GW25-e3232
IRAK4 Deficiency Promotes Cardiac Remodeling
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Objectives: To investigate the role of interleukin-1 receptor-associated kinase-4 (IRAK-4), which plays an essential role in the innate immune system, in cardiac remodeling induced by pressure overload and elucidate the underlying mechanisms.

Methods: In vivo studies were performed using IRAK4 heterozygous knockout (IRAK4) mice and wild type (IRAK4) mice. Models of cardiac remodeling were induced by aortic banding (AB). Cardiac remodeling was evaluated by echocardiography, catheter-based measurements of hemodynamic parameters and histological analysis. In vitro studies were performed using H9c2 cardiomyoblasts with over-expression of IRAK4 or not. Models of cardiomyoblast hypertrophy were induced by Angiotensin II (AngII) treatment. Cardiomyoblast hypertrophy was evaluated by immunofluorescent staining of α-actin. mRNA and protein expression were detected by real-time PCR and Western Blot analysis.

Results: IRAK4 was upregulated in pressure overload induced mice heart. IRAK4+/- mice exhibited exacerbated cardiac hypertrophy, dysfunction and fibrosis after 4 weeks of aortic banding (AB) compared with that in wild-type mice. Furthermore, enhanced activation of the MEK/ERK-1/2, p38 and NFκB pathways was found in IRAK4+/- mice compared to WT mice.

While IRAK4 overexpression significantly attenuated cardiomyoblasts hypertrophy induced by AngII, the increased NO level and eNOS protein as well as the decreased ICAM-1 and VCAM-1 protein expression were observed after treatment with sphinogine-1-phosphate (SIP) significantly increased in serum of diabetic rats. Sphinogine-1-phosphate receptor type 2 (SIPR2) was the overwhelming SIP receptor expressing in human coronary artery endothelial cells (HCAECs) exposed to high glucose condition. The present study explored the role of SIP/SIPR2 in diabetic vascular endothelial dysfunction and underlying mechanism, which may provide SIP/SIPR2 pathway as therapeutic target to prevent and treat vascular endothelial dysfunction in diabetes mellitus.

Methods: No level, endothelial nitric oxide synthase (eNOS), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) mRNA and protein expression were measured in HCAECs by commercial kit and western blot, respectively. miRNA-31 level was detected by real-time PCR. After treatment by different methods including SIPR2 specific antagonist, small molecular RNA interference, overexpression of SIPR2, transfection of miRNA mimic and inhibitor respectively, the above indexes of endothelial cells function were investigated in HCAECs cultured under 25mM high glucose condition. The target gene of miRNA-31 was predicted using microRNA on-line analysis software and validated by dual-luciferase reporter gene system and western blot.

Results: No level and eNOS protein level were decreased, ICAM-1 and VCAM-1 protein level were increased significantly in HCAECs by SIP treatment or SIPR2 overexpression. The enhanced NO level and eNOS protein as well as the decreased ICAM-1 and VCAM-1 protein expression were observed after treatment with sphinogine-1-phosphate inhibitor DMS, SIPR2 specific antagonist JTE-013 and specific SIPR2-siRNA respectively in HCAECs induced by SIP under high glucose condition. MiRNA-31 expression in indicated cells induced by SIP was also inhibited by JTE-013 and SIPR2-siRNA. Moreover, the HCAECs co-transfected SIPR2-siRNA and miRNA-31 mimic were shown the decreased in NO level and eNOS protein level while the expression of adhesion molecule protein expression including ICAM-1 and VCAM-1. The reversal effects were found in HCAECs overexpressed SIPR2 and transfected miRNA-31 inhibitor together. Additionally, microRNA on-line analysis software was used to predict the target of miRNA-31 and found that there was possible binding evidence of miRNA-31 in human eNOS mRNA 3’-UTR. Furthermore, the result of dual luciferase reporter gene experiment showed that the luciferase activities were inhibited by overexpressed miRNA-31 at the presence of the wild type human eNOS mRNA 3’-UTR, which can be abrogated by mutations in seed complementary site. Protein expression of eNOS in miRNA-31 transduced cells was examined to be suppressed after miRNA-31 transfection. Together, our results demonstrated that eNOS was direct target of miRNA-31.

Conclusions: SIP/SIPR2 mediated vascular endothelial cells dysfunction by upregulating miRNA-31 expression in HCAECs exposed to high glucose.

