plaques with thicker fibrous caps only [54]. This discrepancy may be due to the different stage of atherosclerotic development investigated. Nevertheless, due to the importance of this HSP70 in pro-survival signaling, the observed differences suggest caution andmore experimental studies are necessary to clarify the exact role of HSP70 in atherosclerosis before translating its overexpression in a clinical scenario.

5. Gene therapy targeting angiogenesis

Among target genes for HIF, PR39, a proline (P)- and arginine (R)-rich peptide with 39 aminoacids, is of great interest because of its effects in limiting cardiac injury through induction of angiogenesis [55]. Muinck and colleagues [56] investigated the role of PR39 in defense against IRI. The authors proved that intramyocardial delivery of recombinant adenoviral vectors encoding for PR39 in a murine model of myocardial ischemia–reperfusion injury, induced by ligation and release of the left anterior descending coronary artery, resulted in a significant reduction of myocardial infarct size [56]. Since this effect was completely abolished after

cotransfection with a PR39 adenovirus plus plasmid encoding a HIF-1alpha dominant negative construct (PR39 + HIF- 1alpha-dn), the authors showed that cardioprotection by PR39was likely conveyed by protective metabolic and survival responses through HIF- 1alpha stabilization and not by angiogenesis. Despite pro-fibrotic effects, the active form of TGFbeta has been shown to exert cardioprotection in in vivo models of IRI [56], and also to decrease cell injury in an in vitro model of hypoxia-reoxygenation [57,58]. Dandapat and colleagues [34] investigated whether upregulation of a TGFbeta active form in vivo using adeno-associated virus type 2 would induce cardioprotection from IRI. Adenovirus-TGFbeta (AAV/ TGFbeta active) or adenovirus-GFP was injected into 4 different sites in the left descending artery territory. After 6 weeks of intramyocardial injections rats were subjected to 60 min left descending artery ligation followed by 60 min of reperfusion. Following ischemia–reperfusion, AAV/TGFbeta active-overexpressing rats had a much smaller infarct size. In investigating the involvement of lipid peroxidation (measured by malondialdehyde levels in the myocardial tissues) in IRI, authors showed that overexpression of AAV/TGFbeta active significantly reduced IRI-induced lipid peroxidation,whichwas also related to reduced activation of NADPH oxidase and NF-kB. These data demonstrate that overexpression of TGFbeta by AAV can protect cardiac tissue from reperfusion injury, possibly via an antioxidant mechanism, suggesting the potential of TGFbeta active gene therapy for cardioprotection from IRI. Sphingosine kinase 1 (SPK1) has been identified as a central mediator of ischemia preconditioning; it plays a protective role in ischemia/reperfusion- induced cardiomyocyte death. Duan and colleagues showed that intramyocardial delivery of recombinant adenoviral vectors encoding for SPK1 in a rat model of myocardial IRI, resulted in significant reduction of myocardial infarct size [59]. Marked improvement of several functional parameters, such as left ventricular (LV) systolic pressure, LV end-diastolic pressure, and peak velocity of contraction (dP/ dt), was observed in the heart injected with SPK1, compared to saline and adenoviral control [59].

6. Gene therapy targeting antiapoptotic and autophagy genes

Since IRI has been shown to elicit apoptosis of cardiomyocytes via the production of reactive oxygen species and mitochondrial damage, up-regulation of antiapoptotic genes or autophagy would be a further therapeutic approach for preventing myocardial damage. Autophagy is a housekeeping process that helps in maintaining cellular energy homeostasis and removes damaged organelles. In the heart, autophagy is an adaptive process that is activated in response to stress including acute and chronic ischemia [60]. Ischemia preconditioning has been shown to be causally associated with an increased number of autophagosomes in cardiomyocytes in hearts undergoing three cycles of 5 min regional ischemia alternating with 5 min reperfusion [61]. Cardioprotection from autophagy during ischemia preconditioning could be inhibited with dominant negative mutation of the autophagy protein Atg5 [61]. Several studies have shown that both the intrinsic and extrinsic apoptotic cell-death pathways are induced in the ventricular cardiomyocyte after myocardial ischemia/reperfusion, and that they converge with each other in the activation of caspase-3 [62–64]. It has been reported that cardiomyocytes can acquire resistance to IRI after brief ischemia through induction of anti-apoptotic genes, such as Bcl-2 and Bcl-xL [25]. Kossmehl and colleagues [64] examined the expression of FasL, Bcl-2, Bax, caspase-3 and p53 in an isolated hemoperfused porcine working heart model of acute ischemia (2 h), followed by reperfusion (4 h). The authors also analyzed the influence of the renin–angiotensin system (RAS) on an ischemia/reperfusion-induced programmed cell. In this model, Fas, Bax, Bcl-2, caspase-3 and p53 proteins were increased during ischemia/reperfusion in the hearts, and further elevated by the administration of angiotensin I. The RAS inhibitor quinaprilat reduced Bcl-2, Bax, caspase-3 and p53 expression, but had no

