The remote ischemic preconditioning stimulus modifies gene expression in mouse myocardium

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Background: We have recently demonstrated that remote ischemic preconditioning reduces ischemia-reperfusion injury in animal models. The mechanisms by which the remote ischemic preconditioning stimulus exerts its effect remain to be fully defined, and its effect on myocardial gene expression is unknown. We tested the hypothesis that remote ischemic preconditioning modifies myocardial gene expression immediately after the remote ischemic preconditioning stimulus (early phase) and 24 hours later (late phase).

Methods: Twenty male (C57BL/6) 10- to 12-week-old mice were randomized into 4 groups: group 1 (control, early phase; n = 5), group 2 (remote ischemic preconditioning, early phase; n = 5), group 3 (control, late phase; n = 5), and group 4 (remote ischemic preconditioning, late phase; n = 5). Groups 2 and 4 underwent remote ischemic preconditioning induced by 6 cycles of 4 minutes of occlusion and 4 minutes of reperfusion of the femoral artery. Groups 1 and 2 were killed 15 minutes after completion of sham procedure or remote ischemic preconditioning, and the hearts were removed and frozen in liquid nitrogen. Groups 3 and 4 were killed 24 hours after remote ischemic preconditioning, and the hearts were harvested in the same fashion. Gene expression was assessed by using the Affymetrix MG-430A chip (Affymetrix, Santa Clara, Calif).

Results: Data filtering ($P < .05$, analysis of variance) and hierarchic 2-way clustering identified significant differences in gene expression among the 4 groups. Genes involved in protection against oxidative stress (eg, Hdhsc, Prdx4, and Fabp4) and cytoprotection (Hsp73) were upregulated, whereas many proinflammatory genes (eg, Egr-1 and Dusp 1 and 6) were suppressed.

Conclusion: A simple remote ischemic preconditioning stimulus modifies myocardial gene expression by upregulating cardioprotective genes and suppressing genes potentially involved in the pathogenesis of ischemia-reperfusion injury.

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Ischemic preconditioning (IPC) is a potent innate mechanism of defense against ischemia-reperfusion (IR) injury. Both an early and a late phase of local ischemic IPC can be induced in mice, with a significant reduction in subsequent myocardial infarction size in response to prolonged ischemia.¹ Modification of myocardial gene expression has been described in murine models of local IPC,^{2,3} but there are no previous data examining genomic responses to IPC induced by a remote stimulus. It is now known that myocardial IPC can be induced as a result of brief ischemia of an organ or tissue remote from the heart. In previous rodent models remote IPC (rIPC) was induced by using renal and mesenteric ischemia,⁴ as well as infrarenal occlusion of the aorta.^{5,6} Although very effective at reducing myocardial infarction, these methods are not easily applicable experimentally or in the clinical arena. We have recently described a much simpler and noninvasive method of

Abbreviations and Acronyms

CNBP	= cellular nucleic acid-binding protein
CPB	= cardiopulmonary bypass
DUSP	= dual-specificity phosphatase
FABP	= fatty acid-binding protein
GAPDH	= glyceraldehyde-3-phosphate dehydrogenase
HADHSC	= L-3-hydroxyacyl-CoA dehydrogenase
HSP	= heat shock protein
IPC	= ischemic preconditioning
IR	= ischemia-reperfusion
KIF	= kinesin superfamily protein
PCR	= polymerase chain reaction
PDGFRB	= platelet-derived growth factor receptor β
PPAR	= peroxisome proliferator-activated receptor
rIPC	= remote ischemic preconditioning
ROS	= reactive oxygen species
SER	= sarcoendoplasmic reticulum

