Supplementary materials and methods

Antibodies

The anti-LMPTP antibody was generated by immunizing rabbits with a GST fusion of human LMPTP protein [41]. GAPDH, NF-κB and PKA antibodies were from Santa Cruz (California, USA), PLCδ, Ephrin Receptor (5A) and Gαq/11 from Abcam (Cambridge, USA), Tubulin, Bclxl, insulin receptor β, anti-ERK(1-2), anti-pERK (1-2), anti-Bax, anti-Akt and anti-pAkt (Ser473) antibodies were from Cell Signaling Technology (Beverly, USA). Anti-pTyr antibody was from Millipore (Billerica, MA, USA). Anti-CaMKIIδ specific antibody was a gift from Dr Donald Bers (University of California Davis, California). Anti-pCaMKIIδ Thr286 was from Genescript (USA). Anti-pp38 (pThr179/Tyr181), PLCβ and anti-wheat germ agglutinin/lectin antibodies were purchased from (Sigma, USA). Anti-phosphoLamban and anti-phospho-phospholamban antibodies were from Millipore.

Animals

Generation of *Acp1* knockout mice

Acp1 gene-trapped mice were generated by microinjection of genetically manipulated 129/Ola ES cells (obtained from Baygenomics) [22] into C57Bl/6 blastocysts. One 95% male chimera was bred with C57Bl/6 females to assess germline transmission, then *Acp1* $^{+/-}$ mice were backcrossed for at least 10 generations onto the Balb/c background.

Transverse aortic constriction

Male Balb/c and *Acp1* KO mice 13-15 weeks old were subject to trans-aortic constriction by ligation of the aorta between the innominate artery and the left common carotid artery using a 7-0

polypropylene suture (Ethicon) and an overlying 27-gauge needle to produce a discrete stenosis. Sham operations were performed by opening and closing of the chest.

Echocardiography and analysis

A Vevo 770 High-Resolution *In-Vivo* Imaging system and GE VividE9 system were used to measure cardiac cardiac dimensions and function after Sham or TAC surgery in mice. During echocardiographic analysis, mice were subjected to 1-2% isoflurane before assessing cardiac function. Left ventricular dimensions were imaged along a parasternal short-axis view and recorded in M-mode. End diastolic diameter (EDD) and end systolic diameter (ESD) were measured in order to calculate fractional shortening (FS) and ejection fraction (EF) and to determine left ventricular volume during diastole and systole. At study end-point, hearts were collected after opening the pericardial cavity and placed in liquid nitrogen.

Primary neonatal rat cardiomyocytes isolation and treatment

Primary neonatal rat cardiomyocytes were isolated using Worthington Neonatal CardioMyocytes System (Worthington, USA) according to the manufacturer's instructions. Briefly, 2-3 days old pups were sacrificed by decapitation and after opening the thorax, hearts were quickly removed. Cardiomyocytes were isolated by digestion with 0.5 mg/ml Type II collagenase and 0.5% Trypsin. After dissociation, the cells were pre-plated for 30-45 min to allow fibroblasts to attach and then plated on 100-mm cell culture dishes at a density of $2x10^6$ cells in DMEM (Invitrogen) supplemented with 10% FBS and antibiotics (100 U/ml penicillin, 10 mg/ml streptomycin solution). The cells were maintained in a humidified 5% CO_2 chamber at 37°C

Protein extraction

Grinded whole heart or harvested whole cell extracts were suspended in high salt lysis buffer (1M Hepes, 5M NaCl, 0.5M EDTA and 2M sucrose) containing protease (0.2 mM AEBSF, 2 µM E64, 2.6 mM aprotinin, 1 µM pepstatin) and phosphatase inhibitors (Thermo scientific, Philadelphia, PA, USA). The suspensions were homogenized and incubated in ice for 30 min. The soluble fraction was extracted by centrifugation at 12,000 rpm at 4°C for 15 min.

Co-immunoprecipitation

800 µg of whole cell extracts from hearts were immunoprecipitated using anti-insulin receptor β antibody at 10 µg/ml according to the manufacturer's instructions (Pierce Classic IP kit). 30 µl of the anti-insulin receptor beta antibody immunoprecipitated samples were analyzed by immunoblotting using anti-pTyr antibody. The membrane was then stripped and incubated with anti-insulin receptor beta antibody.

Western blotting

Protein lysate concentrations were measured by Bradford assay (Bio-Rad). Typically, 40 µg of whole heart or cell extract was analyzed by Western blot. Western blot analysis was performed by running a 4-12 % NuPAGE® Novex® Bis-Tris Gels (Invitogen, USA). After transfer of the proteins onto PVDF membrane, the membrane was blocked in TBS–Tween (50 mM Tris, 138 mM NaCl, 2.7 mM KCl, 0.05% Tween 20, pH 8.0) containing 5 % milk at room temperature for 1h. The membrane was then incubated with primary antibodies (1:1000 dilution) in TBS–Tween overnight at 4ºC. The membrane was then washed three times in of TBS–Tween and incubated for 1 h with a secondary antibody. Imaging and quantification were performed using ImageQuant LAS 4000 mini analyzer (GE Healthcare) and Adobe Photoshop CS6.