effect on Fas protein [64]. Gomez et al. [65] studied which of the Fas/FasL or the mitochondrial pathway has a predominant role in cardiomyocyte death after ischemia/reperfusion injury. The authors used amodel of genetic inactivation of the Fas receptor (lpr mice), pretreated with either saline or cyclosporin A [a desensitizer of the mitochondrial permeability transition pore (mPTP)], undergoing 25 min of ischemia followed by 24 h of reperfusion [65]. The authors found that control and lpr hearts exhibited a comparable increase in caspase-3 activity, while cyclosporin A treatment significantly reduced caspase-3 activity in control and lpr hearts, suggesting that the Fas pathway likely plays a minor role, whereas mitochondria are preferentially involved in mice cardiomyocyte death after a lethal insult [47,65]. Huang and colleagues [33] investigated whether adenovirusmediated Bcl-xL gene transfer could provide a direct cardioprotective effect in the context of cardiac IRI. Adenovirus-mediated gene transfer into the heart was carried out by direct injection of adenoviral vector encoding human Bcl-xL (AdBclxL) into the anterior wall of the left ventricle four days prior to ischemia–reperfusion injury. Three experimental groups were evaluated: saline control group injected with saline alone, LacZ group injected with adenoviral vector encoding LacZ, and Bcl-xL group injected with Bcl-xL gene. Rats were sacrificed 4 days after gene-transduction. 30 min ischemia followed by 30 min reperfusion at 37 °C were applied to the excised heart. In the in vivo protocol of ischemia–reperfusion, 4 days after the injection into the heart the left anterior descending coronary artery was ligated. After 30 min the suturewas released and reperfusionwas carried out. Ratwere sacrificed 24 h after reperfusion and infarct size was evaluated. In this model, adenoviral gene transduction did not affect cardiac performance such as coronary flow and dp/dt after ischemia–reperfusion protocol. Marked improvement of dp/dt recovery and coronary flow was observed in the heart injectedwith Bcl-xL, in comparisonwith saline and adenoviral control. A decrease of caspase-3 after ischemia–reperfusion injury was observed in the heart injected with Bcl-xL in comparison with saline and adenoviral control. This effect was accompanied by decreased apoptosis at the surrounding area outside the Bcl-transduced myocardium. In the in vivo protocol, a significant reduction of serum creatine kinase levels was observed in the animals treated with the myocardial Bcl-xL gene transduction.

7. Gene therapy targeting PI3K–Akt pathway

Dephosphorylation of FoxOs following deactivation of the PI3K–Akt pathway induces expression of cell death-related genes, including FasL and Bim[66], which are phosphorylated by Akt. It is known that activation of the PI3K–Akt pathway by growth factors such as HGF has the potential to limit infarct size and determine cardioprotection, preventing or attenuating the activation of intrinsic and extrinsic apoptotic cell death pathways. This is potentially clinically relevant at the time of MI and post-MI reperfusion. Among growth factors with cardioprotective effects through the activation of the Akt pathway, IGF-1 and HGF are of great interest because of their effects in promoting cell growth and viability [32,67,68], in reducing apoptosis after transient ischemia in vivo [69–71], in reducing fibrosis [72] and inducing angiogenesis [73,74]. In an ex vivo model of IRI hearts Buerke and colleagues investigated whether adenovirus-mediated IGF transfer could provide cardioprotection. Adenovirus-IGF or LacZ were injected into the left ventricle apex and 48 h later rats were subjected to 30 min LAD ligation and 24 h reperfusion. LAD ligation produced a significant area of anteroapical infarction, which was not different among saline and LacZ, but significantly reduced in adenovirus-IGF injected hearts. HGF, also known as scatter factor (SF) because of its property to induce the scattering of epithelial cells, consists of six domains: an amino-terminal domain (N), four kringle domains (K1–K4) and a serine proteinase homology (SPH) domain [75]. By using a rabbit model of ischemia– reperfusion injury, Chen and colleagues demonstrated that HGF/SF gene transfer is cardioprotective through its multiple beneficial

actions, such as angiogenesis, Bcl-2 overexpression, and decreasing hydroxyl radicals, deoxyuride-5'-triphosphate biotin nick end labeling (TUNEL)-positive myocytes, and fibrotic area [76]. The authors performed intraventricular injection of recombinant adenovirus encoding for HGF/SF (Ad.HGF/SF), followed by 30 min ligation of the left coronary artery and reperfusion for a minimum of 30 min to a maximum of 14 days. The authors showed a significant reduction of infarct size in Ad.HGF-injected hearts compared with saline- and Ad.LacZ-treated hearts [76]. At 14 days after reperfusion, HGF gene transfer improved left ventricular ejection fraction and fractional shortening, reduced the fibrotic area, and increased the capillary density in the risk area. At 4 h after reperfusion, Bcl-2 protein was overexpressed and the incidence of TUNEL-positive myocytes was significantly decreased in the risk area [76].

