Intrinsic remote conditioning of the myocardium as a comprehensive cardiac response to ischemia and reperfusion

Noemi Pavo[1], Dominika Lukovic[1], Katrin Zlabinger[1], David Lorant[2], Georg Goliasch[1], Johannes Winkler[1], Dietmar Pils[3,4], Katharina Auer[5], Hendrik Jan Ankersmit[4], Zoltán Giricz[6], Márta Sárközy[7], András Jakab[8,9], Rita Garamvölgyi[10], Maximilian Y. Emmert[11,12,13], Simon P. Hoerstrup[11,12,13], Derek J Hausenloy[14,15], Péter Ferdinandy[6,16], Gerald Maurer[1], Mariann Gyöngyösi[1]

[1] Department of Cardiology, Medical University of Vienna, Vienna, Austria
[2] Department of Anaesthesiology, Medical University of Vienna, Vienna, Austria
[3] Center for Medical Statistics, Informatics, and Intelligent Systems (CeMSIIS), Medical University of Vienna, Vienna, Austria
[4] Department of Surgery, Medical University of Vienna, Vienna, Austria
[5] Department of Obstetrics and Gynecology - Molecular Oncology Group, Medical University of Vienna, Vienna, Austria
[6] Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary
[7] Department of Biochemistry, Faculty of Medicine, University of Szeged, Szeged, Hungary
[8] Department of Biomedical Imaging and Image-guided Therapy, Medical University of Vienna, Vienna, Austria
[9] Center for MR-Research, University Children's Hospital Zurich, Zurich, Switzerland
[10] Institute of Diagnostic Imaging and Radiation Oncology, University of Kaposvar, Kaposvar, Hungary
[11] Swiss Centre for Regenerative Medicine, University of Zurich, Zurich, Switzerland
[12] Division of Surgical Research, University Hospital of Zurich, Zurich, Switzerland
[13] Clinic for Cardiovascular Surgery, University Hospital of Zurich, Zurich, Switzerland
[14] The Hatter Cardiovascular Institute, University College London, London, UK
[15] Cardiovascular and Metabolic Disorders Program, Duke-NUS Graduate Medical School, Singapore, Singapore
[16] Pharmahungary Group, Szeged, Hungary

Address for correspondence:
Mariann Gyöngyösi MD PhD
Department of Internal Medicine II / Division of Cardiology
Waehringer Guertel 18-20
1090 Vienna, Austria
Tel.: +43-1-40400-46140
Fax: +43-1-40400-42160
E-mail: mariann.gyongyosi@meduniwien.ac.at

Keywords: cardioprotection, gene expression, NOGA mapping, ischemia/reperfusion, LV remodelling

Abstract

We have previously shown that distal anterior wall ischemia/reperfusion (I/R) induces gene expression changes in the proximal anterior myocardial area, involving genes responsible for cardiac remodeling. Here we investigated the molecular signals of the ischemia non-affected remote lateral and posterior regions and present gene expression profiles of the entire left ventricle (LV) by using our novel and straightforward method of 2D and 3D image reconstruction.. Five or 24h after repetitive 10min I/R without subsequent infarction, pig hearts were explanted and myocardial samples from 52 equally distributed locations of the LV were taken. Expressional changes of seven genes of interest (HIF-1α; caspase-3, transcription factor GATA4; myocyte enhancer factor 2C /MEF2c/; hexokinase 2 /HK2/; clusterin /CLU/ and excision repair cross-complementation group 4 /ERCC4/) were measured by qPCR. 2D and 3D gene expression maps were constructed by projecting the fold changes on the NOGA anatomical mapping coordinates. Caspase-3, GATA4, HK2, CLU, and ERCC4 were up-regulated region-specifically in the ischemic zone at 5 h post I/R injury. Overexpression of GATA, CLU and ERCC4 persisted after 24 h. HK2 showed strong up-regulation in the ischemic zone and down-regulation in remote areas at 5 h, and was severely reduced in all heart regions at 24 h. These results indicate a quick onset of regulation of apoptosis-related genes, which is partially reversed in the late phase of I/R cardioprotection, and highlight variations between ischemic and unaffected myocardium over time. The NOGA 2D and 3D construction system is an attractive method to visualize expressional variations in the myocardium.

