GW28-e1021

Myocardial reparative functions of exosomes from mesenchymal stem cells are enhanced by hypoxia treatment of the cells via transferring microRNA-210 in an nSMase2-dependent way

Jinyun Zhu,[1,2] Hong Yu[1,2]
[1]Cardiovascular Key Lab of Zhejiang Province; [2]Department of Cardiology, the Second Affiliated Hospital, College of Medicine, Zhejiang University

OBJECTIVES Stem cell-derived exosomes have been shown to have therapeutic effects on cardiac regeneration. Since hypoxia could modulate mesenchymal stem cells to have better paracrine effect, we investigated whether exosomes derived from hypoxia-treated MSCs (Exo^H) have superior properties than those from normoxia-treated MSCs (Exo^N) for myocardial repair.

METHODS Mouse bone marrow-derived MSCs were cultured under hypoxia or normoxia for 24 hours, and the conditioned media were collected for exosome purification by ultracentrifugation. Exosomes were identified by protein marker expression, transmission electron microscopy and nanoparticle tracking analysis. In vivo study, we divided mice into four groups including Sham, PBS, Exo^H, and Exo^N group. Exosomes and PBS are delivered intramyocardially into adult C57BL/6J mice at the border of an ischemic region following ligation of the left anterior descending coronary artery. Echocardiography was performed to evaluate cardiac function at baseline and 3,7,14 and 28 days after myocardial infarction (MI). Heart sections were stained with picrosirius red staining for fibrosis quantification. Immunofluorescence staining with endothelial marker (CD31) and artery marker (aSMA) reflected angiogenesis in the peri-infarct areas of hearts at day 28 after MI. We also evaluated proliferation of endogenous Sca-1^+cardiac progenitor cells (CPCs) after MI 7days with marker Ki67. For in vitro, we took tube formation in human umbilical vein endothelial cells (HUVEC) for detection angiogenesis and done TUNEL and AnnexinV-PI for apoptosis in neonatal mouse cardiomyocytes. In addition, miRNA array was done to detect the hypoxia-specific microRNA in the exosomes. We test the biological functionality of the microRNA-210 in H9C2 and HUVEC cells through transfected with microRNA-210 mimic or siRNA210.

RESULTS Exo^H had significantly better effects on promoting endothelial cells for tube formation and protecting cardiomyocytes from apoptosis under oxidative stress in vitro than Exo^N. Intramyocardial injection of Exo^H into the infarcted heart of C57BL/6 mouse resulted in significantly higher mice survival rate, smaller scar size and better recovery of cardiac functions (n=24 per group). In comparison with Exo^N treatment, Exo^H had superior capability to increase vascular density, decrease cardiomyocyte apoptosis, reduce fibrosis, recruit cardiac progenitor cells, and enhance cell proliferation in the infarcted heart. MicroRNA array of exosomes showed significantly higher level of microRNA-210 (miR-210) in Exo^H. Transfection of endothelial cells and cardiomyocytes with miR-210 mimic also resulted in similar biological effects as Exo^H did, whereas inhibiting miR-210 in MSCs diminished the superior pro-angiogenesis and anti-apoptosis effects of thereafter derived Exo^H. Hypoxia treatment of MSCs increased the cellular expression of neutral sphingomyelinase 2 (nSMase2) which is crucial for exosome secretion. The expression of nSMase2 is also increased by adding DMOG (a stabilizer of Hif-1a) to MSCs under hypoxia condition. Blocking the activity of nSMase2 by inhibitor GW4869 resulted in reduced miR-210 secretion after hypoxia treatment and abrogated the beneficial effects of Exo^H.

CONCLUSIONS Hypoxic culture augments miR-210 and nSMase2 activities in recipient MSCs and their secreted exosomes, and this is responsible at least in part for the enhanced cardioprotective actions of exosomes derived from hypoxia-treated cells.

GW28-e1029

Methylseleninic Acid Promotes the Ox-LDL Transportation though Cultured Endothelial Cell Monolayers by Increasing Calpain Activity

Zhihui Cai,[1] Yanqing Zhang,[1] Zhi Huang[1]
[1]Jinan University

OBJECTIVES Atherosclerosis is a chronic inflammatory disease which initiates by the accumulation of low density lipoprotein (LDL) in the artery wall. Methylseleninic acid (MSA, CH3SeO2H), a metabolite of selenium from animal cells, has exhibited anti-oxidative and anti-cancer activity. However, the effect of MSA on atherosclerosis has not been reported by far. In this study, we investigated the effect of MSA on endothelial integrity.