8. Vectors useful for gene therapy

The introduction of exogenous genes can be performed either by directly introducing the delivery vector [such as lentivirus or adenoassociated viral (AAV) vectors] into the anatomical site (in vivo) or by harvesting cells from the patient, transferring the gene(s) to the cells in tissue culture and then transferring the genetically modified cells back into the patient (ex vivo) (reviewed in [77]). The ex vivo approach typically relies on transplanting cells, such as stem cells, lymphocytes, fibroblasts, or – alternatively – the cells of interest, that are removed from the body and injected after therapeutic transgene modifications. This ex vivo approach allows for targeting of specific cells for gene delivery, supplies cells that may directly participate in the regenerative process, allows for both autocrine and paracrine effects fromthe expressed growth factor, and avoids the safety risks of directly injecting viral vectors or transfection reagents in vivo. This approach, however, involves an extra step to manipulate and expand cells in culture, and has the risk of contamination. Additionally, the ex vivo approach does not eliminate the possibility of retroviral vectors causing insertional activation of other genes, the over-expression of which may cause cancer. The in vivo gene delivery approach is more straightforward, but is limited by inefficient gene delivery, nonspecific cellular targeting, the complexity of cloning and integrating the gene into the target cells and safety and efficiency of transduction. With respect to the delivery of growth factors (GFs), a possible drawback of the use of lentiviral and AAV vectors for delivering genes that encode for GFs might be that they can cause a chronic overexpression of the protein,with an uncertain therapeutic effect. Short-term gene expression of the GF gene (by plasmid transfection or adenoviral vectors) would be desirable if the goal is to deliver a secreted protein, such as IGF-1, vascular endothelial growth factor (VEGF) and HGF, while long-term expression would be preferable if the goal is to express membrane proteins such as receptors for growth factors that require stable expression. Limitations of these strategies are the low transfection efficiency with plasmids and the immunogenic response of the host against the adenoviruses.

9. Conclusions and perspectives

Gene therapy may offer a novel, effective strategy for the management of cardioprotection in ischemic cardiac disease. Preclinical studies have shown the potential of gene therapy for protecting the heart against acute myocardial ischemia through mimicking ischemic conditioning. AAV seems particularly suitable to address the last requirement, since they have shown specific tropism for cardiomyocytes, and a capability to drive the expression of the delivered genes in these cells for a long time. However, to date, there have been no well-controlled long-term studies in the preclinical area. Hence, we cannot make any conclusive statements about the clinical suitability/efficacy of gene delivery in humans. The crucial issues that are still to be resolved prior to

achieving clinical success include the understanding of whether protected cells might be obtained as a result of the delivery of a single factor orwhether protected cells might require more complex gene combinations, the identification of the proper timing of therapeutic gene expression and, probably, the development of more efficacious gene delivery tools that allow a time-controlled gene delivery and expression. If resolved, cellmediated synthesis of cardioprotective gene modulators might offer more efficient targeting of receptors and, consequently, a more robust and predictable approach in cardioprotection. Nonetheless, accumulating data from the genomic studies of ischemic conditioning encourage investigators to further identify novel gene targets for cardioprotection.

Acknowledgements

Thisworkwas supported by grants fromthe National Research Fund of Hungary (OTKA ANN 107803, and K 109737). PF is a Szentágothai fellow (National Excellence Program of Hungary, TAMOP 4.2.4.A/2-11- 1-2012-0001). ZG is a János Bolyai fellow of the Hungarian Academy of Sciences.

References

[1] Z. Varga, Z. Giricz, P. Bencsik, R. Madonna, M. Gyogyosi, R. Schulz, M. Mayr, T. Thum, L. Puskas, P. Ferdinandy, Functional genomics of cardioprotection by ischemic conditioning and the influence of comorbid conditions: implications in target identification, Curr. Drug Targets (2015 Apr 27).

[2] T. Kuzuya, S. Hoshida, N. Yamashita, H. Fuji, H. Oe, M. Hori, T. Kamada, M. Tada, Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia, Circ. Res. 72 (1993) 1293–1299.

[3] M.S. Marber, D.S. Latchman, J.M.Walker, D.M. Yellon, Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associatedwith resistance tomyocardial infarction, Circulation 88 (1993) 1264–1272.