Introduction

Metabolism and gene expression patterns are changing intensively in the ischemic regions of an infarcted heart, but also remote heart regions respond to the injury quickly after the onset of infarction. We have recently shown that both acute and chronic ischemia alters the molecular signals of the ischemia non-affected, but adjacent regions, termed as intrinsic remote conditioning against adverse left ventricular (LV) remodeling [1, 2].

Conditioning of the heart against ischemic injury is one of the most potent mechanisms to prevent the heart from ischemic damage [3-7] . Single or repetitive brief intervals of ischemia and reperfusion induce cardioprotective effects against a subsequent ischemic insult. The protective effect is characterized by two time windows: Early effects last a few hours, and are conferred by the rapid release of transmitter molecules such as bradykinin and prostaglandins. The second window of protection is mainly relying on transcriptional regulation, mediated by activation of kinases and transcription factors, and subsequent effects of proteins generated *de novo*. The transcriptional changes are initiated quickly after r-I/R, and are fully effective about one to three days later. Although clinical translation of ischemic preconditioning is difficult to achieve for practical reasons, elucidation of the underlying mechanisms might lead to identification of potential modulating agents and molecular targets for the development of novel therapeutic strategies [7].

For the investigation of molecular changes in the myocardium in heart diseases, precise sampling of tissue sections, including documentation, is necessary for facilitating understanding of regional variations between directly affected and remote tissue [8, 9]. In many rodent models and experiments, the entire heart is often analyzed as a whole due to its small size. In large animals, the affected heart tissue, and usually a randomly selected sample of remote heart tissue (often used for “normal” control sample) is used for analyses by histology, or for gene expression or protein abundance. Exact locations of sampling are usually not reported, and the potential impact of the sampling locations on the analysis outcome is often neglected.

The three-dimensional NOGA® (Biologics Delivery Systems, a Johnson & Johnson company, Irwindale, CA, USA) mapping system is equipped for simultaneous measurement of electrical and mechanical activities of the myocardium and distinguishes between viable and non-viable

myocardium [10]. The NOGA and the CARTO systems have the same principles to construct 2D and 3D real-time display of the myocardial viability, wall motion and electrical activity, and are currently the only imaging technologies available for the clinics. In addition the NOGA system can be used for guided intramyocardial injections of biologicals.

Here, we show the use of the NOGA system, employed as a transcardial mapping *in vitro*, for precise documentation of locations of tissue samples, and for creating a map of gene expression levels. By replacing the voltage values by the respective fold changes of qPCR quantification, it is possible to build color-coded two- or three-dimensional images, which aptly visualize spatial gene expression. In order to demonstrate the utility of this approach, we determined and compared gene expression values in pig hearts with and without repetitive ischemia and reperfusion (r-I/R).

Results and Discussion

Data visualization using the NOGA system is straightforward: analytical data gathered by qPCR-based quantification of expression can be entered at the locations which correspond to the samples, overriding the original voltage or local activation values. The intrinsic color coding and 2D and 3D capabilities allow for simple and attractive data visualization and thus identification of regions with high or low expression levels. In particular, the sampling location of the remote myocardium is important for the gathered molecular data, as for some of the examined genes their expression differs significantly within unaffected myocardial tissue sections. However, it must be noted that expression in basal tissue segments needs to be interpreted with some caution, since the tissue composition differs (mixture of fibrotic and muscular tissue).

We collected a total of 52 tissue samples for each pig hearts and compared the individual expression levels of seven genes of interest – HIF-1α, caspase-3, transcription factor GATA4, myocyte enhancer factor 2C (MEF2c), hexokinase 2 (HK2), clusterin (CLU) and excision repair cross-complementation group 4 (ERCC4) between control animals and pigs that underwent r-I/R (Figure 1). The gene selection was based on earlier NGS analyses of ischemic and remote tissue areas, and we primarily selected genes with currently incompletely elucidated functions in ischemic injury [2]. Two distinct time points were investigated; five hours after r-I/R, to examine quick transcriptional regulation, and

24 h after r-I/R, to gain information on transcriptional changes relevant for later, sustained cardioprotection, called second window of protection (SWOP). After filling the values obtained by qPCR to the NOGA software, two- and three-dimensional visualizations were readily obtained (Figure 1).

