OBJECTIVES Downregulated NDUFA13, an accessory subunit of complex I, has been shown to be able to render the tumor cells more resistant to apoptosis. In the present study, therefore, we aimed to investigate whether and how NDUFA13 downregulation in the heart protects against I/R injury.

METHODS We generated Myh6Cre+ER^{Tam}NDUFA13$^{flox/flox}$ mice and demonstrated that heterozygous NDUFA13 knockout, i.e. Myh6-Cre+ER^{Tam}NDUFA13$^{flox/-}$ mice exhibited normal cardiac morphology and function at normal state, and were more resistance to apoptosis when exposed to ischemia-reperfusion (I/R) injury, resulting in a significant decrease in infarct size (IS).

RESULTS Interestingly, a decrease in substrate driven oxygen consumption of complex I was observed, however, a compensation for the total oxygen consumption by complex I and complex II was observed, which was associated with an increase in residual oxygen consumption (ROX) when complex III inhibitor, Antimycin A, was used. Interestingly, less superoxide generation was observed in heterozygous knockout mice compared with normal control, which may attenuate the injury caused by ischemia reperfusion.

CONCLUSIONS In conclusion, heterozygous NDUFA13 knockout attenuated infarct size after ischemia reperfusion through reduced superoxide production in mitochondria.

GW28-e0891

miR-21 Reduces Hydrogen Peroxide-Induced Apoptosis in c-kit+Cardiac Stem Cells In Vitro through PTEN/PI3K/Akt Signaling

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OBJECTIVES miR-21 reduces hydrogen peroxide- (H2O2-) induced apoptosis in c-kit+ CSC and estimated the contribution of PTEN/PI3K/Akt signaling to this oxidative circumstance.

METHODS CSCs were isolated from rat atrial appendage and purified using anti-rabbit secondary antibody conjugated magnetic beads. Harvested CSCs were treated with H_2O2 100 uM for 2 h. miR-21 mimics, miR-21 inhibitor, and their control scrambles with transfection reagent Lipofectamine 2000,Early apoptosis and necrosis of CSCs were determined by flow cytometry using Annexin V-FITC/PI staining assay . miR-21 and PTEN mRNA levels were determined by using quantitative RT-PCR. Western blot analysis of apoptosis-related protein from c-kit+ cell.

RESULTS miR-21 mimics efficiently reducedH2O2-induced apoptosis in c-kit+ CSC, as evidenced by the downregulation of the proapoptosis proteins caspase-3 and Bax and upregulation of the antiapoptotic Bcl-2. In addition, the gain of function of miR-21 in c-kit+ CSC downregulated the protein level of PTEN although its mRNA level changed slightly; in the meantime, miR-21 overexpression also increased phospho-Akt (p-Akt).The antiapoptotic effects of miR-21 were comparable with Phen (bpV), the selective inhibitor of PTEN, while miR-21 inhibitor or PI3K's inhibitor LY294002 efficiently attenuated the antiapoptotic effect of miR-21.

CONCLUSIONS these results indicate that the anti-H2O2-induced apoptosis effect of miR-21 in c-kit+ CSC is contributed by PTEN/PI3K/Akt signaling. miR-21 could be a potential molecule to facilitate the c-kit+ CSC therapy in ischemic myocardium.

GW28-e0894

Impaired autophagosome clearance triggers myocardial necroptosis in ischemia/reperfusion injury

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OBJECTIVES Necroptosis is crucially involved in severe cardiac pathological conditions, whereas efficient autophagic flux is essential for myocardial survival. However, whether impaired autophagosome processing contributes to myocardial necroptosis after ischemia/reperfusion (MI/R) is unknown. Here we demonstrate that autophagosome accumulation enhances MI/R triggered myocardial necroptosis in aged hearts.

METHODS Young (3-4 mon) and aged (22-24 mon) mice were subjected to MI/R *in vivo*. The isolated hearts of each group were perfused and subjected to MI/R in vitro and treated with necroptosis inhibitor necrostain-1 to restrain necroptosis. Aged mice were injected with metformin to restore autophagy flux.