inducing rIPC using transient limb ischemia induced by tourniquets or a standard blood pressure cuff.^{7,8} This novel method prevented IR-induced endothelial dysfunction in human subjects⁷ and reduced the extent of myocardial infarction in a porcine model by 40%.⁸ It also renders significant protection against IR injury in murine models.^{9,10} More germane to cardiac surgery are our preliminary experimental data regarding the ability of rIPC to modify responses to cardiopulmonary bypass (CPB)-induced myocardial, neuronal, and lung injury¹¹ and our recent study showing that rIPC of the recipient reduces IR injury in the transplanted heart.¹² The mechanisms by which rIPC exerts its effects are unknown. We have recently demonstrated that this rIPC stimulus modifies inflammatory gene expression in circulating human leukocytes,¹³ but there are no data regarding potential myocyte genomic responses to the rIPC stimulus. In the present study we hypothesized that transient lower-limb ischemia produces significant changes in myocardial gene expression (ie, remotely from the site of ischemia).

Methods

We performed a cross-sectional study in inbred mice to identify myocardial responses to rIPC. The study protocol was approved by the Animal Care and Use Committee of Toronto General Hospital and was performed in accordance with the Guidelines of the Canadian Council for Animal Care. Twenty inbred (C57BL/6), male, 10- to 12-week-old mice weighing from 22.5 to 26 g (median, 24.1 g) were randomized into 4 groups. All mice were anesthetized at the same time with a single intraperitoneal injection of 90 mg/kg ketamine and 19 mg/kg xylazine (Rompun). All animals were placed supine on the same warming blanket to keep the body temperature constant. Group 1 (control, early phase; n =

5) and group 2 (rIPC, early phase; n = 5) were studied to evaluate the early effect of the rIPC stimulus. Group 3 (control, late phase; n = 5) and group 4 (rIPC, late phase; n = 5) were studied to evaluate the late effect of the rIPC stimulus. To that end, groups 2 and 4 underwent 6 cycles of 4 minutes of femoral artery occlusion and 4 minutes of reperfusion. Unilateral limb ischemia was achieved noninvasively by applying direct pressure to the femoral vessels at the inguinal level without skin transgression. Venous congestion was observed during occlusion, followed rapidly by brisk reactive hyperemia during reperfusion, with 3.5 $\times$ magnifying surgical glasses. The reproducibility and reliability of the method of mice lower-limb ischemia has been verified by means of pulse oxymetry modified for application in mice. The timing of this IPC protocol was chosen to reproduce a previously reported effective local IPC method, which was shown to reduce infarction size in a mouse by 75% when the 30-minute left anterior descending coronary artery occlusion was performed 10 minutes after the local IPC and by 48% when the left anterior descending coronary artery occlusion was performed 24 hours after the IPC.¹ Animals in groups 1 and 2 were killed 15 minutes after completion of the rIPC or sham procedure. The chest was opened promptly. The heart was removed and frozen in liquid nitrogen. There was no overt limb damage in the remaining 10 animals (groups 3 and 4), and they had uneventful recovery. Animals in groups 3 and 4 were killed 24 hours later immediately on induction of the same anesthesia, and the heart was harvested.

RNA Isolation

Total RNA was isolated from 20 heart samples with Trizol Reagent (GIBCO/BRL), according to the manufacturer's protocol. The quality of total RNA was assessed with the Agilent 2100 Bioanalyzer (version A.02.01S1232, Agilent Technologies). Only RNA with an optical density ratio of 1.99 to 2.0 at 260/280 was used.

