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Late ischemic preconditioning of the myocardium alters the expression of genes involved in inflammatory response

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Abstract Myocardial ischemic preconditioning (IPC) is a potent endogenous mechanism of cardioprotection against ischemia–reperfusion injury. In this study we focused on the second phase of IPC as the most interesting in terms of therapeutic implementations. We aimed at the detection of genes, which are differentially expressed at 16 h after reperfusion. Preconditioning of canine myocardium was initiated by 5 min occlusion of the left anterior descending coronary artery with subsequent reperfusion. cDNA representational difference analysis in combination with microarray hybridization and reverse transcription polymerase chain reaction were used to reveal the changes in gene expression in canine hearts. We found that functionally related genes for tristetraproline (TTP), selectin E, matrix metalloproteinase 9, and tumor necrosis factor-α were highly upregulated at the late phase of IPC. The upregulation of TTP gene at the late phase of IPC, reported here for the first time, may represent a cardioprotective mechanism, which could be a promising perspective in clinical interventions against ischemia–reperfusion injuries of the heart.
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1. Introduction

Preconditioning of the myocardium with short periods of sublethal ischemia and intermittent reperfusion renders it more resistant to a subsequent potentially lethal ischemic insult [1]. The immediate protective effect of myocardial ischemic preconditioning (IPC) is transient and is lost over the ensuing 1–2 h. However, the initial preconditioning stimulus also initiates a second window of protection (SWOP) that becomes apparent 12–24 h later and lasts for 3–4 days [2,3]. Unlike early IPC, the SWOP protects not only against myocardial infarction but also against myocardial stunning [3,4]. Because of this, and because of its sustained duration, considerable interest has recently focused on the SWOP and on its clinical relevance. Once the mechanism of this adaptive alteration is elucidated, it should be possible to exploit it to protect the ischemic myocardium in patients [5].

The mechanism of SWOP is not clear in detail, but may involve a complex cascade of cellular events. First, myocardial ischemia and subsequent reperfusion induce major metabolic perturbations, which result in a production of chemical signals, such as nitric oxide, reactive oxygen species, adenosine, etc., triggering the development of IPC [3]. These initiators cause an increase in the rate of myocardial protein synthesis. Whereas in early IPC existing proteins are activated, late phase requires increased de novo protein synthesis [6]. So far, attention has focused on a small number of protein products as potential distal mediators of delayed preconditioning. These include heat shock proteins, inducible nitric oxide synthase, aldose reductase, ATP-sensitive potassium channel, cyclo-oxygenase 2, and antioxidant enzymes [3]. The progression of IPC also involves activation of different transcription-regulatory mechanisms (kinase signaling pathways/transcription factors) and posttranscriptional modulators [7].

It is now evident that SWOP is a complex multi-factor process, which probably includes the alteration of many genes at the RNA level [3]. We used cDNA representational difference analysis (RDA) to make an inventory of genes up- or downregulated at the different phases of IPC in canine hearts. The RDA technique offers a means for the identification of candidate genes in a highly specific manner. In the present study, we focused on differential gene expression during the late phase of preconditioning. The ultimate goal of our work was to reveal the genes that may present an interest as potential pharmacological targets for clinical implementations.

2. Materials and methods

2.1. Materials

IPC was simulated in anesthetized, open-chest dogs. The animals were subjected to 5 min of coronary occlusion of the left anterior descending coronary artery perfusing the anterior wall followed either by 4, 8, or 16 h of reperfusion. In the canine model, even a shorter (90 s to 2.5 min), single occlusion is capable of preconditioning the heart [8–10]. Dogs in a second, control group were sham-operated, but not subjected to coronary occlusion. During the experiments, systemic hemodynamics was measured regularly. In addition, arterial blood gases and hematocrit were monitored regularly. Body temperature, which is a major factor for myocardial contractile function, was

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measured periodically, and it was maintained between 37 and 38°C with the aid of a heating/cooling table. Following the experimental protocol, transmural blocks of myocardial tissue from the center of the anterior wall and the posterior control wall were taken and immediately frozen at −80°C.