[4] R. Bolli, The late phase of preconditioning, Circ. Res. 87 (2000) 972–983.

[5] R. Bolli, Preconditioning: a paradigm shift in the biology of myocardial ischemia, Am. J. Physiol. Heart Circ. Physiol. 292 (2007) H19–27.

[6] G.F. Baxter, P. Ferdinandy, Delayed preconditioning of myocardium: current perspectives, Basic Res. Cardiol. 96 (2001) 329–344.

[7] D.J. Hausenloy, H. Erik Botker, G. Condorelli, P. Ferdinandy, D. Garcia-Dorado, G. Heusch, S. Lecour, L.W. van Laake, R. Madonna, M. Ruiz-Meana, R. Schulz, J.P. Sluijter, D.M. Yellon, M. Ovize, Translating cardioprotection for patient benefit: position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology, Cardiovasc. Res. 98 (2013) 7–27

[8] P. Ferdinandy, D.J. Hausenloy, G. Heusch, G.F. Baxter, R. Schulz, Interaction of risk factors, comorbidities and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning, Pharmacol. Rev. 66 (4) (2014) 1142–1174.

[9] J.P. Sluijter, G. Condorelli, S.M. Davidson, F.B. Engel, P. Ferdinandy, D.J. Hausenloy, S. Lecour, R. Madonna, M. Ovize, M. Ruiz-Meana, R. Schulz, L.W. Van Laake, Novel therapeutic strategies for cardioprotection, Pharmacol. Ther. 144 (2014) 60–70.

[10] J.D. McCully, M.K. Bhasin, C. Daly, M.C. Guerrero, S. Dillon, T.A. Liberman, D.B. Cowan, J.D. Mably, F.X. McGowan, S. Levitsky, Transcriptomic and proteomic analysis of global ischemia and cardioprotection in the rabbit heart, Physiol. Genomics 38 (2009) 125–137.

[11] H.K. Kim, S.W. Kang, S.H. Jeong, N. Kim, J.H. Ko, H. Bang, W.S. Park, T.H. Choi, Y.R. Ha, Y.S. Lee, J.B. Youm, K.S. Ko, B.D. Rhee, J. Han, Identification of potential target genes of cardioprotection against ischemia–reperfusion injury by express sequence tags analysis in rat hearts, J. Cardiol. 60 (2010) 98–110.

[12] K.M. Black, R.J. Barnett, M.K. Bhasin, C. Daly, S.T. Dillon, T.A. Libermann, S. Levitsky, J.D. McCully, Microarray and proteomic analysis of the cardioprotective effects of cold blood cardioplegia in themature and aged male and female, Physiol. Genomics 44 (2012) 1027–1041.

[13] V. Sawhney, S. Brouilette, D. Abrams, R. Schilling, B. O'Brien, Current genomics in cardiovascular medicine, Curr. Genomics 13 (2012) 446–462.

[14] J.A. Lee, What was genomics? Lancet Oncol. 4 (2003) 584.

[15] P. Ferdinandy, R. Schulz, G.F. Baxter, Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning, Pharmacol. Rev. 59 (2007) 418–458.

[16] B.Z. Simkhovich, S. Abdishoo, C. Poizat, S.L. Hale, L.H. Kedes, R.A. Kloner, Gene activity changes in ischemically preconditioned rabbit heart gene: discovery array study, Heart Dis. 4 (2002) 63–69.

[17] A. Onody, A. Zvara, L. Hackler Jr., L. Vigh, P. Ferdinandy, L.G. Puskas, Effect of classic preconditioning on the gene expression pattern of rat hearts: a DNA microarray study, FEBS Lett. 536 (2003) 35–40.

[18] B.Z. Simkhovich, P. Marjoram, C. Poizat, L. Kedes, R.A. Kloner, Brief episode of ischemia activates protective genetic program in rat heart: a gene chip study, Cardiovasc. Res. 59 (2003) 450–459.

[19] A. Vincent, G. Gahide, C. Sportouch-Dukhan, A. Covinhes, A. Franck-Miclo, F. Roubille, C. Barrere, J. Adda, C. Dantec, C. Redt-Clouet, C. Piot, J. Nargeot, S. Barrere-Lemaire, Down-regulation of the transcription factor ZAC1 upon pre- and postconditioning protects against I/R injury in the mouse myocardium, Cardiovasc. Res. 94 (2011) 351–358.

[20] C. Csonka, G. Szucs, Z. Varga-Orvos, P. Bencsik, T. Csont, A. Zvara, L.G. Puskas, P. Ferdinandy, Ischemic postconditioning alters the gene expression pattern of the ischemic heart, Exp. Biol. Med. (Maywood) 239 (2014) 141–150.

