Supplementary Data

Supplementary Materials and Methods

Isolation and culture of rat skeletal myoblasts (MY). Rat MY were isolated from the hind-limbs of male Fischer-344 rats as described previously [1]. The present study conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes (NIH Publication No. 85-23, revised 1985) and protocol approved by the Institutional Animal Care and Use Committee, University of Cincinnati.

In Vitro Studies

Preconditioning of MY

The cells were grown in Petri dishes at a density of $3.5x10^4$ cells/cm^2. One day later, the cells were randomly assigned to four experimental groups: (i) $^{non\text{-}PC}$MY (ii) ^{PC}MY treated with 200 μmol/L diazoxide (Dz; US Pharmacopeia) 30-min. (iii) ^{PC}MY pretreated with 30 μM Wortmannin (Sigma) for 1-h before preconditioning (iv) ^{PC}MY pretreated with 100 μM PD098059 for 1-h with (Calbiochem) before DZ PC (v) ^{PC}MY pre-transfected with IL11 specific siRNA. The cells from various treatment groups were later used either for changes in molecular expression of IL11, Erk1/2, Stat3 and miR-21 or for the assessment of cytoprotective effects of preconditioning under oxidant stress.

For IL11 siRNA transfection, the cells were seeded a day prior to start of experiment at a density of $2x10^4$ cells/cm^2 in DMEM supplemented with 10% FBS. The cells were transfected with 10nM of ON-TARGET plus SMART pool rat IL11 siRNA (Dharmacon Cat. No. L-092679-01-0010) using Lipofectamine-2000 (Invitrogen) per instructions of the manufacturer at 37°C in a CO_2 incubator. For control, the cells were

transfected with scrambled siRNA. The cells were later preconditioned with 200μM DZ for 30 min at 37 °C in a CO_2 incubator.

In order to mimic the effects of preconditioning induced IL11, in another set of experiments, MY were seeded in 35 mm^2 at a cell density of $3.5x10^4$ cells/cm^2 and were treated with recombinant IL11 protein (rIL11). In another group of treatment, the cells were pre-treated with IL11Rα before treatment with rIL11. Using untreated MY as control, the cell samples were used to observe changes in Erk1/2, pStat3, and miR-21 expression and its effect on cell survival under oxidant stress.

For cytoprotection studies, they were subjected to oxidative stress injury of 100 μM H_2O_2 for 2-h. For western blot analysis, the cells were lysed for protein extraction.

Lactate dehydrogenase leakage (LDH)

Intracellular LDH leakage, a well-known indicator of cell membrane integrity and viability, was measured in the cell-conditioned medium samples using a CytoTox-ONE Homogenous Membrane Integrity Assay (Promega) per instructions of the manufacturer.

Reverse-transcription polymerase chain reaction

Isolation of total RNA from the different treatment groups of MY, and their subsequent first-strand cDNA synthesis, was performed using an RNeasy kit (Qiagen) and an Omniscript Reverse Transcription kit (Qiagen), respectively, per instructions of the manufacturer. IL11 (PPR06463A) primer pairs from SuperArray Bioscience Corporation were used for PCR.

Western immunoblotting

Total protein lysate from cells of various treatment groups were extracted and then fractionated and analyzed on an SDS gel electrophoresis and immunoblotting as

described previously [1]. Antibodies used in this study include IL11 (sc-7924; Santa Cruz Biotechnology), actin (sc-1616; Santa Cruz Biotechnology), pStat3 (Ser727) (9134; Cell Signaling Technology), pAkt (Ser473) (9271; Cell Signaling Technology), ImmunoPure Peroxidase Conjugated goat anti-rabbit IgG (H+L) (Pierce) and rabbit anti goat IgG-HRP (sc-2768; Santa Cruz Biotechnology).

In Vivo Studies

Cell labeling.

PKH-26 labeling of MY

The cells were labeled with PKH26 cell tracker dye (Sigma) to study their fate post-transplantation per instructions of the supplier.

*nls*LacZ reporter gene labeling of MY

The cells were also labeled with *nls*LacZ reporter gene (LacZ reporter gene with nuclear localization signal) to allow monitoring of cell fate after transplantation. HEK-293 cells were infected with adenoviral vector encoding for *nls*LacZ gene and the cells were cultured to confluence for 4-5 days in 10% FBS containing DMEM before their supernatant was collected, filtered through a 0.2 μm filter and used for transduction of MY. For transduction of MY with *nls*LacZ reporter gene, MY were incubated with neat concentration of the filtered supernatant from HEK-293 cells for 8h on two consecutive days to achieve optimum transduction efficiency.