GW25-e5186
A genetic association study for polymorphisms of platelet membrane glycoprotein and coronary artery disease
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Objectives: To study the association of platelet membrane glycoprotein polymorphism (GP IaC807T) and eNOS gene polymorphism (eNOS 894GG) with coronary artery disease (CAD).

Methods: A case-control study design was adopted. The study group consisted of 153 individuals (mean age=65.6±10.8 years) with coronary artery disease identified by coronary angiography and 153 individuals (mean age=65.6±10.8 years) without coronary artery disease. A newly-developed technology named improved Multiplex Ligation Detection Reaction, iMLDR, was used to detect target genes and SNPs. The risk factors for coronary artery disease were analyzed by logistic regression.

Results: (1) For GP IaC807T, the comparisons of genotype and allele distributions between cases and controls were significant statistically (X²=6.640, P=0.036 and X²=5.725, P=0.017, respectively). But for GP IaC807T, the comparisons of genotype and allele distributions between cases and controls were not different significantly between cases and controls respectively. (2) Even adjusted by gender, blood glucose, fibrinogen and triglyceride, still the results from logistic analysis showed that more than 4 times risks for coronary artery disease were increased by TT genotype compared with CC genotype (OR=4.2% vs. ADSCs: 3.4% and MI+PBS: 3.7% vs. MI+ADSCs: 5.6%; MI+PBS: 3.7% vs. MI+ADSCs: 5.6%; MI+ADSCs: 3.1% vs. MI+ADSCs: 5.6%). None of the ADSCs gave rise to cardiomyocytes, and ADSC-induced cardioprotection was mainly induced by paracrine function. The conditioned medium from shPHD2-ADSCs decreased cardiomyocyte apoptosis. Insulin-like growth factor-1 (IGF-1) levels were 3.8 times higher in the conditioned medium of shPHD2-ADSCs than ADSCs, and depletion of IGF-1 attenuated the cardioprotective effects of shPHD2-ADSC-conditioned medium. NF-kappaB activation was induced by shPHD2 to stimulate IGF-1 secretion via binding to the IGF-1 gene promoter. A combination of HIF-1alpha silencing and IGF-1 neutralization blocked the beneficial effects of shPHD2-ADSCs for MI. Similar findings were observed with BM-MSCs.

Conclusions: PHD2 silencing promotes stem cell survival in infarcted hearts and enhances their paracrine function to protect cardiomyocytes. Its prosurvival effect on stem cells is HIF-1alpha dependent, while it enhances stem cell paracrine function through NF-kappaB-mediated IGF-1 upregulation. PHD2 silencing in stem cells may be a novel strategy for enhancing the effectiveness of stem cell therapy after MI.
AngII-induced inflammation were partly related to TLR4. Co-treatment of CFs with pioglitazone and AngII revealed no effect on AngII TypeI receptor (AT1), but downregulated AT1-dependent ERK1/2 phosphorylation. Furthermore, pioglitazone inhibited AngII-induced MCP-1 and TNF-α production and overexpression of TLR4, Myd88 and NF-κB, and increased nuclear-PARP1/2 production in CFs.

**Conclusions:** These results suggest that TLR4 is involved in the AngII-induced inflammatory responses in CFS, and pioglitazone provides its anti-inflammatory and anti-bacterial effect which is partly dependent on interfering with the TLR4-dependent signaling pathway (AT1/ERK1/2/TLR4/Myd88/NF-κB) to prevent the hypertension induced venile remodeling.

**GW25-e0069**

**Ambulatory Blood Pressure Monitoring of Healthy Chinese Children Aged 5-12 Years**

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**Objectives:** We studied the characteristics of ambulatory blood pressure (ABP) and their in-fluences in healthy Chinese children.

**Methods:** Casual blood pressure (BP), ABP, body height, body mass index (BMI), personal and familial medical histories were recorded in 338 Chinese children (aged 5-12 years). Data were treated by ABP database system and analyzed by SPSS 20.0 including correlations, regressions and conventional statistical methods.