[21] Z.V. Varga, A. Zvara, N. Farago, G.F. Kocsis, M. Pipicz, R. Gaspar, P. Bencsik, A. Gorbe, C. Csonka, L.G. Puskas, T. Thum, T. Csont, P. Ferdinandy, MicroRNAs associated with ischemia–reperfusion injury and cardioprotection by ischemic pre- and postconditioning: protectomiRs, Am. J. Physiol. Heart Circ. Physiol. 307 (2014) H216–227.

[22] Z. Giricz, Z.V. Varga, T. Baranyai, P. Sipos, K. Paloczi, A. Kittel, E.I. Buzas, P. Ferdinandy, Cardioprotection by remote ischemic preconditioning of the rat heart is mediated by extracellular vesicles, J. Mol. Cell. Cardiol. 68 (2014) 75–78.

[23] A.L. Barabasi, Z.N. Oltvai, Network biology: understanding the cell's functional organization, Nat. Rev. Genet. 5 (2004) 101–113.

[24] R. Bolli, S.Manchikalapudi, X.L. Tang, H. Takano, Y. Qiu, Y. Guo, Q. Zhang, A.K. Jadoon, The protective effect of late preconditioning against myocardial stunning in conscious rabbits is mediated by nitric oxide synthase. Evidence that nitric oxide acts both as a trigger and as a mediator of the late phase of ischemic preconditioning, Circ. Res. 81 (1997) 1094–1107.

[25] Y.Guo, W.K. Jones, Y.T. Xuan, X.L. Tang, W. Bao, W.J.Wu, H.Han, V.E. Laubach, P. Ping, Z. Yang, Y. Qiu, R. Bolli, The late phase of ischemic preconditioning is abrogated by targeted disruption of the inducible NO synthase gene, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 11507–11512.

[26] P. Bencsik, K. Kupai, Z. Giricz, A. Gorbe, J. Pipis, Z. Murlasits, G.F. Kocsis, Z. Varga-Orvos, L.G. Puskas, C. Csonka, T. Csont, P. Ferdinandy, Role of iNOS and peroxynitrite-matrix metalloproteinase-2 signaling in myocardial late preconditioning in rats, Am. J. Physiol. Heart Circ. Physiol. 299 (2010) H512–518.

[27] Q. Li, Y. Guo, Y.T. Xuan, C.J. Lowenstein, S.C. Stevenson, S.D. Prabhu,W.J.Wu, Y. Zhu, R. Bolli, Gene therapy with inducible nitric oxide synthase protects against myocardial infarction via a cyclooxygenase-2-dependent mechanism, Circ. Res. 92 (2003) 741–748.

[28] Y.L. Tang, K. Qian, Y.C. Zhang, L. Shen, M.I. Phillips, A vigilant, hypoxia-regulated heme oxygenase-1 gene vector in the heart limits cardiac injury after ischemia– reperfusion in vivo, J. Cardiovasc. Pharmacol. Ther. 10 (2005) 251–263.

[29] Q. Li, R. Bolli, Y. Qiu, X.L. Tang, Y. Guo, B.A. French, Gene therapy with extracellular superoxide dismutase protects conscious rabbits against myocardial infarction, Circulation 103 (2001) 1893–1898.

[30] J. Jayakumar, K. Suzuki, I.A. Sammut, R.T. Smolenski, M. Khan, N. Latif, H. Abunasra, B. Murtuza, M. Amrani, M.H. Yacoub, Heat shock protein 70 gene transfection protects mitochondrial and ventricular function against ischemia–reperfusion injury, Circulation 104 (2001) I303–307.

[31] D.D. Belke, B. Gloss, J.M. Hollander, E.A. Swanson, H. Duplain, W.H. Dillmann, In vivo gene delivery of HSP70i by adenovirus and adeno-associated virus preserves contractile function in mouse heart following ischemia–reperfusion, Am. J. Physiol. Heart Circ. Physiol. 291 (2006) H2905–2910.

[32] W. Chao, T. Matsui, M.S. Novikov, J. Tao, L. Li, H. Liu, Y. Ahn, A. Rosenzweig, Strategic advantages of insulin-like growth factor-I expression for cardioprotection, J. Gene Med. 5 (2003) 277–286.

[33] J. Huang, Y. Ito, M. Morikawa, H. Uchida, M. Kobune, K. Sasaki, T. Abe, H. Hamada, Bcl-xL gene transfer protects the heart against ischemia/reperfusion injury, Biochem. Biophys. Res. Commun. 311 (2003) 64–70.