For β-galactosidase expression analysis, MY transduced with *nls*LacZ or croyopreserved tissue sections of the heart were fixed with 0.5% glutareldehyde for 15-min at room temperature. After twice rinsing with PBS for 5min each, the sections were incubated overnight at 37°C with 1.3 mg/mL 5-bromo-4-chloro-3indoyl-β-D-

galactopyranosid (Xgal; Bio-Rad), 30 mM potassium ferricyanide, 30 mM potassium ferrocyanide, 0.02% Nonidet P-40 and 2 mM magnesium chloride in PBS. At the end of the incubation period, the sections were rinsed in PBS and then observed under light microscope for bluish green stained nuclei, indicative of the presence of transplanted cells [2].

Experimental rat model of acute myocardial infarction and cell transplantation

Experimental rat heart model of acute myocardial infarction was developed in young female Fisher-344 rats by permanent coronary artery ligation as described earlier [3]. The animals were grouped to receive intramyocardial injections of 70 µL of basal DMEM without cells (group-1) or containing $1.5x10^6$ $^{Non\text{-}PC}$MY (group-2) or ^{PC}MY (group-3). The injections were performed at multiple sites (average of 4 to 5 sites/animal) in the free wall of the left ventricle (LV) under direct vision. The chests of animals were sutured, and animals were allowed to recover.

Statistical analysis

All data were described as mean±SEM. To analyze the data statistically, we performed 1-way ANOVA with post hoc analysis and considered a value of $p<0.05$ as statistically significant.

Supplementary References

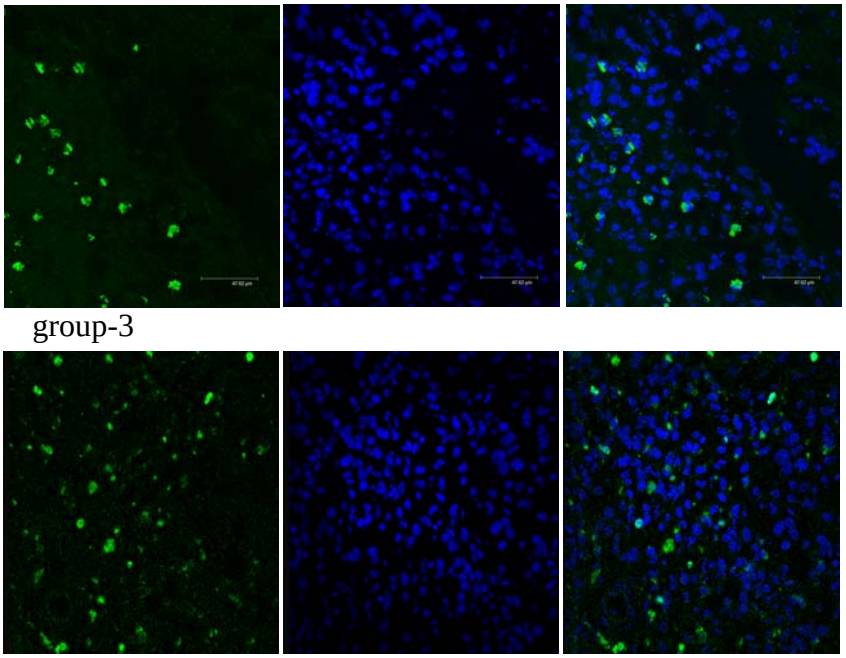
1. Niagara MI, Haider H, Jiang S, Ashraf M. Pharmacologically preconditioned skeletal myoblasts are resistant to oxidative stress and promote angiomyogenesis via release of paracrine factors in the infarcted heart. ***Circ Res*****.** 2007;100:545-55.

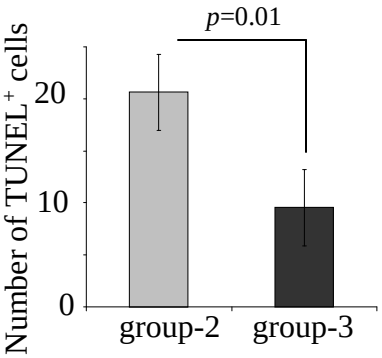
2. Ye L, Haider H, Jiang S, Ling LH, Ge R, Law PK, Sim EK. Reversal of myocardial injury using genetically modulated human skeletal myoblasts in a rodent cryoinjured heart model. ***Eur J Heart Fail.*** 2005;7:945-52.

3. Jiang S, Haider H, Idris NM, Salim A, Ashraf M. Supportive interaction between cell survival signaling and angiocompetent factors enhances donor cell survival and promotes angiomyogenesis for cardiac repair. ***Circ Res.*** 2006;99:776-84.

Legend to the Supplementary Figures

Supplementary Figure-SI: TUNEL staining on heart tissue sections on day-7 post-transplantation of cells shows that preconditioning significantly decreased apoptosis (green= TUNEL positive cells and blue=DAPI nuclear staining).


group-3
group-2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group-2
group-3