Objectives: As the most common sustained cardiac arrhythmia, atrial fibrillation (AF) is regarded as a heritable disease partially. Most former researches were concentrated in heterozygous mutations or single nucleotide polymorphisms (SNPs) in the exon coding regions. The relationship between AF occurrence and SNPs in nonprotein-coding micro-RNAs is still mysterious. Our study focused on this filed.
Methods: A total of 123 participants were enrolled from genetically unrelated ethnic Han Chinese living in Nantong city of Jiangsu Province. 65 atrial fibrillation patients (Male: 53.85%; Age: 69.75±13.44) were confirmed with electrocardiogram or Holter, 58 normal individuals (Male: 48.28%; Age: 63.36±14.11) were assigned to control group. Genotypes of the pre-miRNA SNP rs11614913 in miR-196a2 were distinguished using the method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The primers used for amplification were 5'-TCCA GATAGATGCAAAGCTGAA-3' (forward) and 5'-ATAGGTTGAGAGGACGGCA TAA-3' (reverse). PCR products were digested by specific restriction enzyme Hpy188I, and then separated by 2% agarose gel electrophoresis. For hsa-mir-196a2 rs11614913 C/T, allele T is cuttable, yielding two fragments of 81 bp and 219 bp, allele C is uncuttable and the fragment is still 300 bp. Hardy-Weinberg equilibrium were evaluated by chi-square test. Student's t test for continuous variables and chi-square test for categorical variables were used. Odds ratio (OR) and 95% confidence intervals (CI) were reported to estimate the associations between genotypes and AF risk using logistic regression analyses. Differences were considered statistically significant at $P<0.05$.
Results: The genotype frequencies were 15.4% (TT), 46.1% (TC), and 38.5% (CC) for the AF patients, while 39.7% (TT), 46.5% (TC), and 13.8% (CC) for the control participants. Significantly increased AF risk was found to be associated with C allele ($P<0.0001$; OR, 2.716; 95% CI, 1.620-4.553). Compared with miR-196a2 rs11614913 wild-type homozygote TT, significantly increased AF risks exist in variant homozygote CC (OR, 8.625; 95% CI, 2.939-25.316) and heterozygote TC (OR, 2.556; 95% CI, 1.032-6.326). After Adjusting for age and sex, participants with TC/CC genotypes still show high risk for AF statistically (OR, 3.614; 95% CI, 1.537-8.497).
Conclusions: Our study provides the first evidence that the SNP rs11614913 in miR-196a2 may be associated with increased risk of atrial fibrillation in Han Chinese population. It may be used as candidate biomarker for AF susceptibility.

GW25-e0432

Qiliqiangxin inhibits angiotensin II-induced transdifferentiation of rat cardiac fibroblasts through suppressing interleukin-6

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Objectives: Qiliqiangxin (QL), a traditional Chinese medicine, had long been used to treat chronic heart failure. Recent studies revealed that differentiation of cardiac fibroblasts (CFs) into myofibroblasts played an important role in cardiac remodeling and development of heart failure, however little was known about whether QL ameliorated myocardial remodeling via regulating CFs differentiation. The present study aimed to investigate the effects of QL on angiotensin II (AngII)-induced CFs transdifferentiation and the underlying mechanism.
Methods: Study was performed on in vitro cultured CFs from Sprague-Dawley rats. Cells were stimulated by AngII (100nM) for 24 h in the presence and absence of pretreatment with QL (0.5mg/ml) or Olmesartan (OLM 10nmol/L). Small-interfering RNA (siRNA) was used to knockdown interleukin-6 (IL-6). CFs transdifferentiation was examined by the expression of transforming growth factor-β_1 (TGF-β_1), α-smooth muscle actin (α-SMA), IL-6, collagen typeI and typeIII.
Results: Results showed that AngII induced significant increases of TGF-β_1 and α-SMA in CFs, which could be attenuated by QL, or by blocking AT1 receptor with OLM. In addition, AngII-induced upregulation of IL-6 was reduced by QL, and knocking down of IL-6 in CFs also suppressed the expression of AngII-induced TGF-β_1 and α-SMA, suggesting QL-mediated downregulation of IL-6 might be centrally involved in AngII-induced CFs transdifferentiation. Further data showed that enhanced type I and type III collagens in response to AngII were inhibited by QL, OLM or IL-6-siRNA.
Conclusions: These data provided evidence that CFs transdifferentiation could be reversed by QL through regulating IL-6 signaling.