RESULTS Autophagosome clearance was impaired in aged hearts and autophagosome abundance was further increased after MI/R. In young hearts, the autophagy substrate Sequestosome1 (p62/SQSTMl) was increased in ischemia and was degraded in reperfusion, while aged hearts exhibited increased p62 at basal and further accumulation during MI/R because of disabled degradation. Aged mice subjected to MI/R endured greater myocardial necroptosis, as evidenced by reductions in lactate dehydrogenase (LDH) release and Evans blue dye (EBD) penetration. We found that overload of p62 conjugated with the RIP1 and promoted massive RIP1-RIP3 complex (necrosome) during MI/R. Necrosome phosphorylated MLKL and promoted the translocation of MLKL from cytoplasm to membrane, which eventually wrecked the cell membrane. Treatment of necrostain-1 inhibited the RIP1-RIP3-MLKL signaling, which ameliorated MI/R injury compare with vehicle in aged hearts. Down-regulation of p62 significantly reduced p62-RIP1-RIP3-MLKL signaling cascade in the aged hearts. Meanwhile, treatment of metformin restored the autophagy flux in the senescent hearts and decreased p62 level, which protected aged hearts from MI/R-induced RIP3-dependent necroptosis.

CONCLUSIONS Our findings show that accumulation of p62 is sufficient to promote RIP1-RIP3-MLKL cascade by triggering the formation of p62-RIP1, thereby enhancing MI/R induced necroptosis in aged hearts. These findings confirm the new mode of autophagic dysfunction promoted necroptosis and provide new views for aging-related myocardial vulnerability.

GW28-e0907

Insulin reverses MCP-1 suppressed cholesterol efflux to HDL3/ApoA1 through up-regulation of ABCA1, ABCG1 and SR-BI in differentiated 3T3-L1 adipocytes

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OBJECTIVES Insulin has been reported to influence cholesterol removal from different cells, but the results have been controversial. Based on our previous study, which we found that insulin promote cholesterol efflux from HepG2 cells and can reverse the decreased cholesterol efflux to HDL though PI3K-Akt pathway, we further investigate the effects of insulin on damaged cholesterol efflux by MCP-1 in this report.

METHODS 3T3-L1 preadipocytes were obtained from the Cell Bank of the Chinese Academy of Sciences and differentiated into adipocytes as described previously. Fully differentiated adipocytes (day 13) seeded in collagen-coated 24-well plates were starved for 6 h and labeled with ^{3}H-cholesterol (1 Ci/ml) (Perkin-Elmer Analytic Sciences, Boston, MA) for 24 h. Cellular cholesterol efflux was initiated by the addition of DMEM containing 0.2% BSA with 20μg/ml human apoA1 or 50 μg/ml HDL_3 with the indicated dose of MCP-1 for the indicated period of time in the presence or absence of insulin. After incubation, the radioactivity of the medium and cells was measured using a liquid scintillation counter. Cholesterol efflux was calculated as the percentage of total [^{3}H]-cholesterol released into the medium after subtraction of the values obtained in the absence of a cholesterol acceptor. 3T3-L1 adipocytes were harvested for Real-time PCR, western blotting, cell-surface protein assays and Confocal microscopy. The data were statistically analyzed using SPSS 13.0, and the results are expressed as the means ± SD.

RESULTS 1.MCP-1 reduced cholesterol efflux to HDL3 and apoA1 in differentiated 3T3-L1 adipocytes. 2. In differentiated 3T3-L1 adipocytes, MCP-1 reduced cholesterol efflux to HDL_3 mainly by inhibiting SR-BI and ABCG1 and to apoA1 mainly by inhibiting ABCA1. 3. MCP-1 decreased ABCA1 and SR-BI mRNA expression but not ABCG1. 4. MCP-1 decreased total and cell surface ABCA1, ABCG1, and SR-BI protein expression as shown by Western blotting and confocal microscopy in differentiated 3T3-L1 adipocytes. 5. Insulin increased MCP-1 suppressed cholesterol efflux to HDL_3 and apoA1 depending on Akt phosphorylation. 6. Insulin reversed MCP-1 suppressed ABCA1 and SR-BI mRNA expression, and ABCA1, ABCG1 and SR-BI protein expression via PI3K/Akt pathway.

CONCLUSIONS Insulin reverses the suppressed cholesterol efflux to HDL3 and apoA1 by MCP-1 through up-regulation of ABCA1, ABCG1 and SR-BI through PI3K/Akt pathway in 3T3-L1 adipocytes, which