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Isolation of RNA from heart tissue and cDNA synthesis

Total RNA from shock-frozen left ventricular myocardium was extracted using the standard guanidinium thiocyanate method, followed by centrifugation in a cesium chloride gradient (Pharmacia LKB, Freiburg, Germany). Poly(A^+) RNA was isolated by using oligo(dT) coupled to magnetic beads (Dynabeads, Dynal, Norway) according to the manufacturer's instructions. To overcome individual alterations in gene expression, mRNA samples from three animals of the same experimental groups were pooled. Double-stranded cDNA was synthesized from mRNA by oligo(dT) priming using the Super Choice cDNA synthesis kit (Invitrogen) as recommended by the manufacturer.

2.3. RDA experiments

RDA was carried out by the protocol of Hubank and Schatz [11] with slight modifications. Representations were generated after digestion of 1.5 μg double-stranded cDNA with *Dpn*II restriction enzyme, ligation of R-Bam adapters (14°C, 12 h) and amplification using R-Bam-24 as a primer (5 min at 72°C; 20 cycles of 1 min at 95°C and 3 min at 72°C). Purifications were always done with Qiaex spin columns (Qiagen, Germany); difference products (DP) 1–3 were generated following a standard protocol [11] with aliquots analyzed by gel electrophoresis (compare Fig. 1). For generation of the driver 300 μg of control representation was digested with 750 U of *Dpn*II. For generation of the tester 10 μg of representation was digested with 50 U of *Dpn*II and ligated to J-Bgl-24 using J-Bgl-12 as adapter oligonucleotide.

First subtractive hybridization was done with 40 μg of digested driver and 400 ng of tester (1:100 ratio). Subsequent polymerase chain reactions (PCRs) were done as described in [11] using J-Bgl-24 as primer, resulting in DP1. Adapters were removed by complete digestion with *Dpn*II before N-Bgl oligomers were ligated.

In subsequent steps the adapters were changed before each round of RDA (N-Bgl-12/24 for second and J-Bgl-12/24 for third round again) and using the corresponding 24-mers as PCR primers. Further rounds of subtraction hybridization and amplification were done in a similar manner with tester to driver ratios of 1:800 at the second round and 1:400 000 at the third round of RDA.

Determination of DNA concentration was done by fluorometry. Cloning of DP3 was in the pGEM-T Easy vector system in *Escherichia coli* DH10b cells. Individual colonies from the subtracted libraries were picked into 384-well liquid cultures. Sequencing of the clones followed standard procedures for the automated sequencer (Applied Biosystems, USA). BLASTN and BLASTX analyses were used to screen for nucleotide and protein homologies of the sequences identified [12].