METHODS We used HUVECs to build up cultured endothelial cell monolayers.

RESULTS MSA treatments at 5 μM could increase the transportation of ox-LDL through monolayers. Immunofluorescence results showed that MSA could disrupt the formation of adherens junction by VE-cadherin. Western blot results further showed that MSA could induce the proteolysis of VE-cadherin in a time-dependent manner. We also found that MSA increased calpain activity by up-regulation of calpain-1 and its membrane localization. Additionally, increase of ox-LDL permeability and proteolysis of VE-cadherin could be attenuated by co-treating with the calpain inhibitor II (ALLM) or calpain inhibitor IV (Z-LLY-FMK).

CONCLUSIONS In conclusion, our results suggest that exogenous MSA promote ox-LDL transport through the leak of cultured endothelial cell monolayers, which caused by the proteolysis of VE-cadherin and the lift of calpain activity.

GW28-e1030

Endothelial and Platelet microparticles in cardiac syndrome X

Yousef Rasmi,[1] Fereshteh Ghaffari,[1] ShahramSeyedi,[1] MirHossein SeyedMohammadzad,[2] Alireza Rostamzadeh,[2] Elmira Roshani[1]
[1]Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran; [2]Department of Cardiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

OBJECTIVES Cardiac syndrome X [CSX] is characterized by the presence of chest pain, positive exercise test and normal angiography in coronary arteries. Although the cause of the syndrome is still unknown, studies suggest that microvascular coronary dysfunction plays a crucial role in its pathogenesis. Research has shown that circulating endothelial microparticles (EMPs) are novel marker for endothelial dysfunction that they contribute to the severity and progression of vascular disease. The aim of this study was to compare the level of endothelial microparticles among patients with cardiac syndrome x and healthy control subjects.

METHODS Forty patients with CSX and 19 healthy controls were enrolled. Two groups were matched for BMI and sex. We evaluated the level of EMP markers by flowcytometery using CD31, CD41 and CD62 monoclonal antibodies in the peripheral blood of patients and control subjects. Clinical and laboratory factors associated with EMPs were assessed.

RESULTS CD31+/41- endothelial microparticle counts and percent were 4.43±3 counts/μL and 0.262±0.29 in CSX patients versus 1.56±0.8 counts/μL and 0.058±0.04 in healthy control ($p<0.05$). Percent of CD62E in CSX were significantly higher than control subjects ($p<0.05$). CD31+41+ platelet microparticle counts and percent were significantly higher in CSX patients compared with control subjects. There was no significantly correlation between risk factors of cardiovascular disease and EMPs in CSX patients but levels of hs-CRP correlated with PMP positively.

CONCLUSIONS Our findings show that the circulating level of EMPs and PMPs increase in CSX patients.

GW28-e1051

Inhibition of HDAC3 prevents diabetic cardiomyopathy in OVE26 mice via epigenetic regulation of DUSP5-ERK1/2 pathway

Zheng Xu,[1] Jian Sun[1,2,3]
[1]Cardiovascular Center, the First Hospital of Jilin University, Changchun, China; [2]Cardiovascular Center, the First Hospital of Jilin University, Changchun, China; [3]Pediatric Research Institute, Department of Pediatrics, the University of Louisville, Louisville, KY, USA

OBJECTIVES The present study was designed to determine whether DCM can be prevented by specific inhibition of HDAC3 and to elucidate the mechanisms by which inhibition of HDAC3 prevent DCM.

METHODS Type 1 diabetes OVE26 and age-matched wild-type mice were given the selective HDAC3 inhibitor RGFP966 or vehicle for 3 months. These mice were then sacrificed immediately or 3 months later for cardiac function and pathological examination.