Affymetrix GeneChip Hybridization and Staining

A total of 20 hybridizations were performed on the MG-430A mouse GeneChip set (Affymetrix) with the 20 total RNAs from heart samples of 5 mice from each of the 4 different groups (5 early-phase control, 5 early-phase rIPC, 5 late-phase control, and 5 late-phase rIPC). Samples were prepared for hybridization according to standard Affymetrix instructions and performed at the Genomic core center at the Hospital for Sick Children. Briefly, a primer encoding the T7 RNA polymerase promoter linked to oligo-dT₁₇ was used to prime double-stranded cDNA synthesis from each mRNA sample by using Superscript II RNase H reverse transcriptase (Life Technologies). Each purified (Qiaquick kit, Qiagen) double-stranded cDNA was transcribed in vitro by using T7 RNA polymerase (T7 kit, Enzo Biochemicals), incorporating biotin-UTP (Enzo) into the cRNAs followed by purification with RNEasy (Qiagen), and quantitated by measuring absorption at 260 nm/280 nm. Samples were fragmented and hybridized to the GeneChip for 16 hours at 45°C and scanned (GeneArray scanner, Affymetrix). MicroArray Suite Version 5 (Affymetrix) was used to scale intensities across the GeneChip sets to 150 fluorescence units and to determine expression values for each gene on the chip. The expression value for each gene was determined by calculating the

Figure 1. Hierarchic clustering of differentially expressed genes in control animals and after remote ischemic preconditioning (IPC). Data filtering and 2-way hierarchic cluster analysis identified 166 genes that were differentially expressed. Gene expression levels are depicted as color variation from red (high expression) to blue (low expression). The color in each cell of the figure displays the level of expression for each gene (columns) in the myocardium of each individual animal (row).

average of differences (perfect match intensity minus mismatch intensity) of the probe pairs in use for the gene.

Experimental design, gene lists, hierarchic trees, chip hybridization, and statistical analysis were done in compliance with the Minimum Information About a Microarray Experiment guidelines.¹⁴

Affymetrix GeneChip Data Analysis

Gene analysis software. Data analysis was performed with GeneSpring (Silicon Genetics).

Data analysis. Scanned raw data were processed with Affymetrix Microarray Suite version 5.0 software. The average intensity value for each probe set, which directly correlates with mRNA abundance, was calculated as an average of fluorescence differences for each perfectly matched versus single nucleotide-mismatched probe. To test the integrity of the starting RNA, we examined the signal intensity ratio for the 3' probe set over the 5' probe set for the housekeeping genes β -actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). For the 13 arrays used in this study, the 3'/5' ratios were 1.3 ± 0.07 and 0.97 ± 0.06 for β -actin and GAPDH, respectively. Once sample quality was demonstrated, those genes with consistently present calls were considered. Data obtained from MAS 5.0 absolute analyses of all the individual arrays were analyzed and clustered with GeneSpring to monitor the expression of chosen genes over the different experimental time points (<http://www.silicongenetics.com>).

GeneSpring 6.1 was then used for normalization, each sample was normalized to its control (untreated), and then genes were filtered first on the basis of a *P* value of less than .05, 1-way analysis of variance (not equal variance) was performed on these genes, and finally 1.5-fold upregulated or downregulated genes in the rIPC groups versus the control groups generated 166 genes, which were used for hierarchic clustering (Figure 1). Each sample

was analyzed individually, and none of the methods was applied to pooled data. Global normalization was performed, and all intensities across the GeneChips were scaled. In addition to global normalization, we normalized each sample to its control to remove the biologic variation.

Microarray Validation

The microarray results were confirmed by using real-time quantitative polymerase chain reaction (PCR) on 4 randomly selected genes that demonstrated significantly altered gene expression. Predesigned FAM-labeled TaqMan primer sets for mice were ordered against the following: dual-specificity phosphatase (DUSP) 1 (Mm00457274_m1), heat shock 70-kd protein 5 (Mm00517691_m1), peroxiredoxin 4 (Mm_00450261_m1), and L-3-hydroxyacyl-coenzyme A dehydrogenase (Mm_00492535_m1; Applied Biosystems).

Amplicon abundance was determined in real time normalized against a GAPDH control. Fold changes were determined as a ratio of sample biopsy RNA to that of the average RNA expression in preischemic biopsy specimens.