2.4. Microarraying of RDA products

Arrays representing the DP3 clones were produced for back-hybridization with individual clones to reduce the redundancy within this library and for back-hybridization with representations of the cDNA populations. Plasmid inserts of all picked clones were PCR-amplified using DP3-specific RDA primer J-Bgl-24. PCR was performed by adding 0.5 μl saturated growth liquid culture to 100 μl PCR reactions containing 10 mM Tris (pH 9.0), 50 mM KCl, 150 μM dNTP, 3 M betain, 30 mM cresol red, and 2.0 U Taq DNA polymerase (MBI Fermentas, Germany) in 96-well plates. Thermal cycling conditions consisted of an initial denaturation at 95°C for 3 min, followed by 35 cycles of 95°C for 1 min and 72°C for 3 min, with a final 10 min extension at 72°C in PTC100 thermal cycler (MJ Research). Five microliters of each PCR were examined on an agarose gel. Each PCR product was purified by ethanol precipitation. Subsequent spotting on custom polylysine slides was carried out with ESI robotic spotter (Engineering Services) using betain spotting solution according to the protocol described by Diehl et al. [13]. PCR products of individual clones or RDA amplicons (1 μg each) were fluorescently labeled with either Cy3 or Cy5 dye. Maximum incorporation of fluorescent nucleotide was accomplished with 100 μM dGTP, dTTP and dATP and 10 μM unlabeled dCTP: dUTP-Cy3 or dUTP-Cy5 (Amersham) was added to a final concentration of 20 μM and incorporated into DNA using random primer labeling as per the manufacturer's recommendations (MBI Fermentas). Labeled RDA probes were purified through Qiagen columns (Qiagen, Germany), dried in a Speed-Vac, and resuspended in a total volume of 16 μl 3×sodium saline citrate (SSC), 1% sodium dodecyl sulfate (SDS), 5×Denhardt's solution, 100 μg/ml sheared salmon sperm DNA, 50% formamide and 10% dextran sulfate. The probe was denatured in the hybridization solution at 80°C for 10 min and applied to the arrayed/denatured slide at 42°C for 12 h in a humidified chamber (Telechem). Hybridized slides were washed in 2×SSC, 0.1% SDS for 5 min at room temperature, and in 0.2×SSC for 5 min prior to scanning by laser ScanArray5000 fluorometer (GSI Lumonics). For comparative hybridizations with the cDNA representations separate images with 10 μm per pixel resolution were captured for each of two fluorophores used. GenePix ver. 3.0 (Axon Instruments) software was used to quantitate the signal at each spot. Signal normalization was performed by using internal standards (canine cyclophilin A and β-actin genes) and external controls prepared from the custom set of non-cross-hybridizing *Arabidopsis thaliana* genomic DNA clones.

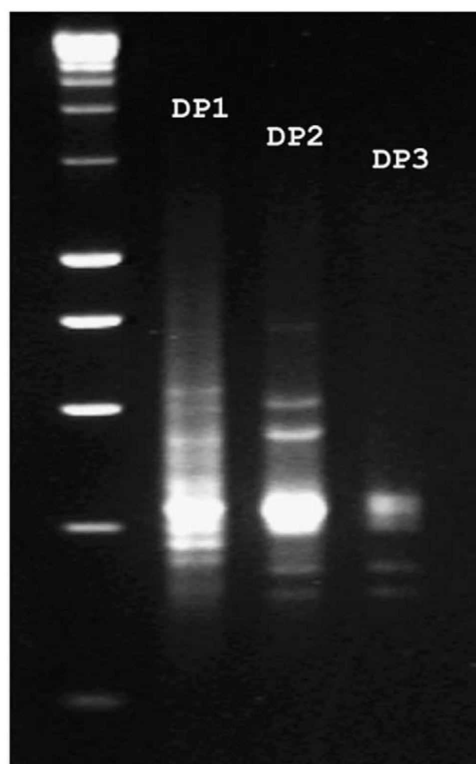

Fig. 1. Comparison of the three DPs of the RDA procedure on 2.5% agarose gel. Leftmost lane is GeneRuler™ DNA Ladder marker (MBI Fermentas) with 100 bp increments (size of the lower band is 100 bp). Whereas in DP1 a relatively complex DNA can be seen, a reduction of complexity towards few bands in DP2 and DP3 becomes visible.

2.5. Reverse transcription (RT)-PCR

cDNA samples synthesized from the starting RNA were used as templates for RT-PCR reactions. Amplification was performed in 1×PCR buffer (50 mM KCl, 10 mM Tris–HCl, 0.1% Triton X-100), with a total reaction volume of 50 μl. This contained final concentrations of 200 μM of each dNTP, 2.5 mM of $MgCl_2$, 500 μM of each primer, and 1.5 U/μl of Taq DNA polymerase (MBI Fermentas). The structures of the primers for the amplification of the genes found among RDA difference products were derived from the DNA sequences obtained by us. The sequences of canine tumor necrosis factor-α (TNF-α) and cyclophilin A genes were extracted from the EMBL nucleotide database. Primer pairs were designed using Primer3