RESULTS HDAC3 activity was significantly increased in the heart of diabetic mice. Administration of RGFP966 significantly prevented

DCM, as evidenced by improved diabetes-induced cardiac dysfunction, hypertrophy and fibrosis, along with diminished cardiac oxidative stress, inflammation, and insulin resistance, not only in the mice sacrificed immediately or 3 months later following the three-month treatment. Furthermore, phosphorylated extracellular signal-regulated kinases (ERK) 1/2, a well-known initiator of cardiac hypertrophy, was significantly increased, while dual specificity phosphatase 5 (DUSP5), an ERK1/2 nuclear phosphatase, was substantially decreased in diabetic hearts. Both of these changes were prevented by RGFP966. Chromatin immunoprecipitation assay showed that HDAC3 inhibition elevated histone H3 acetylation on the *DUSP5* gene promoter at both two-time points.

CONCLUSIONS These findings suggest that diabetes-activated HDAC3 inhibits DUSP5 expression through deacetylating histone H3 on the primer region of *DUSP5* gene, leading to the derepression of ERK1/2 and the initiation of DCM. This study indicates the potential application of HDAC3 inhibitor for the prevention of DCM.

GW28-e1058

Trimetazidine Prevents Cardiac Rupture in Mice with Myocardial Infarction by Suppressing Oxidative Stress

Wei Gong,[1] Shaoping Nie[1]
[1]Beijing Anzhen Hospital, Capital Medical University, Beijing, China

OBJECTIVES Cardiac rupture (CR) is a catastrophic complication of acute myocardial infarction (MI). At present, there are no effective pharmacological strategies for preventing post-MI rupture. Here we investigated the effect of trimetazidine (TMZ) on post-MI cardiac rupture and its underlying mechanisms of action.

METHODS MI models were induced by left coronary artery ligation in male C57BL/6 mice, with shams undergoing the same operation without coronary artery ligation. The operated mice were randomly divided into 4 groups: sham+saline, sham+TMZ, MI+saline and MI+TMZ (20mg/kg/day) treatment group. Animals allocated to the rupture incidence were closely monitored for 7 days; autopsy was performed once animals were found dead to determine the reason of death, and infarct size was determined. Heart function was detected by echocardiography. Oxidative stress markers were analyzed by Western blotting. Cultured neonatal cardiomyocytes and H9c2(2-1) were exposed to normoxia or anoxia and treated with TMZ.

RESULTS Since CR in mice mostly happen within the first week after MI, we observed the incidence of CR of MI mice for one week. We found that the CR rate of mice treated with TMZ was significantly lower than the saline-treated group (34.4% vs. 19.4%, $P<0.05$). To investigate the mechanism of the effect of TMZ on CR, we examined the expression of MMP2, MMP9 in the cardiac tissues of the sham-operated and MI groups of mice. The results showed that the MMP2, MMP9 expression in the TMZ-treated group was significantly lower than the saline-treated group. Further, we found that ROS and H_2O_2 level increased dramatically after MI, and TMZ treatment abolished level of ROS and H_2O_2. To further examine the action of TMZ on MMP2, MMP9 expression, we pretreated H9c2(2-1) and primary cardiomyocytes with anoxia and found that TMZ treatment increased expression of MMP2, MMP9. TMZ pretreatment markedly decreased the expression of MMP2, MMP9, and reduced level of ROS and H_2O_2 by anoxia.

CONCLUSIONS TMZ prevents cardiac rupture through inhibition of oxidative stress, which is attributable to the down-regulation of MMP2, MMP9 expression. Our findings suggested that early administration of TMZ to patients with acute MI is a potential preventive approach for CR.

FOOTNOTES This study was supported by grants from the National Natural Science Foundation of China (81600213), Beijing Natural Science Foundation (7141003).

GW28-e1060

Involvement of histone methylation in macrophage apoptosis and unstable plaque formation in methionine induced hyperhomocysteinemic ApoE-/- mice

Guangzhi Cong,[1] Ru Yan,[1] Hui Huang,[1] Kai Wang,[1] Shaobin Jia[1]
[1]General Hospital of Ningxia Medical University

OBJECTIVES Hyperhomocystinemia(Hhcy) is an independent risk factor of atherosclerosis and promotes the unstable plaque formation. Epigenetic mechanisms play an important role in the pathogenesis of atherosclerosis induced by Hhcy. However, the exact mechanism still undefined. The present study sought to determine the hypothesis whether histone methylation modification is involved in macrophage apoptosis and unstable plaque formation in the condition of Hhcy.