Total cDNA was synthesized in a 2-step reverse transcriptase-PCR protocol by using Superscript First-Strand Synthesis System for reverse transcriptase-PCR (Invitrogen catalog no. 11904-018). RNA (1.5 μ g) was added to 1 μ L of 10 mmol/L deoxyribonucleoside triphosphate mix and Oligo(dT)₁₂₋₁₈ (0.5 μ g/ μ L) up to a 10- μ L volume with water treated with diethylpyrocarbonate. Primer annealing was initiated by heating the reaction mix to 65°C for 5 minutes and cooling to 4°C, after which 9 μ L of 10 $\times$ RT Buffer, 25 mmol/L MgCl₂, 0.1 mol/L dithiothreitol, and RNase Out was added and incubated at 42°C for 2 minutes. The final step was initiated by adding 1 μ L of SuperScript II RT and allowed to run for 50 minutes at 42°C.

Quantitative real-time PCR was performed on 3.6 ng of total cDNA per well. For each gene of interest, TaqMan Assay-on-

TABLE 1. Myocardial gene expression patterns after remote ischemic preconditioning

GenBank no.	Affymetrix no.	Gene name	Gene symbol	Group 1 Control, early	Group 2 IPC, early	Group 3 Control, late	Group 4 IPC, late
Immediate early response genes							
NM_007913	1417065	Early growth response 1	Egr-1	0.74 ± 0.32	0.76 ± 0.16	2.82 ± 0.98	1.15 ± 0.17
AV299745	1437850	Cellular nucleic acid-binding protein	Cnbp	0.71 ± 0.16	1.37 ± 0.15	0.85 ± 0.18	1.42 ± 0.39
Phosphatase genes							
NM_013642	1448830	Dual-specificity phosphatase 1	Dusp1	0.88 ± 0.21	0.78 ± 0.16	2.4 ± 0.45	1.14 ± 0.34
NM_026268	1415834	Dual-specificity phosphatase 6	Dusp6	0.69 ± 0.19	0.89 ± 0.18	1.67 ± 0.2	1.25 ± 0.2
BB816770	1456462	Protein phosphatase 1, cb	Ppp1cb	0.81 ± 0.21	1.16 ± 0.3	0.72 ± 0.18	1.35 ± 0.19
Heat shock protein genes							
NM_022310	1416064	Heat shock 70-kd protein 5	Hspa5,Grp78	0.96 ± 0.16	0.75 ± 0.14	2.56 ± 0.87	1.23 ± 0.63
AK010861	1423151	DnaJ (Hsp40) homolog	Dnajb11	0.92 ± 0.16	0.97 ± 0.2	2.96 ± 0.66	1.15 ± 0.33
BC006722	1420622	Heat shock 70-kd protein 8	Hspa8,Hsp73	0.75 ± 0.15	1.77 ± 0.39	0.82 ± 0.23	1.13 ± 0.4
Oxidative stress response genes							
BF319868	1423423	Glucose-regulated protein 58	Grp58	1.04 ± 0.16	0.85 ± 0.14	2.28 ± 0.59	1.16 ± 0.55
NM_016764	1416166	Peroxiredoxin 4	Prdx4	0.72 ± 0.4	1.33 ± 0.39	0.74 ± 0.45	1.4 ± 0.41
BB114220	1436756	L-3-hydroxyacyl-CoA dehydrogenase	Hadhsc	0.4 ± 0.27	1.41 ± 0.2	0.49 ± 0.37	1.35 ± 0.27
NM_00880	1417148	Platelet-derived growth factor receptor	Pdgfrb	1.21 ± 0.35	0.59 ± 0.18	1.08 ± 0.2	0.87 ± 0.25
BC002148	1451263	Fatty acid-binding protein 4	Fabp4	0.67 ± 0.16	1.29 ± 0.18	0.77 ± 0.17	1.36 ± 0.22
Mitochondrial function gene							
AK014590	1418428	Kinesin family member 5B	Kif5b	0.75 ± 0.24	1.53 ± 0.36	0.75 ± 0.26	1.39 ± 0.33
TNF signaling gene							
NM_013749	1418572	TNF receptor 12	Tnfrsf12a	1.86 ± 0.28	1.33 ± 0.8	1.28 ± 0.45	0.62 ± 0.32
Genes with unknown function							
BB483357	1438651	Angiotensin receptor-like 1	Agtr1	0.71 ± 0.25	0.54 ± 0.18	2.14 ± 0.35	1.38 ± 0.58
AK014338	1428112	Arginine rich	Armet	0.98 ± 0.16	0.79 ± 0.15	6.13 ± 2.4	2.25 ± 1.9
AK018378	1423420	Adrenergic receptor, β 1	Adrb1	0.6 ± 0.26	1.14 ± 0.24	1.04 ± 0.52	1.21 ± 0.41
NM_02638	1416187	DNA segment, Chr4	D4Bwg05	0.68 ± 0.17	1.46 ± 0.2	0.68 ± 0.18	1.46 ± 0.23