GW25-e0557

Effects of Atorvastatin on Pregnancy Associated Plasma Protein-A Level in Vascular Smooth Muscle Cells Induced by Oxidized-low Density Lipoprotein

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Objectives: Both pregnancy-associated plasma protein A (PAPP-A) and vascular smooth muscle cells (VSMCs) play roles in the development of atherosclerosis (AS). In this study, we investigated the effect of atorvastatin on PAPP-A level in VSMCs induced by oxidized-low density lipoprotein (ox-LDL) and its potential mechanism in AS.
Methods: Different concentrations of ox-LDL (75, 150, 300μg/ml) were added into the culture medium for different processing time (2, 12 and 24h) to induce rat VSMCs oxidative injury model. The level of PAPP-A mRNA and protein were evaluated by RT-PCR and Western Blotting. Then we observed the change in PAPP-A mRNA and protein expression after treatment with atorvastatin.
Results: Compared with control group, PAPP-A mRNA and protein levels increased in a dose and time-dependent manner when VSMCs were induced by ox-LDL. After exposure to ox-LDL (300 μg/ml) for 12 and 24h, PAPP-A mRNA and protein levels were dramatically increased ($P<0.01$). However, PAPP-A mRNA and protein levels were strongly decreased after atorvastatin treatment.
Conclusions: Atorvastatin can regulate the expression of PAPP-A mRNA and protein induced by ox-LDL, which demonstrate that PAPP-A is probably involved in the anti-As mechanism of atorvastatin.

GW25-e1129

Toll-like Receptor 4 Knockout Protects Against Isoproterenol-induced Cardiac Fibrosis: The Role of Autophagy

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Objectives: Toll-like receptor 4 participates in the process of acute heart injury. The underlying mechanisms of its protection are multi-factorial, but we hypothesized that Toll-like receptor-mediated autophagy control plays a vital role. The purpose of this study was to clarify the effect of autophagy on cardiac fibrosis.
Methods: Cardiac fibrosis was induced by subcutaneous isoproterenol (ISO) injection, and rapamycin was simultaneously administered orally for 14 days. Animal echocardiography was then used to evaluate the success of the cardiac fibrosis model, and the mice were sacrificed after the echocardiography examination.
Results: Toll-like receptor-4 knockout (TLR4 KO) mice had better heart function than did wild-type (WT) mice ($P<0.05$). Rapamycin treatment reduced the left ventricular ejection fraction to 23.5% ($P<0.05$), and the collagen volume fraction of the ISO and ISO plus rapamycin groups was 5.9% and 25.9%, respectively, in TLR4 KO mice. Compared with the WT mice, Beclin1 and autophagy were downregulated in TLR4 KO mice ($P<0.05$); however, the ISO plus rapamycin group had higher autophagy activity than did the ISO group in TLR4 KO mice ($P<0.05$).
Conclusions: Our results suggest that TLR4 knockout-induced cardioprotection against isoproterenol-induced cardiac fibrosis is associated with reduced autophagy induction. Cardiac fibroblast autophagy participates in its own activation. The moderate inhibition of autophagic activity may be a new strategy for treating cardiac fibrosis.

GW25-e1455

Keratin and ankyrin repeat domain 1 protein identified by proteomic analysis may be involved in coronary artery disease

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Objectives: Circulating endothelial microparticles (EMPs) are recently suggested as a promising biomarker of endothelial function and target for cardiovascular disorders. However, increased studies reported that the biological effects of EMPs are ambivalent. The objective of this study is to identify proteins differentially expressed between patients with coronary artery disease (CAD) and control subjects.
Methods: Different EMPs populations were generated from patients (n=5) with CAD and control subjects (n=5) for proteomic analysis by LC/MS. For the in vitro assessment, differential gene expression of identified proteins was validated by RT-PCR in HUVECs stimulated with TNF-α or ox-LDL. For further confirmation of candidate proteins in vivo, a total of 82 patients with CAD and 36 age- and sex-matched healthy controls were recruited. Detection of identified proteins in serum was carried out with ELISA.
Results: By applying proteomic analysis, two proteins, namely, keratin and ankyrin repeat domain 1 protein (ANKRD1), were suggested as candidates. For further identification, exposure of HUVEC to TNF-α or ox-LDL up-regulated the expression of the mRNA levels of keratin and ANKRD1 in a concentration-dependent manner respectively ($P<0.05$). As for the in vivo experiment, keratin levels in the patients of acute coronary syndromes (ACS) and stable angina pectoris (SAP) groups were significantly higher than those of the control group ($P<0.05$), while ANKRD1 could distinguish patients with ACS from patients with SAP and control subjects, respectively.
Conclusions: Increased levels of keratin and ankyrin repeat domain 1 protein are found in patients with CAD, rendering these two proteins as novel biomarkers and potential drug target for cardiovascular diseases. The specific molecular mechanisms require future studies.

GW25-e1650

Uncoupling protein 2 knockout exacerbates salt-induced cardiovascular and renal remodeling

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