The function of HIF-1α for cardioprotection is well characterized [11, 12]. In ischemic preconditioning, it is essential for protection from consequent ischemia in both the acute and delayed (SWOP) phases of protection. HIF-1α is a powerful transcriptional regulator and among its target genes are VEGF, EPO, inducible nitric oxide synthase (iNOS), and angiopoetin 1 and 2. The regulated genes and the functional consequences are to a certain degree cell-type dependent [13] and include increase of angiogenesis, vascular remodeling, and glucose metabolism [12]. In pig hearts, HIF-1α was upregulated in ischemic and most of the remote myocardium after 5h (Figure 2). At 24h, we observed unchanged HIF-1α expression compared to controls in those heart region, but upregulation in the remote zone that had shown unchanged expression levels at the earlier time point. This expressional pattern indicates a short term strong activation of HIF-1α in the entire myocardium after r-I/R likely starting at the directly affected region with a slightly delayed reaction of remote areas. Interestingly, transcriptional upregulation of HIF-1α seems to be restricted to the early phase, but it is documented that HIF-1α protein is additionally stabilized after preconditioning [14], and thus the downstream targets of HIF-1α are active also in the late phase of cardioprotection.

The effector caspase-3 is involved in both the intrinsic and extrinsic apoptosis pathways. It is an important apoptosis mediator in myocardial infarction, and is detected in human serum after an infarct, after escaping into the bloodstream following myocardial cell death [15]. Caspase-3 mRNA levels were substantially elevated in ischemia-affected myocardium (Figure 2) 5h after I/R, but were reduced back to baseline levels at the later time point. In remote areas, caspase transcription was largely unchanged, with only marginal down regulation after 24 h. These data indicate that a certain degree of apoptosis occurs shortly and temporarily after ischemia only in directly ischemic-injury affected tissue.

The stress-associated transcription factor GATA4 is an essential regulator of cardiac gene expression in general and modulates adaptive responses after injury [16]. It confers regenerative effects and is

critical for the regenerative capability of neonatal mice hearts [17]. Together with transcription factors Mef2c and Tbx5 (the combination of the three factors is termed GMT), it was reported to play a role in cardiac reprogramming [18, 19]. In injured heart tissue, GATA4 was increasingly upregulated over 24h after r-I/R. Similarly to Caspase-3, its upregulation was limited to the infarcted area (Figure 2). The time-sequence of up regulation suggests that expression of pro-survival genes such as GATA4 follow an initial activation of pro-apoptotic genes such as caspase-3. In contrast, the expression level of the cardiac transcription factor Mef2c was found to be largely unchanged (Figure 2). This indicates that transcriptional activation of Mef2c is not playing a major role in SWOP induced by r-I/R.

Hexokinase-2 is an important enzyme in the glucose metabolism, namely the phosphorylation of glucose as one of the initiating steps of glycolysis. In addition, HK-2 has been shown to be implicated in oxidative stress and the production of ROS, and mitochondrial binding of HK-2 promotes cell survival. Decreased levels of HK-2 after r-I/R resulted in altered remodeling with higher rates of cell death and fibrosis and lower angiogenesis [20]. In mice, HK-2 knockdown exaggerated cardiac hypertrophy after induction of pressure overload [21]. In r-I/R pig hearts, spatial analyses of expression showed short-term up-regulation of HK-2 in the ischemic area, with a pronounced down regulation in some remote areas 5h after r-I/R and in all heart areas 24 h after r-I/R (Figure 3 and Supplementary Animations). The quick up regulation after ischemic injury may be indicative of an activation of pro-survival signaling, and the reduction in all heart areas after 24 h is likely to have an important impact of cell energy metabolism, but also ROS production, and cardiomyocyte survival, and indicates an onset of the molecular processes leading to cardiac remodeling.

The cytoprotective chaperone clusterin/apolipoprotein J (CLU) is produced and secreted in response to stress signals, and its plasma levels are increased in several disorders, including neurodegenerative diseases and neoplasms, but also atherosclerosis and myocardial infarction [22]. Interestingly, CLU was recently reported to be associated with survival in patients with heart failure [23]. In the myocardium, CLU protects against apoptosis, modulates matrix metalloproteinase expression and stimulates angiogenesis. In a complex with the proteohormon leptin, it binds to the leptin receptor, which results in transcriptional activation of intracellular pathways including the JAK/STAT pathway [24]. We encountered a marked increase in CLU expression already 5h after ischemic injury in the

affected tissue, and a less pronounced, but considerable elevation of expression in the remote zone 24h after r-I/R (Figure 4). These data are an indication that CLU plays a role in restoring cell function, cardioprotection and might be an important mediator of intrinsic remote conditioning.