[34] A. Dandapat, C.P. Hu, D. Li, Y. Liu, H. Chen, P.L. Hermonat, J.L. Mehta, Overexpression of TGFbeta1 by adeno-associated virus type-2 vector protects myocardium from ischemia– reperfusion injury, Gene Ther. 15 (2008) 415–423.

[35] Q. Li, Y. Guo,W. Tan, A.B. Stein, B. Dawn,W.J.Wu, X. Zhu, X. Lu, X. Xu, T. Siddiqui, S. Tiwari, R. Bolli, Gene therapywith iNOS provides long-termprotection against myocardial infarction without adverse functional consequences, Am. J. Physiol. Heart Circ. Physiol. 290 (2006) H584–589.

[36] Q. Li, Y. Guo, W.J. Wu, Q. Ou, X. Zhu, W. Tan, F. Yuan, N. Chen, B. Dawn, L. Luo, E. O'Brien, R. Bolli, Gene transfer as a strategy to achieve permanent cardioprotection I: rAAV-mediated gene therapy with inducible nitric oxide synthase limits infarct size 1 year later without adverse functional consequences, Basic Res. Cardiol. 106 (2011) 1355–1366.

[37] R. Bolli, Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research, J. Mol. Cell. Cardiol. 33 (2001) 1897–1918.

[38] X.Y. Li, P.B. McCay, M. Zughaib, M.O. Jeroudi, J.F. Triana, R. Bolli, Demonstration of free radical generation in the “stunned”myocardiumin the conscious dog and identification of major differences between conscious and open-chest dogs, J. Clin. Invest. 92 (1993) 1025–1041.

[39] H. Takano, S. Manchikalapudi, X.L. Tang, Y. Qiu, A. Rizvi, A.K. Jadoon, Q. Zhang, R. Bolli, Nitric oxide synthase is the mediator of late preconditioning against myocardial infarction in conscious rabbits, Circulation 98 (1998) 441–449.

[40] P. Pagliaro, C. Penna, Redox signalling and cardioprotection: translatability and mechanism, Br. J. Pharmacol. (2014) (vol.).

[41] S.C. Francis, M.K. Raizada, A.A. Mangi, L.G. Melo, V.J. Dzau, P.R. Vale, J.M. Isner, D.W. Losordo, J. Chao, M.J. Katovich, K.H. Berecek, Genetic targeting for cardiovascular therapeutics: are we near the summit or just beginning the climb? Physiol. Genomics 7 (2001) 79–94.

[42] L.G. Melo, R. Agrawal, L. Zhang, M. Rezvani, A.A.Mangi, A. Ehsan, D.P. Griese, G. Dell'Acqua, M.J. Mann, J. Oyama, S.F. Yet, M.D. Layne, M.A. Perrella, V.J. Dzau, Gene therapy strategy for long-term myocardial protection using adenoassociated virus-mediated delivery of heme oxygenase gene, Circulation 105 (2002) 602–607.

[43] X. Liu, A.S. Pachori, C.A. Ward, J.P. Davis, M. Gnecchi, D. Kong, L. Zhang, J. Murduck, S.F. Yet, M.A. Perrella, R.E. Pratt, V.J. Dzau, L.G. Melo, Heme oxygenase-1 (HO-1) inhibits postmyocardial infarct remodeling and restores ventricular function, FASEB J. 20 (2006) 207–216.

[44] A.S. Pachori, L.G. Melo, L. Zhang, S.D. Solomon, V.J. Dzau, Chronic recurrent myocardial ischemic injury is significantly attenuated by pre-emptive adenoassociated virus heme oxygenase-1 gene delivery, J. Am. Coll. Cardiol. 47 (2006) 635–643.

[45] X. Liu, J.A. Simpson, K.R. Brunt, C.A.Ward, S.R. Hall, R.T. Kinobe, V. Barrette, M.Y. Tse, S.C. Pang, A.S. Pachori, V.J. Dzau, K.O. Ogunyankin, L.G. Melo, Preemptive heme oxygenase-1 gene delivery reveals reduced mortality and preservation of left ventricular function 1 yr after acute myocardial infarction, Am. J. Physiol. Heart Circ. Physiol. 293 (2007) H48–59.

[46] T. Date, S. Mochizuki, A.J. Belanger, M. Yamakawa, Z. Luo, K.A. Vincent, S.H. Cheng, R.J. Gregory, C. Jiang, Expression of constitutively stable hybrid hypoxia-inducible factor-1alpha protects cultured rat cardiomyocytes against simulated ischemia–reperfusion injury, Am. J. Physiol. Cell Physiol. 288 (2005) C314–320.

[47] S.G. Ong, W.H. Lee, M. Huang, D. Dey, K. Kodo, V. Sanchez-Freire, J.D. Gold, J.C. Wu, Cross talk of combined gene and cell therapy in ischemic heart disease: role of exosomal microRNA transfer, Circulation 130 (2014) S60–69.