Table 1
Sequences of the primers used for RT-PCR

Molecule	Forward 5′→3′	Reverse 5′→3′	Product length (bp)
Cyclophylin A	agagaagggattcggttacaaag	atggtgatcttcttgctggtct	348
Selectin E	gttagaacagggattcggagatt	acagagagcactcctctggttc	265
TTP	ctggtggagagtccaagctgt	ggcagagttcggtcttgtactt	262
TNF-α	atcatcttctcgaaccccaag	ggttgacctttgtctggtagga	272
MMP9	ccccttccttatcaccgacac	gatccacggtctgcgtcttc	275
SAA	tgacatgagagaagccaactaca	agagccaagaggaagctcagt	290
Cysteine dioxygenase	ggaggtgaacgtagaggaggt	acgtgacagccagacagaagt	347
Metastasis-associated gene (Mta1)	gaagctgtgctccggtaccta	cccctactgggcatcaagag	213
Phosphoglucomutase-related protein (PGMRP)	cctccattcaagtgatgacg	tgtatgcagagagctatgagagg	150
Amphiregulin (AR)	ggaagaagaagaatccctgtg	tttcgttcttcagcttctcct	301
Collagen type IV α 1 chain (COL4A1)	tgtatgcagagagctatgagagg	cctccattcaagtgatgacg	150
RNA polymerase II subunit	agtgcagaccacaccagactac	ccttgaagcacccagtacaa	343
Iroquois homeobox protein 3 (Irx3)	cctcaagaaggagaacaagatga	acttcataaagggtcgggaga	259
Mutant desmin (MUTDESM)	gaggagtggtacaagtcgaagg	tgtcaacatctgtgtgctgct	202
TIS21	gacccctatgaggtgtcctac	ggagactgccatcacatagttt	159
Ran binding protein 13 (RANBP13)	ccaacttggatgctgtgtct	ttccttcaccatctccttcac	314
Helix-loop-helix protein (Id-2)	tccaaataggagattgcgttg	tctgtggattcgttgttgttg	284

software [14]. The record of primers used in the experiments is given in Table 1. PCR reactions were performed under conditions in which the linearity of the amplification was confirmed. The concentration of cDNA templates in all PCR reactions was equalized. Additionally, amplification of the cyclophilin A gene with the same templates served as an internal reference because it is expressed at a relatively constant level in virtually all tissues [15]. RT-PCR results were visualized on agarose gel.

3. Results

To detect genes differentially regulated at the SWOP we performed cDNA RDA using mRNA isolated 16 h after reperfusion as a tester and at 8 h after reperfusion as a driver. The RDA resulted in a straight reduction of cDNA population complexity in each round of subtractive hybridization and amplification (Fig. 1). After cloning of DP3, picking of 384 clones and the sequencing of selected clones, 44 DNA contigs were identified. The search in public databases resulted in the identification of 15 different known genes (of which three were represented by more than one contig), five repetitive sequences, and 16 sequences unidentified so far (Table 2).

Difference products representing unique DNA sequences were PCR-amplified, spotted onto glass slides, and hybridized with Cy3/Cy5-labeled amplicons. After subtracting the background signal and normalization, the spot intensities were calculated. The genes were considered differentially expressed if the ratio of the medians (R_m) of Cy3/Cy5 signals of the corresponding spots were higher than 2.5. Three genes appeared to be highly upregulated: tristetraproline (TTP) ($R_m > 8$), matrix metalloproteinase-9 (MMP9) ($R_m > 6$), and selectin E ($R_m > 7$).