**Results:** Means of 24-Hour SBP/DBP/PP (systolic BP/diastolic BP/pulse pressure) were 112.7±10.2/76.5±6.7/46.6±7.3 and 110.9±9.8/66.5±5.5/44.6±6.0 mmHg in normal weight 118 boys and 139 girls, and 113.8±11.3/67.9±6.5/50.7±8.7 and 111.5±10.6/65.7±5.5/49.7±7.1 mmHg in overweight 46 boys and 35 girls, respectively. BMI, weight and gender were primary influencing factors on ABP-derived parameters. Overweight children had higher means (P<0.01 or <0.05) of systolic BP (SBP) and pulse pressure (PP), and higher hypertensive prevalence (35% vs. 15% for boys and 40% vs. 18% for girls) than normal weight children. Boys had higher (P<0.01) means of PP (24-Hour /Daytime) than girls in normal weight children. Age related negatively with DBP and body height correlated with heart rate. Circadian variations of ABP (diaper pattern) were seen in most of children (76%). Familial histories of hypertension showed no influence on ABP.

**Conclusions:** BMI, weight and gender were the main influencing factors on ABP in Chinese children and our data provides basic information of ABP in Chinese children.

**GW25-e0249**

**Treatment of Myocardial Infarction by Transplantation of Adiponectin Gene-Modifed Stromal Vascular Fraction cells from Adipose Tissue**

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**Objectives:** The aim of this study was to investigate the effect of the human adiponectin (Hapm1) gene-modified stromal vascular fraction (SVF) cells (Hapm1-SVF) transplantation on the cardiac function and histological changes of heart tissue in rats with myocardial infarction.

**Methods:** Rats were divided into four groups (n=8): sham-operated (sham), untreated myocardial infarction (MI), SVF treated (SVF), and Hapm1-SVF (SVF carrying human adiponectin gene and labeled with EGF) treated (Hapm1-SVF) groups. The MI model was established by ligation of the left anterior descending coronary artery. SVF were injected into the infarcted border zone of MI rats. EF and FS were detected by echocardiography 4 weeks after the cells transplantation. LVSP and LVDp were measured by PowerLab biological signal analytical system. Vascular density was determined by immunochemical staining.

**Results:** It was shown that LVSP and LVDp were significantly increased in MI rats treated with SVF cells or Hapm1 gene-modified SVF cells as compared with untreated MI rats, but still not reach the normal level (LVSP: sham (99.35±7.45 vs MI (72.83±3.57) VS SVF (77.85±8.72) VS Hapm1-SVF (84.23±5.86) mmHg, P<0.05). EF and FS of rats in Hapm1-SVF or SVF groups were significantly higher than that of MI rats, but still not reach the normal level (EF: sham (81.85±4.63% vs MI (43.21±3.10%) VS SVF (64.93±4.13%) VS Hapm1-SVF (74.56±3.60%), P<0.05). Meanwhile, the density of vessels in Hapm1-SVF group was higher than that in SVF or MI group, but still lower than that in sham group.

**Conclusions:** In summary, transplantation of SVF cells carrying hapa1 gene may stimulate recovery of cardiac function after myocardial infarction in rats.

**GW25-e0435**

**Klotho Deficiency Causes Hypertension and Renal Damage and Its Mechanism**

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**Objectives:** Klotho (KL) is a recently discovered aging-suppressing gene. Insertional mutation of KL gene resulted in a premature aging syndrome. Overexpression of KL gene extended the lifespan by 20% and reduced other aging disorders. Hypertension is a common aging-related diseases. The purpose of this study is to assess if KL deficiency affects blood pressure (BP) and renal damage as well as the underlying mechanism.