The excision repair cross-complementation group 4 (ERCC4/XPF) gene is a subunit of the ERCC1/XPF endonuclease which has a function in repair of DNA damage [25]. It cleaves nucleic acids specifically at junctions between double- and single stranded DNA and is a component of the machinery for nucleotide excision repair, and others. It is taking part in stress response. We identified ERCC4 to be upregulated after r-I/R in an NGS dataset and our spatiotemporal analysis shows that the up regulation is focused on the tissue directly affected by ischemia (Figure 5). While a deficiency of ERCC1/ERCC4 has been linked to carcinogenesis and cancer progression, its role in the myocardium is currently unknown. The upregulation of ERCC4 might be a consequence of cell and nucleic acid damage.

Exploiting the 3D reconstruction technology of the NOGA endocardial (here epicardial) electroanatomical mapping system, we demonstrate a new and straightforward methodology with which to display gene expression patterns in 2D and 3D, without the need for an extensive bioinformatics background or training. The image-omics that we present of the ischemic preconditioned heart integrates genomic data with biomedical imaging, to facilitate exploration and visualization of relevant gene expression patterns that underlie the SWOP. These data provide biological insight into cardioprotective mechanisms, that are essential for better understanding of the complexity of I/R injury.

As reported earlier, the NGS-based analysis of global gene expression patterns in ischemic, border, and remote zones [2] showed distinct changes of several pathways and a number of genes that were previously not linked to ischemic preconditioning. The gene expression patterns show that a quick onset of regulatory response after r-I/R in the directly affected tissue with up regulation of genes involved with stress response and apoptosis. The more detailed spatial expression patterns reported here corroborate the NGS results and also highlight the role of intrinsic remote conditioning: that remote heart areas (unaffected by ischemia) can have varying expression levels of genes playing important roles in cardioprotection and prevention of adverse left ventricular remodeling. For

investigating mechanisms such as cardiac remodeling on the molecular level, it may thus be advisable to harvest tissue sections from a few spots of the remote zone. More importantly, we show that the NOGA system is particularly useful, yet very facile to use for constructing 2D and 3D representations of expression patterns. Since externally gathered values of biopsies that were taken with the system can be simply entered into the software, this approach can likewise be employed for any other readout, for example from histological analysis or from proteomics data.

Conclusion

Using 2D and 3D visualization (3D image-omics) of temporal and spatial gene expression maps of the heart, we demonstrate that r-I/R stimuli provoke distinct alterations in gene expression profiles in different regions of the myocardium. We employed a clinically relevant closed-chest pig model to highlight transcriptional regulations induced by r-I/R. The analysis of multiple tissue samples at several time points with the novel methodology described herein increases our understanding of r-I/R mechanisms. The reported data indicate a short term stress response, which is followed by prolonged expressional alterations, including transcriptional regulators and survival signals, which are essential for the second window of cardioprotection after ischemic preconditioning in the ischemia affected but also in the non-affected myocardial areas.

Material and Methods

Ethical Statement

Animal investigations were carried out in accordance with the "Position of the American Heart Association on Research Animal Use," as adopted by the AHA on November 11, 1984. The study was approved by the Ethics Committee on Animal Experimentation at the University of Kaposvar, Hungary. The study design is displayed in Figure 1A. The study corresponds to the ARRIVE guidelines [26].

Porcine Model of Ischemic Preconditioning

Domestic pigs (male, 15 kg, n=20, randomized into r-I/R[5h], n=6, r-I/R[24h], n=6, and sham operated controls, n=8) underwent cardiac catheterization under general anaesthesia. The r-I/R

protocol consisted of three repetitive cycles of 10 min I/R via percutaneous balloon occlusion and deflation in the mid left anterior descending coronary artery (LAD) as described previously [2].