To confirm the results of microarray hybridization we used RT-PCR as an independent method of measuring levels of

Table 2
List of the canine sequences identified among the RDA difference products[a]

Number	Gene ID	Database ID	Score bits)	*E* value	Similarity (%)	Accession number[b]
1	*Canis familiaris* serum amyloid A protein (SAA)	M59175	410	e-112	96	BQ172873
2	*Homo sapiens* cysteine dioxygenase	D85777	298	2e-78	92	BQ172874
3	*Homo sapiens* metastasis-associated gene (Mta1)	U35113	430	e-118	91	BQ172875
4	*Homo sapiens* phosphoglucomutase-related protein (PGMRP)	L40933	302	1e-79	91	BQ172876
5	*Bos taurus* tristetraproline (TTP)	L42319	246	3e-63	91	BQ172881
6	*Canis familiaris* E-selectin	L23087	283	2e-74	95	BQ172882
7	*Homo sapiens* amphiregulin (AR)	M30704	344	2e-92	86	BQ172885
8	*Canis familiaris* collagen type IV α 1 chain (COL4A1)	U50933	687	0.0	98	BQ172889
9	*Mus musculus* RNA polymerase II subunit	D85818	163	5e-38	93	BQ172896
10	*Canis familiaris* MMP9	AF169244	678	0.0	98	BQ172897
11	*Mus musculus* iroquois homeobox protein 3	Y15001	186	3e-45	86	BQ172898
12	*Homo sapiens* mutant desmin (MUTDESM)	AF137053	276	4e-72	89	BQ172909
13	*Homo sapiens* helix-loop-helix protein (Id-2)	M97796	60	5e-07	85	BQ172892
14	*Mus musculus* TIS21	M64292	301	5e-80	92	BQ172883
15	*Homo sapiens* Ran binding protein 13 (RANBP13)	AF267987	280	4e-73	94	BQ172907
16	Repetitive sequences	Accession numbers[b]: BQ172877, BQ172879, BQ172886, BQ172891, BQ172906				
17	Sequences without homology in GenBank database	Accession numbers[b]: BQ172878, BQ172880, BQ172884, BQ172887, BQ172888, BQ172890, BQ172893, BQ172894, BQ172895, BQ172899, BQ172901, BQ172902, BQ172903, BQ172904, BQ172905, BQ172908				

[a]IDs of nearest homologs from GenBank database are given. Sequences with *E* values more than 0.005 were taken as unidentified. The interpretation of bit scores and *E* values is given in BLAST tutorial available at http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/Blast_output.html#Scores.
[b]Accession numbers of the sequences of RDA differential products in dbEST GenBank database.

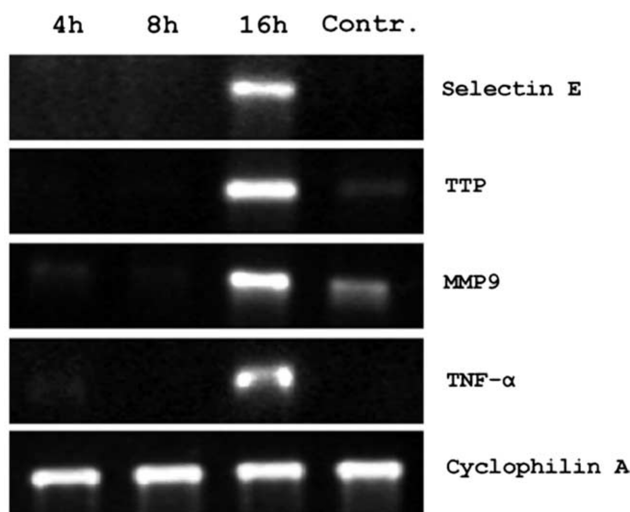

Fig. 2. RT-PCR analysis of expression-selected genes. Upregulation of TTP, TNF-α, selectin E, and MMP9 genes is visible at 16 h after reperfusion. Constitutively expressed cyclophilin A gene served as normalization reference. No overexpression of the respective genes can be seen in the control, neither at 4 nor at 8 h after reperfusion.

