GW27-e0816

Long non coding RNA (LncRNA) with Enhancer-like Function modulates WNT signal pathway of atherosis genes in HUVECs

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OBJECTIVES Long non-coding RNA (LncRNA) has been reported to be involved in the coronary artery disease (CAD). However, the knowledge on the association of LncRNAs is still lacking. The study is to investigate the implication of lncRNA NONHSAT112178 (lncRNAPPARσ) in the modulation of gene sets directly involved in CAD.

METHODS A microarray was performed to discovery lncRNAPPARσ. Then, a real-time fluorescence quantitative PCR (RT-qPCR) was used to verify the results. Race and Northern blot were explored to obtain the full length, and the distribution was investigated by RNA FISH. And RNA pull down and RIP were performed to study the direct binding site for lncRNA and beta-catenin.

RESULTS RT-qPCR was performed to validate the microarray results, which indicated the expression of lncRNAPPARσ, neighboring protein-coding gene PPARσ, in circulating EC increased more than 4 times compared with the control group. Then, we designed and tested siRNA sequences to specifically target the transcript and successfully knocked down the expression of lncRNAPPARσ in HUVECs. Unexpectedly, we found an enhancer-like function for the lncRNAPPARσ in HUVECs, depletion of the lncRNA led to decreased activity of the neighboring protein-coding gene PPARσ, implying the expression of its target gene ADRP and ANGPTL4 reducing, moreover, due to PPARσ's trans-suppression, increasing matrix metalloproteinase-9 (MMP-9), LEF1, ICAM-1 and IL-6 production. What's more, affecting the PPARσ's pathway, the upstream was also investigated, the abundance of TCF4 was decreased significantly. The observed transcriptional enhancement by lncRNAPPARσ was mediated through the physical association with beta-catenin. This interaction is required for proper genomic localization of lncRNAPPARσ in activation of gene expression and regulation of PPARσ mediates inflammation and extra-cellular matrix remodeling in CAD.

CONCLUSIONS These results reveal an unanticipated role for lncRNAPPARσ in activation of critical genes involved in extra-cellular matrix remodeling and inflammatory response to finally impact in the risk of CAD.

GW27-e0841

Hyperhomocysteinemia promotes unstable plaque formation in ApoE-/- mice: A role of Endoplasmic Reticulum Stress induced macrophage apoptosis

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OBJECTIVES Hyperhomocysteinemia(Hhcy) promotes unstable atherosclerotic plaque formation. Macrophage apoptosis and plaque necrosis are the two hallmarks of advanced plaque. Endoplasmic reticulum stress plays a critical role in macrophage apoptosis and plaque instability induced by homocysteine. The present study was sight to explore the mechanisms macrophage apoptosis mediated by endoplasmic reticulum stress in the the pathogenesis of unstable plaque in Hhcy ApoE double knock (ApoE-/-) mice.

METHODS Four week old C57BL/6J mice and ApoE-/- mice were received a control (n= 12) or methionine (2%) diet (n= 12). After 16 weeks on the diets, mice were sacrificed, and the levels of total plasma homocysteine, plasma cholesterol and triglycerides were analyzed. Parameters of advanced plaque progression including necrotic core size, collagen content and macrophage apoptosis in both en face aortae and aortic valve sections were quantified. Meanwhile, the makers of endoplasmic reticulum stress were detected in aortas of mice. Furthermore, Raw264.7 macrophage were challenged with homocysteine (50, 100,200 or 500 μmol/L) in the absence or presence of ox-LDL (50ug/ml) or PBA(an ER stress inhibitor). The apoptosis and makers of endoplasmic reticulum stress were comprehensively accessed.

RESULTS Hhcy was observed in all groups supplemented with methionine, compared to diet-matched control groups($P < 0.05$). The differences of plasma total cholesterol levels or lipid profiles were not observed between methionine-supplemented groups and the diet-matched control groups ($P > 0.05$). Atherosclerotic lesions were only observed in both en face aortae and aortic valve sections in ApoE-/- mice mice but not C57BL/6J mice even though the methionine diet. The methionine-supplemented ApoE-/- mice showed significantly increased atherosclerotic lesions area ($P < 0.05$). Necrotic core area and lesion apoptotic cells was significantly increased and collagen content reduced in plaques of ApoE-/- mice supplemented with methionine ($P < 0.05$). The expression of endoplasmic reticulum stress makers included BIP, ERO1 α and IRE1α were also increased in the methionine-supplemented ApoE-/- mice ($P < 0.05$). Homocysteine enhanced foam cell formation with or without OX-LDL in RAW264.7 cell lines in a dose dependent manner ($P < 0.05$). But Homocysteine induced RAW264.7 cell apoptosis only in the presence of ox-LDL (50ug/ml) by AV/PI and Tunnel assays ($P < 0.05$). The expression of endoplasmic reticulum stress makers included BIP, ERO1 α and IRE1α were also increased in the homocysteine challenged RAW264.7 cell in the absence or presence of ox-LDL (50ug/ml) ($P < 0.05$). PBA inhibited foam cell formation, attenuated apoptosis and inhibited expression of ER stress marker ($P < 0.05$).

CONCLUSIONS Hhcy promotes unstable plaque formation in ApoE-/- mice. ER stress may play an important role in macrophage apoptosis and plaque instability induced by Hhcy.