[48] C. Jiang, H. Lu, K.A. Vincent, S. Shankara, A.J. Belanger, S.H. Cheng, G.Y. Akita, R.A. Kelly, M.A. Goldberg, R.J. Gregory, Gene expression profiles in human cardiac cells subjected to hypoxia or expressing a hybrid form of HIF-1 alpha, Physiol. Genomics 8 (2002) 23–32.

[49] C. Soti, E. Nagy, Z. Giricz, L. Vigh, P. Csermely, P. Ferdinandy, Heat shock proteins as emerging therapeutic targets, Br. J. Pharmacol. 146 (2005) 769–780.

[50] S. Okubo, O. Wildner, M.R. Shah, J.C. Chelliah, M.L. Hess, R.C. Kukreja, Gene transfer of heat-shock protein 70 reduces infarct size in vivo after ischemia/reperfusion in the rabbit heart, Circulation 103 (2001) 877–881.

[51] K. Suzuki, Y. Sawa, Y. Kaneda, H. Ichikawa, R. Shirakura, H. Matsuda, In vivo gene transfection with heat shock protein 70 enhances myocardial tolerance to ischemia– reperfusion injury in rat, J. Clin. Invest. 99 (1997) 1645–1650.

[52] K. Suzuki, Y. Sawa, H. Ichikawa, Y. Kaneda, H. Matsuda, Myocardial protection with endogenous overexpression ofmanganese superoxide dismutase, Ann. Thorac. Surg. 68 (1999) 1266–1271.

[53] A.D. Johnson, P.A. Berberian, M. Tytell, M.G. Bond, Differential distribution of 70-kD heat shock protein in atherosclerosis. Its potential role in arterial SMC survival, Arterioscler. Thromb. Vasc. Biol. 15 (1995) 27–36.

[54] J. Madrigal-Matute, O. Lopez-Franco, L.M. Blanco-Colio, B. Munoz-Garcia, P. Ramos-Mozo, L. Ortega, J. Egido, J.L. Martin-Ventura, Heat shock protein 90 inhibitors attenuate inflammatory responses in atherosclerosis, Cardiovasc. Res. 86 (2010) 330–337.

[55] J. Li, M. Post, R. Volk, Y. Gao, M. Li, C. Metais, K. Sato, J. Tsai,W. Aird, R.D. Rosenberg, T.G. Hampton, F. Sellke, P. Carmeliet, M. Simons, PR39, a peptide regulator of angiogenesis, Nat. Med. 6 (2000) 49–55.

[56] E.D. Muinck, N. Nagy, D. Tirziu, M. Murakami, N. Gurusamy, S.K. Goswami, S. Ghatpande, R.M. Engelman, M. Simons, D.K. Das, Protection against myocardial ischemia–reperfusion injury by the angiogenic Masterswitch protein PR 39 gene therapy: the roles of HIF1alpha stabilization and FGFR1 signaling, Antioxid. Redox Signal. 9 (2007) 437–445. R. Madonna et al. / International Journal of Cardiology 191 (2015) 203–210 209

[57] B.C. Yang, D.S. Zander, J.L. Mehta, Hypoxia-reoxygenation-induced apoptosis in cultured adult rat myocytes and the protective effect of platelets and transforming growth factor-beta(1), J. Pharmacol. Exp. Ther. 291 (1999) 733–738.

[58] H. Chen, D. Li, T. Saldeen, J.L. Mehta, TGF-beta(1) modulates NOS expression and phosphorylation of Akt/PKB in rat myocytes exposed to hypoxia-reoxygenation, Am. J. Physiol. Heart Circ. Physiol. 281 (2001) H1035–1039.

[59] H.F. Duan, H. Wang, J. Yi, H.J. Liu, Q.W. Zhang, L.B. Li, T. Zhang, Y. Lu, C.T. Wu, L.S. Wang, Adenoviral gene transfer of sphingosine kinase 1 protects heart against ischemia/reperfusion-induced injury and attenuates its postischemic failure, Hum. Gene Ther. 18 (2007) 1119–1128.

[60] Z. Giricz, R.M. Mentzer Jr., R.A. Gottlieb, Autophagy, myocardial protection, and the metabolic syndrome, J. Cardiovasc. Pharmacol. 60 (2012) 125–132.

[61] C. Huang, S. Yitzhaki, C.N. Perry,W. Liu, Z. Giricz, R.M. Mentzer Jr., R.A. Gottlieb, Autophagy induced by ischemic preconditioning is essential for cardioprotection, J. Cardiovasc. Transl. Res. 3 (2010) 365–373.