gene expression. RT-PCR has an important advantage in comparison to microarray hybridization because it makes it possible not only to detect relative changes in gene expression between two samples, but also to track the level of expression over a time course. We analyzed the expression of 15 identified genes in the heart tissue of the experimental animals at 4, 8, and 16 h after reperfusion and in the sham-operated control. Additionally, TNF-α gene as a crucial regulator was selected for RT-PCR for the considerations mentioned below. Each reaction was repeated at least three times to avoid accidental errors. In all cases, TTP, MMP9, selectin E, and TNF-α genes appeared to be highly overexpressed only at 16 h after the reperfusion, whereas at the other time points and in control no upregulation was observed (Fig. 2). Thus, the genes in focus, identified by RDA, were clearly confirmed as being differentially regulated by two independent methods. Differential expression of the other genes found among the RDA products was not confirmed by both microarray hybridization and RT-PCR, although some of them (serum amyloid A protein (SAA), RNA polymerase II, and collagen IV) were previously reported as being involved in post-ischemic processes in myocardium.

4. Discussion

The cDNA RDA technique combined with microarray hybridization allowed us to identify a group of genes which are highly upregulated at the late phase of IPC (16 h after reperfusion). RT-PCR analysis confirmed this result and additionally revealed that these genes are not overexpressed either in the control group of animals, or at the early stages of IPC. The similarity of expression profiles of TTP, selectin E, MMP9, and TNF-α genes implies their functional interrelations.

Both selectin E and MMP9 are involved in the inflammatory response, which is a common event after myocardial ischemia–reperfusion [16]. It is manifested by the influx of leukocyte cells into the ischemic region due to activation of different signaling pathways and is a prerequisite for myocardium healing and scar formation.

Selectin E is an endothelial-specific cell adhesion molecule that recognizes carbohydrate ligands on circulation leukocytes. It plays a central role in directing neutrophil traffic from the bloodstream into sites of inflammation [17].

MMP9 belongs to a large family of matrix metalloproteinases, which are responsible for collagen degradation and remodeling of the extracellular matrix in a number of physiological processes [18]. Along with some other metalloproteinases, MMP9 is activated during inflammation reaction [19]. It is produced by polymorphonuclear neutrophils and has been suggested to facilitate their migration through the endothelial basement membrane [20].

The expression of both selectin E and MMP9 is regulated primarily at the transcription level by TNF-α, which is one of the principal mediators of inflammatory and apoptosis pathways in mammals. Recent experiments with transgenic mice have demonstrated that cardiac-specific overexpression of TNF-α causes a robust increase in MMP9 activity in vitro [21] and in vivo [22,23].

The regulation of the selectin E expression is mediated by nuclear factor κB (NF-κB), which has three binding sites in the selectin E proximal promoter region. In endothelial cells, NF-κB is retained in an inactive complex with cytoplasmic inhibitor IκB. It was shown that brief exposure of endothelial cells to TNF-α induces degradation of IκB [24]. Thus, TNF-α appears to be a principal regulator of selectin E expression.

Despite its pivotal role in the upregulation of MMP9 and selectin E genes, TNF-α was not found among the DPs in our RDA experiment. However, the results of RT-PCR suggest that TNF-α is overexpressed at 16 h after reperfusion. This reflects that cDNA RDA cannot detect all genes of interest, maybe due to inefficient cloning or absence of amplifiable cDNA fragments.

Another gene, which we identified as being overexpressed at the late phase of IPC, is TTP, also known as TIS11, Nup475 or Zfp36. TTP is the prototype member of zinc finger transcription factors and is widely distributed in many tissues. It was shown that TTP knockout mice display evidence of chronic TNF-α excess, and their macrophages hypersecrete TNF-α [25]. TTP is capable of suppression of TNF-α at the posttranscriptional level by binding to the AU-rich element of the TNF-α messenger RNA [26,27]. Another report suggests that TTP might regulate TNF-α production also at the level of transcription [28]. TTP is activated by the translocation from nucleus to cytoplasm. The amount of TTP in the cytoplasm is increased by the same stimuli that cause increased expression of TNF-α in macrophages, thus leading to the negative feedback loop [26]. This could probably explain the simultaneous upregulation of TTP and TNF-α in our experiments.