**Methods:** One group of heterozygous mutant KL (KL (+/-)) mice and one group of wild type (WT) mice were used to measure BP continuously when they were from the age of 8 months to 13 months old. At the end of month of mice, some sub-groups when the mice were at the age of 15 months, which received eplerenone treatment (6 mg/kg/day, IP) or an equal dose vehicle, respectively. BP was measured and urine was collected during treatment. At the end of the treatment for 3 weeks, the animals were sacriﬁced and blood was collected. Plasma aldosterone level was detected with an aldosterone ELISA kit. Kidney sections were used for periodic acid Schiff (PAS), Masson’s trichrome staining and immunohistochemical staining (CD45, CD3 and CD68). Adrenal sections were used for immunohistochemical staining (Ki67, PPARgamma and TLR4). Plasma kidney area and creatinine level were detected with the quantitative-PCR kit. Urinary albumin concentration was measured with a micro-albuminuria ELISA kit. Western blotting was done to detect the expression of MR, SGK1, NCC, and ATP synthase β in kidneys of KL (+/-) mice. Chronic treatment with eplerenone (aldosterone receptor blocker) decreased hypertension to the control level and prevented the upregulation of SGK1, NCC and ATP synthase β in kidneys of KL (+/-) mice, suggesting that KL deficiency causes hypertension due to plasma aldosterone increase and the subsequent renal sodium retention through SGK1-NCC signaling. Moreover, significant renal structure damage (glomerulus collapse, tubule dilatation) and function decline (Ehrenpreis tubule function and urine albumin excretion) were observed in KL (+/-) mice. Further analysis indicated that several pro-inflammatory cytokines (TNF-α, MCP-1, IL-6 and osteopontin) were upregulated and eculizone (T cell and macrophage) infiltration were increased in kidneys of KL (+/-) mice. Elevated plasma renin activity and kidney damage abolished the activity of renin-angiotensin system.

**Conclusions:** KL is essential to the maintenance of normal BP. KL deficiency caused hypertension and kidney damage via upregulating aldosterone level and consequently increasing inflammation and SGK1-NCC signaling in kidneys.

**GW25-e1138**

**Cellular repressor of EIA-stimulated genes protects against angiotensin II-induced hypertension and vascular remodeling via p38MAPK-mediated regulation of the renin-angiotensin system**

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**Objectives:** Cellular repressor of EIA-stimulated genes (CREG) has been proposed to be a new cardiovascular homeostasis regulator. We hypothesized that CREG is a negative regulator of angiotensin (Ang II)-mediated hypertension and vascular remodeling.

**Methods:** Ten-week-old male heterozygous CREG-deficient (CREG−/−) mice and their littermate wild-type (WT) mice were implanted with osmotic minipumps containing saline, Ang II (1.5 mg/kg, d), or Ang II and recombinant human CREG (rCREG) (15-5000 μg/kg, d) protein for 14 days. Ang II-infused CREG−/− mice were then cotreated with p38 MAPK inhibitor, SB203580 (10 mg/kg, d), beginning 2 days before implantation, to examine blood pressure (SBP) extent of Ang II-induced hypertension remodeling; RNA and protein level of CREG, collagen type III/IV, angiotensin-converting enzyme-2 (ACE2), angiotensin receptor type 1 (AT1R), Ets-1 and MAPK were evaluated. Primary vascular smooth muscle cell (VSMC) culture was performed to assess the mechanism of Ang II-induced CREG down-regulation, as well as CREG-mediated modulation of renin-angiotensin system.

**Results:** CREG levels are high in vascular media under basal conditions but rapidly decreased in response to Ang II. Ets-1 transcription factor expression is upregulated in Ang II-stimulated VSMCs. Chromatin immunoprecipitation analysis showed the interaction of endogenous and exogenous Ets-1 or glutathione S-transferase-tagged Ets-1, bearing only the DNA-binding domain with the authentic CREG promoter. Moreover, Ets-1 siRNA knockdown significantly reduced Ang II-induced repression of CREG expression, indicating Ets-1 mediates Ang II-induced down-regulation of CREG expression. Ang II infusion for 14 days resulted that levels of SBP, increased medial thickness and vascular remodeling of the aorta and mesenteric artery were significantly greater in CREG−/− mice compared with the WT controls. Vascular gene expression level of CREG was lower in Ang II-treated CREG−/− mice than in WT mice, suggesting that CREG deficiency aggravates Ang II-induced hypertension and vascular remodeling. However, daily treatment of Ang II-infused WT mice with rCREG protein improved the above phenotypes. Ang II-vascular remodeling was inhibited by rCREG protein in association with reduced plasma Ang II and increased renal function. Furthermore, the CREG treatment inhibited Ang II-mediated up-regulation of AT1R expression and down-regulation of ACE2 expression by blocking p38MAPK activation. Finally, pharmacological blockade of p38MAPK with SB203580 abolished CREG deficiency mediated the aggravation of Ang II-induced hypertension and vascular remodeling. Therefore, p38MAPK plays a key role in Ang II-mediated hypertension and vascular remodeling, which were exacerbated by CREG deficiency, whereas rCREG protein attenuated Ang II-induced hypertension and vascular remodeling through modulation of balance between