METHODS $ApoE^{-/-}$mice were supplemented with high-methionine (HM) diet for 20 weeks to induce Hhcy. Macrophage Raw 264.7 cells were challenged with homocysteine and histone inhibitor BIX 01294.

RESULTS The hallmark of unstable plaque, lesion apoptotic cells are increased in $ApoE^{-/-}$mice supplemented with high-methionine (HM), accompanied with a decrease expression of histone H3 lysine 9 dimethylation. Hcy increases the apoptosis of macrophage and inhibits the histone H3 lysine 9 dimethylation and the expression of histone methyltransferase G9a in vitro. Inhibition of histone methylation by BIX01294 enhances the macrophage apoptosis and foam cell formation in vitro.

CONCLUSIONS Our data suggests that Hhcy promotes the progression of atherosclerosis via macrophage apoptosis. Histone methylation might involve in macrophage apoptosis and unstable plaque formation in methionine induced hyperhomocysteinemic $ApoE^{-/-}$ mice.

GW28-e1061

The role of LXRα in homocysteine induced foam cell formation

Kai Wang,[1,2] Ru Yan,[2] Ping Jin,[1] Hailin Du,[1] Shaobing Yang,[1,2] Guangzhi Cong,[2] Shaobin Jia[2]
[1]Ningxia Medical University; [2]General Hospital of Ningxia Medical University

OBJECTIVES To investigate the role of Liver X α (LXRα) in homocysteine(Hcy) induced foam cells formation.

METHODS THP-1 monocytes were cultured and induced by ox-LDL to become to foam cell. Then cells were intervened by different concentrations of homocysteine(Hcy) and LXRα agonist T0901317 for 24 hours. Oil red O staining was performed for identification and qualitative analysis. The protein expression of LXRα and its target gene ABCG1 as well as ABCA1 was detected by cyto-immunofluorescence and Western blot.

RESULTS Oil red staining showed that Hcy facilitate foam cell formation. The immunofluorescence and Western blot result suggest that the protein expression of LXRα, ABCG1 and ABCA1 were decreased after the intervention of Hcy and T0901317 could reverse this effect.

CONCLUSIONS Our results suggesting that LXRα might be one of the key role of macrophage lipid metabolism interrupted by Hcy.

GW28-e1062

WWC3 inhibits intimal proliferation after vascular injury through Hippo signaling pathway

Beijia Chen,[1] Liu Guinan[1]
[1]The First Affiliated Hospital of China Medical University

OBJECTIVES To investigate the effect of WWC3 on vascular smooth muscle cells (VSMCs) after injury and its possible mechanism.

METHODS We used platelet-derived growth factor BB (PDGF-BB) as cell injury, rats with balloon injury to make model of rat carotid artery intima injury. Detect the expression of WWC3 in A10 cells (VSMCs) and arteries after injury, and the influence on the proliferation and migration of VSMCs by MTT, Transwell, Western blot, immunohistochemical, and other methods. By regulating WWC3 expression, with luciferase activity assay, immunoprecipitation and other methods, to investigate the molecular mechanism of WWC3 on Hippo signaling pathway.

RESULTS After injury, the expression of WWC3 decreased in A10 cells and rat carotid artery, Hippo signaling pathway activity down-regulated significantly, the expression of YAP (main effector of Hippo signaling pathway), and its main negative downstream target genes such as CTGF increased, enhancing the proliferation WWC3. Over-expressing WWC3, meanwhile stimulating with PDGF-BB, the proliferation and migration of VSMCs were significantly weakened, compared with stimulating with PDGF-BB alone. It is confirmed that WWC3 could interact with LATS1 by co-immunoprecipitation, induce phosphorylation of LATS1, inhibit the nuclear import of YAP with phosphorylation of YAP, thereby upregulate Hippo pathway activity. However, transfecting WWC3-ΔWW, this effect disappeared.

CONCLUSIONS WWC3 expression is down-regulated in VSMCs and the neointimal hyperplasia after the injury (with PDGF-BB or balloon injury), inhibiting the activity of Hippo signaling pathway, enhancing the ability of proliferation and migration of VSMCs. WWC3 can interact with LATS1, to promote the phosphorylation of YAP and reduce its nuclear transference, upregulating Hippo signaling