Of special interest is that TTP activity is primarily regulated by the p38 mitogen-activated protein kinase (p38 MAPK) system [28–30]. Although p38 MAPK is necessary for the induction of TTP [29], several lines of evidence exist that TTP can be deactivated by phosphorylation with p38 MAPK [28,30]. Recent studies revealed that the inhibition of p38 MAPK substantially reduces myocardial ischemia–reperfusion injury [31]. The cardioprotective mechanisms proposed for the explanation of this effect invoke repression of endothelial adhesion molecule expression and blockade of

neutrophil accumulation, i.e. the relief of inflammatory reaction [31].

We assume that the elimination of the posttranscriptional suppression of TTP achieved by p38 MAPK inhibition could play an important role in the cardioprotection due to the ability of TTP to regulate TNF-α expression. Numerous reports indicate that anti-TNF treatment could prevent deleterious consequences of myocardial ischemia and reperfusion (reviewed by Meldrum [32]). On the other hand, several studies suggested that TNF-α activation might have cardioprotective effect in the setting of myocardial ischemia mediated through TNF-induced upregulation of manganese superoxide dismutase [33,34]. Kurrelmeyer et al. [35] proposed that the initial short-term activation of TNF-α during myocardial stress response may be beneficial, but this positive effect is lost if myocardial TNF-α expression becomes sustained and/or excessive.

In our experiments, the expression of TNF-α highly increased 16 h after reperfusion and probably caused the induction of the TTP gene. We suppose that such a feedback upregulation of the TTP gene may represent a part of the delayed preconditioning mechanism.

The ability of TTP to attenuate TNF-α activity may allow the regulation of the downstream inflammatory genes. We suggest TTP-mediated modulation of TNF-α expression as a promising perspective in clinical interventions against ischemia–reperfusion injuries of the heart.