Q-PCR

Total RNA was extract from mouse hearts by using TriZol (Invitrogen) according to manufacturer's protocol. The total RNA quantity and quality was determined by using Nanodrop ND-2000c (NanoDrop Technologies) and Agilent RNA 6000 Nano kit Bioanalyzer (Agilent Technologies). cDNA was synthesized using Superscript III (Invitrogen) using random hexamers. 10 ng of cDNA and primers were added in PCR buffer IQ SYBR Green Supermix (Bio-Rad Laboratories, USA). PCR reactions were run on a CFX96 real-time PCR detection system (Bio-Rad Laboratories, USA). PCR conditions used were: 95°C for 150 s and 70 cycles with 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. Melting curves were measured between 72°C and 94°C with a resolution of 0.5°C. All reactions were conducted in triplicates and the data was analysed using the delta delta C_T method [42,43]. Primers used for fetal cardiac genes, SERCA2A, PLN and GAPDH were as follows:

ANF: sense 5'-CCAAGGCCTCACAAAAGAAC-3'

anti-sense 5'AGACCCAGGCAGAGTCAGAA-3'

β-MHC sense 5'-AGATGAATGCCGAGCTCACT-3'

anti-sense 5'-CTCATCCAAACCAGCCATCT-3'

α-MHC sense 5'-AAATCATTGCCAAGCTGACC-3'

anti-sense 5'-CAGGTCCATGATGCTCTCCT-3'

sk-actin sense 5'-TCG CGA CCT TAC TGA CTA CCT G-3'

anti-sense 5'-GCT TCT CTT TGA TGT CGC GC-3'

GAPDH sense 5'-GGCATTGCTCTCAATGACAA-3'

anti-sense 5'-TGTGAGGGAGATGCTCAGTG-3'

SERCA2A sense 5’- CGAAAACCAGTCCTTGCTGAGGAT-3’

anti-sense 5’-TACTCCAGTATTGGCATGCCGAGA-3’

PLN sense 5’-ATG ACG ACG ATT CAA ATC TCT TGG -3’

anti-sense 5’- TGG GTT TGC AAA GTT AGG CAT AA-3’

Array hybridization

Total RNA was isolated from target tissues/animals (14 male mouse hearts) with standard protocols. Sample handling, cDNA synthesis, cRNA labeling and synthesis, hybridization, washing, array (GeneChip® Mouse Genome 430 2.0 Array, Affymetrix Inc., Santa Clara, CA, USA) scanning, and all related quality controls were performed according to the manufacturer’s instructions. The Affymetrix GeneChip/GCOS software (Affymetrix Inc.) was used to calculate the raw expression value of each gene from the scanned image. The total RNA quality was assessed by the values of the 3’–5’ ratios for actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). DChip [44] outlier detection algorithm was used to identify outlier arrays. 14 samples/chips passed the above-mentioned quality controls and were used for further analyses. The CEL files were utilized for further analysis using dChip [44], MEV [45,46], and PARTEK Genomics Suite (Partek® software, Partek Inc., St. Louis, MO, USA).

Microarray analysis

Following previously described protocols, the transcriptional profiles of 14 samples were probed using Affymetrix’s GeneChip® Mouse Genome 430 2.0 Arrays. The open source R/Bioconductor packages [24] were used for processing and analysis of microarray data. The data was normalized by the GC Robust Multi-array Average (GC-RMA) algorithm

[47,48]. Analysis of Variance (ANOVA) was performed to identify genes varying significantly across different groups. Significantly modulated genes were defined as those with absolute fold change (FC) > 1.5 and unadjusted p-value < 0.05. Gene ontology (GO) and biological term enrichment analyses were performed using DAVID Bioinformatics Resources [49] and Ingenuity Pathways Analysis (IPA) 6.3 (Ingenuity Systems, Mountain View, CA). A right-tailed Fisher's exact test was used to calculate a p-value determining the probability that the biological function assigned to that data set is explained by chance alone. Functional Pathway and network analyses were performed as previously described in Colak et al. [50,51]. Statistical analyses were performed by using SAS 9.2 (SAS Institute, Cary, NC), MATLAB software packages (Mathworks, Natick, MA, USA), and PARTEK Genomics Suite (Partek Inc., St. Louis, MO, USA).

Human subjects

All procedures were performed under approved protocol (RAC# 2100 023) from the Office of Research Affairs at King Faisal Specialist Hospital & Research Centre. Patients with end-stage heart failure secondary to idiopathic dilated cardiomyopathy were followed at King Faisal Heart Centre and were enrolled in our study after informed consent. Hemodynamic function of patients diagnosed with heart failure was performed regularly at King Faisal Heart Centre to monitor the progression of the cardiomyopathy and just prior to the cardiac transplantation. Immediately after the surgery, the explanted hearts were frozen in liquid nitrogen and the Transplant Coordinator contacted our laboratory to transfer the human hearts. Hearts were then stored at -80ºC until collection of all the hearts and until further biochemical analysis. Normal human hearts were obtained from donors who died from accidental death or from cause other than cardiac diseases and that could not be transplanted because of incompatibility with the recipient. Functional data were available for some donor hearts.

Dissection of human and mouse hearts, and histological analyses

Samples from the same region of the left ventricles were dissected from the normal and cardiomyopathic hearts. Total proteins were prepared after grinding of the tissue as described above. Histology was performed in the Pathology Department of King Faisal Specialist Hospital and Research Centre. Briefly, sections were fixed in formalin and embedded in paraffin and 5μM heart sections were stained with Masson's trichrome using standard procedures.

References

(Note: reference numbers correspond to reference list in main article)

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