Briefly, the pigs received an intramuscular injection of 12 mg/kg ketamine hydrochloride, 1 mg/kg xylazine and 0.04 mg/kg atropine, with inhalation anesthesia with isoflurane and O_2. After reaching deep anesthesia, pigs were intubated and the anaesthesia was continued with an anesthetic gas mixture of 1.5-2.5 vol% isoflurane, 1.6-1.8 vol% O_2 and 0.5 vol% N_2O. A 6F introduction sheath (Medtronic Inc, Minneapolis, MN) was placed into the right femoral artery followed by intra-arterial administration of unfractionated heparin (200 IU/kg). A 6F coronary catheter (Medtronic Inc, Minneapolis, MN) was placed into the abdominal aorta and selective angiography of the left coronary arteries was performed. A guidewire (Medtronic Inc, Minneapolis, MN) and then a coronary balloon dilation catheter (2.75 mm diameter, 12 mm length; Medtronic Inc, Minneapolis, MN), were placed into the left anterior descending coronary artery below the origin of the second diagonal branch. In the r-I/R groups (n=12), coronary occlusion was performed with 6 atm inflation pressure. Coronary angiography was done by injecting non-ionic contrast media (Takeda, Zürich, Switzerland) to monitor occlusion and reperfusion of the coronary artery.

At either 5 h or 24 h after the procedure (Figure 1), the animals were sacrificed and hearts were explanted. Myocardial tissue samples distributed equally throughout the entire left ventricle (LV) according to anatomical landmarks (52 samples of each heart) were obtained using a biopsy kit (Acu-Punch, Acuderm, Fort Lauderdale, FL).

In Vitro NOGA-mapping for image-omics.

To enable 2D and 3D displays of the changes in gene expression in the LV, the sampling locations were determined by *in vitro* surface NOGA mapping. The NOGA mapping principles and technique have been described previously [10]. Briefly, using an ultralow magnetic field and a NogaStar® catheter with a magnetic tip (Johnson & Johnson, Diamond Bar, California), the NOGA system displays the heart showing the location of the catheter tip in 3D, and measures the actual electrical signals. In contrast with the real-time use of this system to illustrate location of the catheter tip in relation with viability of that area, for *in vitro* mapping we used only the sampling locations (Figure

1B). Quantitative viability values of the recorded locations were manually replaced with values of fold changes in gene expressions.

Tissue sections were directly inferred to RNAlater and stored at -80°C until RNA isolation. RNA was isolated from the samples using column based extraction (RNeasy, Qiagen, Germany). RNA concentrations were determined using a Nanodrop spectrophotometer (Thermo Fisher) and 500 ng of each sample were reverse transcribed with random hexamer primers to cDNA (Qiagen). Gene expressions were quantified on an Applied Biosystems 7500 Real-Time PCR System(Life Technologies, USA) using Sybr Green (Qiagen) with primers listed in Table 1.

Abbreviations

AMI	acute myocardial infarction
CLU	clusterin
DPP4	dipeptidyl peptidase 4
EPO	erythropoetin
ERCC4	excision repair cross-complementation group 4
GATA4	transcription factor binding to nucleotide sequence GATA
GMT	GATA4, MEF2c and Tbx5
HIF-1α	hypoxia-inducible factor 1α
HK-2	hexokinase-2
JAK	Janus kinase
MEF2c	myocyte-specific enhancer factor 2C
r-I/R	repeated ischemia and reperfusion
RISC	reperfusion injury salvage kinase
ROS	reactive oxygen species
SAFE	survivor activating factor enhancement
STAT	signal transducer of activation and activator of transcription
VEGF	vascular endothelial growth factor

Author Contributions

N.P. and M.G. conceived the study and designed the experiments, N.P., D.Lu., K.Z., A.Z., D.P., K.A., R.G., and M.G. conducted the experiments N.P., D.Lu., K.Z., A.Z., D.Lo., G.G., J.W., D.P., K.A.,H.J.A., Z.G., T.B., M.S., A.J., M.Y.E., S.P.H., D.J.L., P.F., and M.G. analysed and interpreted data, G.M. interpreted data, N.P., J.W., and M.G. drafted the manuscript. All authors reviewed and approved the manuscript.

Conflicts of Interest: None.