IPC, Ischemic preconditioning; CoA, coenzyme A; TNF, tumor necrosis factor.

Demand (Applied Biosystems catalog no. 4331182) was mixed with TaqMan Master Mix, and 15 μ L was loaded per well to a final reaction volume of 25 μ L. Each sample was run in triplicate on the ABI Prism 7000 detection system (Applied Biosystems). Calculation of relative quantities of the various products was determined by using the $\Delta\Delta$ CT method (<http://docs.appliedbiosystems.com/pebi docs/04303859.pdf>) relative to human GAPDH.

Results

General unsupervised clustering of global gene expression in the 20 mice demonstrated clear delineation of each of the 4 groups (Figure 1). Data filtering ($P < .05$, analysis of variance) and hierarchic 2-way clustering identified significant changes in the expression of 166 genes. The quantitative PCR confirmed the microarray results of all 4 randomly selected genes. The changes in expression of 19 genes with the greatest fold change between at least 2 groups are listed in Table 1. It is important to note that the expression of some genes changed significantly after intra-peritoneal injection of anesthetic and the associated stress of anesthesia. For example, gene expression of Egr-1, a gene that has a central role in the pathogenesis of ischemic damage, increased 4-fold in the control group (group 3) 24

hours after anesthesia-induced stress. Conversely, although there was no difference in Egr-1 expression 15 minutes after rIPC compared with control (group 1 vs group 2), Egr-1 gene expression was significantly lower 24 hours after rIPC (group 3 vs group 4, Table 1). There was also significant differential expression of the genes involved in response to oxidative stress, inflammation, and mitochondrial function.

Discussion

This study shows that transient limb ischemia modifies genomic responses in remote organs and, specifically, modifies genes responsible for myocardial responses to inflammatory or oxidation-induced stress. The results of our study are consistent with the recent findings of Li and colleagues,¹⁰ who detected nuclear translocation of nuclear factor κ B in both heart and preconditioned skeletal muscle after transient hind-limb ischemia and demonstrated that gene expression is essential for myocardial protection in mice. The potential relevance of some of the observed genomic responses to the ischemic protection rendered by the rIPC stimulus in coronary ischemic syndromes and the IR injury of cardiac surgery is discussed below.