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References

[1] Murry, C.E., Jennings, R.B. and Reimer, K.A. (1986) Circulation 74, 1124–1136.
[2] Yamashita, N., Hoshida, S., Taniguchi, N., Kuzuya, T. and Hori, M. (1998) J. Mol. Cell. Cardiol. 30, 1181–1189.
[3] Bolli, R. (2000) Circ. Res. 87, 972–983.
[4] Sun, J.-Z., Tang, X.-L., Knowlton, A.A., Park, S.W., Qiu, Y. and Bolli, R. (1995) J. Clin. Invest. 95, 388–403.
[5] Baxter, G.F. and Ferdinandy, P. (2001) Basic Res. Cardiol. 96, 329–344.
[6] Rizvi, A., Tang, X.L., Qiu, Y., Xuan, Y.T., Takano, H., Jadoon, A.K. and Bolli, R. (1999) Am. J. Physiol. 277, H874–H884.
[7] Miller, M.J. (2001) Can. J. Cardiol. 17, 1075–1082.
[8] Li, G.C., Vasquez, J.A., Gallagher, K.P. and Lucchesi, B.R. (1990) Circulation 82, 609–619.
[9] Gross, G.J. and Auchampach, J.A. (1992) Circ. Res. 70, 223–233.
[10] Kloner, R.A. and Yellon, D. (1994) J. Am. Coll. Cardiol. 24, 1133–1142.
[11] Hubank, M. and Schatz, D.G. (1994) Nucleic Acids Res. 22, 5640–5648.
[12] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) J. Mol. Biol. 215, 403–410.
[13] Diehl, F., Grahlmann, S., Beier, M. and Hoheisel, J.D. (2001) Nucleic Acids Res. 29, E38.
[14] Rozen, S. and Skaletsky, H.J. (1996, 1997) Primer3. Code available at http://www-genome.wi.mit.edu/genome_software/other/primer3.html.
[15] Haendler, B. and Hofer, E. (1990) Eur. J. Biochem. 190, 477–482.
[16] Frangogiannis, N.G., Smith, C.W. and Entman, M.L. (2002) Cardiovasc. Res. 53, 31–47.
[17] Chen, X.L., Tummala, P.E., Olliff, L. and Medford, R.M. (1997) Circ. Res. 80, 305–311.
[18] Nagase, H. and Woessner Jr., J.F. (1999) J. Biol. Chem. 274, 21491–21494.
[19] Etoh, T., Joffs, C., Deschamps, A.M., Dowdy, K., Hendrick, J., Baicu, S., Mukherjee, R., Manhaini, M. and Spinale, F.G. (2001) Am. J. Physiol. 281, H987–H994.
[20] Keck, T., Balcom IV, J.H., Fernandez-del Castillo, C., Antoniu, B.A. and Warshaw, A.L. (2002) Gastroenterology 122, 188–201.
[21] Siwik, D.A., Chang, D.L. and Colucci, W.S. (2000) Circ. Res. 86, 1259–1265.
[22] Li, Q., Bolli, R., Qiu, Y., Tang, X.L., Guo, Y. and French, B.A. (2001) Circulation 103, 1893–1898.
[23] Sivasubramanian, N., Coker, M.L., Kurrelmeyer, K.M., MacLellan, W.R., DeMayo, F.J., Spinale, F.G. and Mann, D.L. (2001) Circulation 104, 826–831.
[24] Boyle Jr., E.M., Sato, T.T. and Noel Jr., R.F. (1999) J. Surg. Res. 82, 194–200.
[25] Taylor, G.A., Carballo, E., Lee, D.M., Lai, W.S., Thompson, M.J., Patel, D.D., Schenkman, D.I., Gilkeson, G.S., Broxmeyer, H.E., Haynes, B.F. and Blackshear, P.J. (1996) Immunity 4, 445–454.
[26] Carballo, E., Lai, W.S. and Blackshear, P.J. (1998) Science 281, 1001–1005.
[27] Lai, W.S., Carballo, E., Strum, J.R., Kennington, E.A., Phillips, R.S. and Blackshear, P.J. (1999) Mol. Cell. Biol. 19, 4311–4323.
[28] Zhu, W., Brauchle, M.A., DiPadova, F., Gram, H., New, L., Ono, K., Downey, J.S. and Han, J. (2001) Am. J. Physiol. 281, L499–L508.
[29] Mahtani, K.R., Brook, M., Dean, J.L., Sully, G., Saklatvala, J. and Clark, A.R. (2001) Mol. Cell. Biol. 21, 6461–6469.
[30] Carballo, E., Cao, H., Lai, W.S., Kennington, E.A., Campbell, D. and Blackshear, P.J. (2001) J. Biol. Chem. 276, 42580–42587.
[31] Gao, F., Yue, T.L., Shi, D.W., Christopher, T.A., Lopez, B.L., Ohlstein, E.H., Barone, F.C. and Ma, X.L. (2002) Cardiovasc. Res. 53, 414–422.
[32] Meldrum, D.R. (1998) Am. J. Physiol. 274, R577–R595.
[33] Eddy, L.J., Goeddel, D.V. and Wong, G.H. (1992) Biochem. Biophys. Res. Commun. 184, 1056–1059.
[34] Nakano, M., Knowlton, A.A., Dibbs, Z. and Mann, D.L. (1998) Circulation 97, 1392–1400.
[35] Kurrelmeyer, K.M., Michael, L.H., Baumgarten, G., Taffet, G.E., Peschon, J.J., Sivasubramanian, N., Entman, M.L. and Mann, D.L. (2000) Proc. Natl. Acad. Sci. USA 97, 5456–5461.