Funding

This study was funded by the Ludwig Boltzmann Institute Cluster Cardiovascular Research, The Hungarian Scientific Research Fund (OTKA ANN 107803) and the Austrian Science Fund (KPIO1277FWF). Zoltan Giricz holds a „János Bolyai Fellowship" from the Hungarian Academy of Sciences. Péter Ferdinandy is a Szentágothai Fellow of the Hungarian National Program of Excellence (TAMOP 4.2.4.A/2-11-1-2012-0001). Derek J Hausenloy is funded by the British Heart Foundation (grant number FS/10/039/28270), the Rosetrees Trust, and is supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre.

References

1. Pavo N, Zimmermann M, Pils D, Mildner M, Petrasi Z, Petnehazy O, Fuzik J, Jakab A, Gabriel C, Sipos W, Maurer G, Gyongyosi M and Ankersmit HJ. Long-acting beneficial effect of percutaneously intramyocardially delivered secretome of apoptotic peripheral blood cells on porcine chronic ischemic left ventricular dysfunction. Biomaterials. 2014; 35(11):3541-3550.

2. Pavo N, Lukovic D, Zlabinger K, Zimba A, Lorant D, Goliasch G, Winkler J, Pils D, Auer K, Jan Ankersmit H, Giricz Z, Baranyai T, Sárközy M, Jakab A, Garamvölgyi R, Emmert MY, et al. Sequential activation of different pathway networks in ischemia-affected and non-affected myocardium, inducing intrinsic remote conditioning to prevent left ventricular remodeling. Scientific Reports. 2017; 7:43958.

3. Murry CE, Jennings RB and Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986; 74(5):1124-1136.

4. Bolli R. Preconditioning: a paradigm shift in the biology of myocardial ischemia. American journal of physiology Heart and circulatory physiology. 2007; 292(1):H19-H27.

5. Ferdinandy P, Schulz R and Baxter GF. Interaction of Cardiovascular Risk Factors with Myocardial Ischemia/Reperfusion Injury, Preconditioning, and Postconditioning. Pharmacol Rev. 2007; 59(4):418-458.

6. Heusch G. Molecular Basis of Cardioprotection Signal Transduction in Ischemic Pre-, Post-, and Remote Conditioning. Circ Res. 2015; 116(4):674-699.

7. Hausenloy DJ and Yellon DM. Ischaemic conditioning and reperfusion injury. Nat Rev Cardiol. 2016; 13(4):193-209.

8. Gajarsa JJ and Kloner RA. Left ventricular remodeling in the post-infarction heart: a review of cellular, molecular mechanisms, and therapeutic modalities. Heart Fail Rev. 2011; 16(1):13-21.

9. Gyöngyösi M, Winkler J, Ramos I, Do Q-T, Firat H, McDonald K, González A, Thum T, Díez J, Jaisser F, Pizard A and Zannad F. Myocardial fibrosis: biomedical research from bench to bedside. Eur J Heart Fail. 2017; 19(2):177-191.

10. Gyöngyösi M and Dib N. Diagnostic and prognostic value of 3D NOGA mapping in ischemic heart disease. Nat Rev Cardiol. 2011; 8(7):393-404.

11. Tekin D, Dursun AD and Xi L. Hypoxia inducible factor 1 (HIF-1) and cardioprotection. Acta Pharmacol Sin. 2010; 31(9):1085-1094.

12. Semenza GL. Hypoxia-Inducible Factor 1 and Cardiovascular Disease. Annu Rev Physiol. 2014; 76:39-56.

13. Kelly BD, Hackett SF, Hirota K, Oshima Y, Cai Z, Berg-Dixon S, Rowan A, Yan Z, Campochiaro PA and Semenza GL. Cell Type–Specific Regulation of Angiogenic Growth Factor Gene Expression and Induction of Angiogenesis in Nonischemic Tissue by a Constitutively Active Form of Hypoxia-Inducible Factor 1. Circ Res. 2003; 93(11):1074-1081.

14. Eckle T, Köhler D, Lehmann R, El Kasmi KC and Eltzschig HK. Hypoxia-Inducible Factor-1 Is Central to Cardioprotection. A New Paradigm for Ischemic Preconditioning. 2008; 118(2):166-175.

15. Agosto M, Azrin M, Singh K, Jaffe AS and Liang BT. Serum Caspase-3 p17 Fragment Is Elevated in Patients With ST-Segment Elevation Myocardial Infarction: A Novel Observation. J Am Coll Cardiol. 2011; 57(2):220-221.