Immediately Early Response Genes

The Egr-1 gene is of particular interest because it is a master switch coordinating upregulation of divergent gene families underlying ischemic stress.¹⁵ Growth factors and cytokines, including platelet-derived growth factor, angiotensin II, and tumor necrosis factor, increase Egr-1 gene expression within 15 minutes.¹⁶ Egr-1, in turn, activates transcription of several genes involved in the pathogenesis of ischemic tissue damage.¹⁵ We observed a 4-fold increase in Egr-1 gene expression 24 hours after intraperitoneal injection of anesthetic. Bearing in mind that anesthesia is a relatively minor stress, our results are consistent with the observations of others. For example, a 3-fold increase in Egr-1 gene expression was observed in the ischemic lungs of wild-type mice within 15 minutes, increasing 10-fold by 1 hour of ischemia and 20-fold after 3 hours of reperfusion.¹⁵ Interestingly, rIPC diminished Egr-1 upregulation by approximately 2.5-fold (Table 1). We have previously demonstrated significant upregulation of myocardial Egr-1 during cardiac ischemia in neonates compared with that seen in older children.¹⁷ We speculated that this in part might be one of the factors contributing to increased vulnerability of neonatal myocardium to IR.¹⁸ The current data are also consistent with the previously observed association of Egr-1 induction and activation with renal and cardiac ischemia. Furthermore, lung IR injury, as assessed on the basis of fibrin deposition, leukocyte accumulation, and vascular permeability, is significantly diminished in Egr-1-null mice compared with wild-type mice.¹⁶ Indeed, it was previously suggested, in view of the relatively intact phenotype of Egr-1-null mice, that short-term antagonism of Egr-1 might provide a novel therapeutic target to diminish maladaptive host responses incited by acute ischemia.¹⁶ However, this aim might be achieved primarily by rIPC, which significantly decreased upregulation of Egr-1 gene expression at 24 hours.

We also observed rapid upregulation of cellular nucleic acid-binding protein (CNBP) by rIPC. Its striking conservation in phylogenically diverse organisms suggests that CNBP plays a fundamental biologic role across different species. Consistent with its function as a transcription factor, CNBP protein is located in the nucleus of cells. The physiologic properties of the CNBP gene are unknown.¹⁹ However, it was recently reported that overexpression of CNBP strongly stimulates cell proliferation in mice.¹⁹

Phosphatases

Phosphatases, including protein phosphatases and DUSPs, are key elements in cellular responses to IR injury.^{20,21} The precise role of DUSPs in IR injury, however, is not defined. Nonetheless, it was recently demonstrated that gene expression of DUSP-1 (MKP-1) increased 12-fold in patients immediately after CPB and cardioplegic arrest,²¹ suggesting

involvement of DUSP-1 in CPB-induced systemic inflammatory response. Although DUSP-1 gene expression is upregulated in response to hypoxia and IR,²¹ it might also have some protective properties.²² In contrast, DUSP-6 (MKP-3) is known to mediate dephosphorylation of extracellular signal-regulated kinases 1 and 2, which in turn leads to the degradation of Bcl-2 and increased apoptosis.²³ In the present study DUSP-1 gene expression increased almost 3-fold in response to the anesthesia-induced stress in mouse myocardium, and rIPC significantly attenuated the extent of this upregulation. A similar trend was observed for DUSP-6. Furthermore, myocardial protein phosphatase 1 gene expression was unchanged in control animals but was upregulated after rIPC (Table 1).

Heat Shock Protein Genes

Heat shock 70-kd protein 5 (Hspa 5, Grp78) and protein 8 (Hspa 8, Hsp 73) are 2 important members of the heat shock protein (HSP) 70 family. Involvement of HSP70 gene expression in murine^{2,24} and canine²⁵ hearts by local IPC has previously been demonstrated. In the present study, both Grp78 and Hsp 40 (Dnajb11) were upregulated in response to anesthesia at 24 hours, but much less so after rIPC (Table 1). In contrast, HSP73 was significantly upregulated 15 minutes after rIPC and remained upregulated at 24 hours. Thus our data demonstrate differential expression of members of the HSP70 family by rIPC and are compatible with the local IPC gene expression studies performed by Sergeev and associates²⁶ in rat hearts using similar Affymetrix microarray methodology. The latter study of local IPC revealed trigger-dependent differential transcription of HSPs²⁶ and demonstrated that both remote and local IPC can stimulate expression of different members of the HSP70 family.