[62] M. Wagner, M.A. Siddiqui, Signaling networks regulating cardiac myocyte survival and death, Curr. Opin. Investig. Drugs 10 (2009) 928–937.

[63] M.S. Bhuiyan, K. Fukunaga, Inhibition of HtrA2/Omi ameliorates heart dysfunction following ischemia/reperfusion injury in rat heart in vivo, Eur. J. Pharmacol. 557 (2007) 168–177.

[64] P. Kossmehl, E. Kurth, S. Faramarzi, B. Habighorst, M. Shakibaei, M. Wehland, R. Kreutz, M. Infanger, A.H. J. D., J. Grosse, M. Paul, D. Grimm, Mechanisms of apoptosis after ischemia and reperfusion: role of the renin–angiotensin system, Apoptosis 11 (2006) 347–358.

[65] L. Gomez, N. Chavanis, L. Argaud, L. Chalabreysse, O. Gateau-Roesch, J. Ninet, M. Ovize, Fas-independent mitochondrial damage triggers cardiomyocyte death after ischemia–reperfusion, Am. J. Physiol. Heart Circ. Physiol. 289 (2005) H2153–2158.

[66] P.F. Dijkers, R.H. Medema, J.W. Lammers, L. Koenderman, P.J. Coffer, Expression of the pro-apoptotic Bcl-2 family member Bim is regulated by the forkhead transcription factor FKHR-L1, Curr. Biol. 10 (2000) 1201–1204.

[67] R. Madonna, G. Rokosh, R. De Caterina, R. Bolli, Hepatocyte growth factor/Met gene transfer in cardiac stem cells—potential for cardiac repair, Basic Res. Cardiol. 105 (2010) 443–452.

[68] R. Madonna, C. Cevik, M. Nasser, R. De Caterina, Hepatocyte growth factor: molecular biomarker and player in cardioprotection and cardiovascular regeneration, Thromb. Haemost. 107 (2012) 656–661.

[69] M. Buerke, T. Murohara, C. Skurk, C. Nuss, K. Tomaselli, A.M. Lefer, Cardioprotective effect of insulin-like growth factor I inmyocardial ischemia followed by reperfusion, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 8031–8035.

[70] H. Ueda, Y. Sawa, K.Matsumoto, S. Kitagawa-Sakakida, Y. Kawahira, T. Nakamura, Y. Kaneda, H. Matsuda, Gene transfection of hepatocyte growth factor attenuates reperfusion injury in the heart, Ann. Thorac. Surg. 67 (1999) 1726–1731.

[71] T. Nakamura, S. Mizuno, K. Matsumoto, Y. Sawa, H. Matsuda, Myocardial protection from ischemia/reperfusion injury by endogenous and exogenous HGF, J. Clin. Invest. 106 (2000) 1511–1519.

[72] Y. Taniyama, R. Morishita, H. Nakagami, A. Moriguchi, H. Sakonjo, K. Shokei, K. Matsumoto, T. Nakamura, J. Higaki, T. Ogihara, Potential contribution of a novel antifibrotic factor, hepatocyte growth factor, to prevention of myocardial fibrosis by angiotensin II blockade in cardiomyopathic hamsters, Circulation 102 (2000) 246–252.

[73] Y. Hisaka,M. Ieda, T. Nakamura, K. Kosai, S. Ogawa, K. Fukuda, Powerful and controllable angiogenesis by using gene-modified cells expressing human hepatocyte growth factor and thymidine kinase, J. Am. Coll. Cardiol. 43 (2004) 1915–1922.

[74] Y. Ieda, J. Fujita, M. Ieda, T. Yagi, H. Kawada, K. Ando, K. Fukuda, G-CSF and HGF: combination of vasculogenesis and angiogenesis synergistically improves recovery in murine hind limb ischemia, J. Mol. Cell. Cardiol. 42 (2007) 540–548.

[75] C. Birchmeier, W. Birchmeier, E. Gherardi, G.F. Vande Woude, Met, metastasis, motility and more, Nat. Rev. Mol. Cell Biol. 4 (2003) 915–925.

[76] X.H. Chen, S. Minatoguchi, K. Kosai, K. Yuge, T. Takahashi, M. Arai, N. Wang, Y.Misao, C. Lu, H. Onogi, H. Kobayashi, S. Yasuda, M. Ezaki, H. Ushikoshi, G. Takemura, T. Fujiwara, H. Fujiwara, In vivo hepatocyte growth factor gene transfer reduces myocardial ischemia–reperfusion injury through its multiple actions, J. Card. Fail. 13 (2007) 874–883.

[77] R.Madonna, R. De Caterina, Cell-based gene therapies and stemcells for regeneration of ischemic tissues, In Gene Therapy, Sciyo InTech, 2011.