16. Heineke J, Auger-Messier M, Xu J, Oka T, Sargent MA, York A, Klevitsky R, Vaikunth S, Duncan SA, Aronow BJ, Robbins J, Crombleholm TM and Molkentin JD. Cardiomyocyte GATA4 functions as a stress-responsive regulator of angiogenesis in the murine heart. The Journal of clinical investigation. 2007; 117(11):3198-3210.

17. Malek Mohammadi M, Kattih B, Grund A, Froese N, Korf-Klingebiel M, Gigina A, Schrameck U, Rudat C, Liang Q, Kispert A, Wollert KC, Bauersachs J and Heineke J. The transcription factor GATA4 promotes myocardial regeneration in neonatal mice. EMBO Mol Med. 2017.

18. Inagawa K, Miyamoto K, Yamakawa H, Muraoka N, Sadahiro T, Umei T, Wada R, Katsumata Y, Kaneda R, Nakade K, Kurihara C, Obata Y, Miyake K, Fukuda K and Ieda M. Induction of Cardiomyocyte-Like Cells in Infarct Hearts by Gene Transfer of Gata4, Mef2c, and Tbx5. Circ Res. 2012; 111(9):1147-1156.

19. Zhao Y, Londono P, Cao Y, Sharpe EJ, Proenza C, O'Rourke R, Jones KL, Jeong MY, Walker LA, Buttrick PM, McKinsey TA and Song K. High-efficiency reprogramming of fibroblasts

into cardiomyocytes requires suppression of pro-fibrotic signalling. Nature Communications. 2015; 6:8243.

20. Wu R, Smeele KM, Wyatt E, Ichikawa Y, Eerbeek O, Sun L, Chawla K, Hollmann MW, Nagpal V, Heikkinen S, Laakso M, Jujo K, Wasserstrom JA, Zuurbier CJ and Ardehali H. Reduction in Hexokinase II Levels Results in Decreased Cardiac Function and Altered Remodeling After Ischemia/Reperfusion Injury. Circ Res. 2011; 108(1):60-69.

21. Wu R, Wyatt E, Chawla K, Tran M, Ghanefar M, Laakso M, Epting CL and Ardehali H. Hexokinase II knockdown results in exaggerated cardiac hypertrophy via increased ROS production. EMBO Mol Med. 2012; 4(7):633-646.

22. Trougakos IP, Poulakou M, Stathatos M, Chalikia A, Melidonis A and Gonos ES. Serum levels of the senescence biomarker clusterin/apolipoprotein J increase significantly in diabetes type II and during development of coronary heart disease or at myocardial infarction. Exp Gerontol. 2002; 37(10–11):1175-1187.

23. Koller L, Richter B, Winter M-P, Sulzgruber P, Potolidis C, Liebhart F, Mörtl D, Berger R, Goliasch G, Lang I, Wojta J, Hülsmann M and Niessner A. Clusterin/apolipoprotein J is independently associated with survival in patients with chronic heart failure. J Clin Lipidol.

24. Byun K, Gil SY, Namkoong C, Youn BS, Huang H, Shin MS, Kang GM, Kim HK, Lee B, Kim YB and Kim MS. Clusterin/ApoJ enhances central leptin signaling through Lrp2-mediated endocytosis. EMBO reports. 2014; 15(7):801-808.

25. Manandhar M, Boulware KS and Wood RD. The ERCC1 and ERCC4 (XPF) genes and gene products. Gene. 2015; 569(2):153-161.

26. Kilkenny C, Browne WJ, Cuthill IC, Emerson M and Altman DG. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLOS Biology. 2010; 8(6):e1000412.

Gene	Forward primer	Reverse primer	Amplicon length (bp)
Caspase-3	GGGATTGAGACGGACAGTGG	TGAACCAGGATCCGTCCTTTG	136
clusterin	CATGAAGTTCTACGCGCGTG	AGTAGAAGGGGGAGCTCTGG	92
ERCC4	ATGGGAAGCACTGACCGAAG	GAACACGTCCTGTCGTCACT	114
GATA4	AGAAAACGGAAGCCCAAGAAC	CCACACTGCTGGAGTTGCTG	109
HK-2	CAGCAGAACAGCCTGGATGA	GGATGGCTTCCTTCAGCAGT	106
MEF2c	TAACATGCCGCCATCCGCCC	ATCCTCTCGGTCGCTGCCGT	151

Table 1. Primer sequences and amplicon lengths for qPCR.