Oxidative Stress Response Genes

The expression pattern of several genes involved in the oxidative stress response was modified by rIPC. The third step in the mitochondrial β -oxidation spiral of short-chain fatty acids is catalyzed by short-chain L-3-hydroxyacyl-CoA dehydrogenase (HADHSC). Patients with HADHSC deficiency have defective fatty acid oxidation. HADHSC gene expression was increased more than 3-fold by rIPC and remained at this level at 24 hours (Table 1). This might be a mechanism by which intracellular energy production is preserved during a subsequent IR insult. Peroxiredoxins are potent antioxidants evolved to protect cells from damage by reactive oxygen species (ROS) after IR injury.²⁷ Peroxiredoxin 4 is a secretable protein and might exert its protective function against oxidative damage by scavenging ROS in the extracellular space. We observed rapid upregulation of peroxiredoxin 4 gene expression that persisted for 24 hours after rIPC. Intracellular protection against ROS is mediated

in part by glucose-regulated protein 58 (also known as Erp57), which is a luminal protein of the sarcoendoplasmic reticulum (SER). Glucose-regulated protein 58 modulates the redox state of SER, providing control of SER Ca^{2+} homeostasis.²⁸ Remote IPC reduced the upregulation in response to general anesthesia seen in the control animals at 24 hours. Similarly, platelet-derived growth factor receptor β (PDGFRB) is an important oxidative molecule with serine-threonine kinase activity, which is postulated to play a critical role in the pathogenesis of atherosclerosis.²⁹ Inhibition of PDGFRB results in significant reduction of oxidative stress. Flavonoids of the catechin family, which are found, for example, in red wine, are potent inhibitors of PDGFRB signaling and have been implicated in slowing progression of oxidative endothelial damage.²⁹ We demonstrated, for the first time, that rIPC rapidly suppresses PDGFRB gene expression (2-fold in 15 minutes), and it remains low at 24 hours (Table 1).

Fatty acid-binding protein (FABP) 4 binds fatty acids and transports them to the nucleus, where the FABP4–fatty acid complex activates peroxisome proliferator-activated receptor (PPAR) γ . PPAR γ is a transcription factor that regulates genes involved in lipid and glucose metabolism. Activation of PPAR γ gene expression after local IPC was observed in rat hearts.² FABP4 appears to play an important role in cardiovascular diseases. Its rapid increase after IR injury has been recently used as a prognostic marker.³⁰ In our study FABP4 gene expression was increased 2-fold by the rIPC both early and 24 hours later.

Mitochondrial Locomotion Gene

The kinesin superfamily proteins (KIFs) have been shown to transport organelles, protein complexes, and mRNA to their specific intracellular destinations. Conventional kinesin (KIF5) is a tetramer of 2 kinesin heavy chains and 2 kinesin light chains. Kinesin heavy chains form a structurally and functionally closely related family (KIF5A, KIF5B, and KIF5C). KIF5B is expressed ubiquitously throughout many tissues, whereas KIF5A and KIF5C are specific to nerve tissue. Mouse kinesin family member 5B (KIF5B) is associated with mitochondria. KIF5B-null mice are embryonically lethal, with severe growth retardation at 10 days after conception.³¹ Mitochondria of extraembryonic KIF5B-null mutant cells are abnormally clustered in the perinuclear region.³¹ Similar abnormal perinuclear clustering of mitochondria from an evenly spread distribution throughout the cytoplasm is caused by tumor necrosis factor α in normal cells.³² It was recently shown that activation of tumor necrosis factor receptor 1 results in hyperphosphorylation of kinesin light chains and inhibition of kinesin activity.³² Although there was no detectable change in KIF5B expression in control animals, the KIF5B gene expression doubled

15 minutes after rIPC and remained high in rIPC animals 24 hours later.

Conclusion

In summary, transient limb ischemia induces important global myocardial genomic responses that are compatible with increased resistance to ischemic and oxidative stress both early and late after the stimulus. This study was not designed to describe the nature of the signaling of the rIPC stimulus but provides data on which further studies can be based. Subsequent studies must also address the functional implications of these genomic responses, both at a transcriptional and proteomic level. Nonetheless, this simple method of inducing IPC remotely will facilitate future investigations of this potentially clinically valuable technique.

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