Figure Legends

Figure 1. Principle of NOGA-guided imageomics

A. Timeline of the protocol and the three groups. Gene expression profiles of the whole LV was determined either without intervention (Group Control), or 5h (Group I/R-5h) or 24h (Group I/R-24h) after 3x10 min I/R by repetitive inflation/deflation of an intracoronary balloon placed in the mid part of the porcine left anterior descending coronary artery.

B. Schematic illustration of the image-omics of the gene expression maps. Using the in vivo mapping principles of the myocardial viability map, sampling locations (n=52) were detected in *in vitro* epicardial NOGA-mapping. The voltage values recorded by NOGA in the distinct locations were replaced by the respective values of fold changes in gene expressions gathered from the excised tissue samples.

Figure 2. Spatiotemporal 2D bulls-eye display of HIF-1α, caspase-3, GATA4 and myocyte enhancer factor 2C (MEF2c) gene expression of the entire left ventricle after repetitive ischemia/reperfusion (r-I/R).

Time-dependent presentation of the different gene expression patterns of HIF-1α, caspase-3, GATA4 and MEF2c of the LV of animals in groups control, I/R-5h and I/R-24h after repetitive (3 times) 10 min I/R without consecutive myocardial infarction. Temporary overexpression of HIF-1α and caspase-3 at 5h (red arrow), and mildly increasing upregulation of GATA-4 in the ischemic area at 5 and 24h (red arrows) were detected. Expression levels of HIF-1α and caspase-3 were reduced to baseline levels after 24h, except for part of the remote area with HIF-1α upregulation. No changes in MEFC2 in either the ischemic or the unaffected areas were encountered. Pink and blue colors represent up-regulation of genes; green represents baseline values, while yellow and red areas show down-regulation of the respective genes.

Figure 3. Image-omics (2D and 3D modeling) of the repetitive ischemia/reperfusion (I/R) induced gene expression pattern of hexokinase 2 (HK2).

Representative 3D (top row) and 2D bulls-eye maps (bottom row) of the left ventricle (LV) showing the expression patterns of HK-2. Marked upregulation of HK2 was found in the ischemia-affected apical myocardial region and the border zone with concomitant downregulation in the remote myocardial area (yellow arrow) at 5h. At 24h, HK2 expression was severely downregulated in all myocardial regions.

Figure 4. Image-omics (2D and 3D modeling) of the repetitive ischemia/reperfusion (I/R) induced gene expression pattern of clusterin (CLU).

Representative 3D (top row) and 2D bulls-eye maps (bottom row) of the left ventricle (LV) showing the expression pattern of CLU. Moderate up-regulation was detected at 5h (red arrow), with a higher degree of up-regulation in the ischemia-affected region at 24h (red arrow).

Figure 5. Image-omics (2D and 3D modeling) of the repetitive ischemia/reperfusion (I/R) induced gene expression pattern of excision repair cross-complementation group 4 (ERCC4).

Representative 3D (top row) and 2D bulls-eye maps (bottom row) of the left ventricle (LV) showing the expression pattern of ERCC4. Moderate up-regulation is shown at both 5h and 24h (red arrows), with little changes between the two time points.

Online Animations. Timely 3D display of the hexokinase 2 (HK2) gene expression profile of the whole heart - 3D image-omics model.

Timely 3D display of the hexokinase 2 (HK2), which is responsible for coupling extramitochondrial glycolysis to intramitochondrial oxidative phosphorylation and plays a key role in cellular energy metabolism, expression profile, of the whole heart before repetitive ischemia/reperfusion (r-I/R) (Group Control), at 5h (Group r-I/R[5h]) and at 24h follow-up (Group r-I/R[24h]) using the NOGA principles for 3D construction. Three dimensional

models were constructed by projecting gene expression fold changes of HK2 on NOGA anatomical mapping coordinates. A. Gene expression pattern of HK2 in Group Control. B. Gene expression pattern of HK2 5h after the r-I/R-stimulus. C. Gene expression pattern of HK2 24h after the r-I/R-stimulus. According to the color coding scheme, pink and blue color represent upregulation of the HK2 gene. Green color represents a baseline value, while yellow and red myocardial areas downregulate the HK2 gene.