GW27-e0849

Effects of Neuregulin-1 on autonomic nervous system remodeling post - myocardial infarction on SD rats

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OBJECTIVES To discuss the effect of Neuregulin-1 on cardiac autonomic nervous remodeling post-myocardial infarction on rats and supply the evidences for clinical use.

METHODS 45 SD rats were randomized averagely to three groups: SO, MI and MI+N group. A rat MI model was established by left anterior descending coronary artery ligation. Then, the rats of MI+N group were given neuregulin-1 respectively. After 4 weeks, the rats were given echocardiography test, including left ventricular end-systolic inner diameter (ESD), left ventricular diastolic diameter (EDD), left ventricular end-systolic volume (ESV), left ventricular end-diastolic volume (EDV), left ventricular ejection fraction (EF%), and left ventricular fractional shortening (FS%). Tyrosine hydroxylase (TH), growth associated protein 43 (GAP43), choline acetyltransferase (CHAT) and vesicular acetylcholine transporters (VACHT) mRNA and protein expression were detected in the ischemic zone surrounding by RT-PCR, western blotting, respectively.

RESULTS 1. Compared to SO group, ESD, EDD, ESV, EDV significantly elevated in MI group (0.56±0.04 vs 0.35±0.015, 0.77±0.023 vs 0.62±0.021, 0.41±0.08 vs 0.11±0.015, 0.92±0.12 vs 0.55±0.047, $P<0.05$), while the value of EF, FS significantly decreased (53.28±0.48 vs 80.46±4.35, 23.88±0.21 vs 43.97±4.52, $P<0.05$); Compared with MI group, EF, FS value significantly increase in MI+N group (73.85±4.19 vs 53.28±0.48, 38.02±3.72 vs 23.88±0.21, $P<0.05$), and ESD, EDD, ESV, EDV significantly decreased in MI+N group (0.42±0.017 vs 0.56±0.04, 0.67±0.035 vs 0.77±0.023, 0.18±0.021 vs 0.41±0.08, 0.70±0.02 vs 0.92±0.12, $P<0.05$).

2. In the level of mRNA, compared to SO group, TH, GAP43, CHAT, VACHT value obviously increased in MI group (4.78±0.505 vs 11.04±0.252, 4.94±0.452 vs 1.21±0.453, 3.60±0.797 vs 0.91±0.121, 4.13±0.890 vs 1.00±0.205, $P<0.01$); Compared with MI group, TH, GAP43, CHAT value obviously decreased in MI+N group (3.59±0.280 vs 4.78±0.505, 3.95±0.165 vs 4.94±0.452, 2.14±0.466 vs 3.60±0.797, $P<0.05$); However, VACHT have no difference in statistical (4.22±0.382 vs 4.85±0.450, 2.97±0.424 vs 4.13±0.890, $P>0.05$).

3. In the level of protein, compared to SO group, the TH, GAP43, CHAT, VACHT in MI group markedly elevated (0.678±0.102 vs 0.185±0.076, 0.837±0.043 vs 0.124±0.062, 1.129±0.450 vs 0.409±0.149, 0.749±0.176 vs 0.231±0.096, $P<0.05$); Compared with MI group, TH, GAP43 markedly dropped in MI+N group (0.364±0.141 vs 0.678±0.102, 0.445±0.043 vs 0.837±0.043, $P<0.05$); CHAT, VACHT decreased in MI+N group, but no difference compared to MI group (0.812±0.231 vs 1.129±0.450, 0.693±0.141 vs 0.749±0.176, $P>0.05$).

CONCLUSIONS The data indicated that Neuregulin-1 improved the cardiac function post-myocardial infarction. Neuregulin-1 can obviously inhibit the sympathetic nerve and can not apparently effect on the vagus nerve to modulate auto nervous system remodeling post.

GW27-e0873

Effect of Ginkgo biloba extract 761 on the cardiac fibrosis after myocardial infarction

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OBJECTIVES Myocardial infarction is one of the most serious cardiovascular diseases and is associated with considerable morbidity, mortality and disability rate. Myocardial fibrosis (MF) is an important pathophysiological change after myocardial infarction, which can lead to ventricular dysfunction, irreversible arrhythmia, heart failure, even cardiogenic sudden death. MF after myocardial infarction is tightly associated with the development and prognosis of the disease. However, currently available drugs against MF after myocardial infarction are still very limited. Ginkgo biloba extract is a well-known drug showing high physiological activity in therapies for diseases and is widely used more than 130 countries as a drug or food additive. Ginkgo biloba extract 761 (EGb 761) is a well-defined extract obtained from Ginkgo biloba leaves according to a standardized method. Consequently, a potential use of EGb 761 in lung fibrosis has been proposed. Therefore, this study was performed to investigate the role of EGb 761 in the MF after myocardial infarction.

METHODS Adult SD rats were randomly divided into: sham group, model group, and model + EGb 761 group (25 mg/kg, 50 mg/kg, and 100 mg/kg subgroups). The left anterior descending coronary artery was permanently ligated in the model and model + EGb 761 groups. The sham group was subjected to all procedures excluding permanent coronary artery ligation. The effects of EGb 761 on the MF after myocardial infarction were observed by hyaluronic acid (HA) concentration and Masson staining.

RESULTS Compared with the sham group, HA content was significantly increased in the model group. HA content was significantly decreased in the model + EGb 761 group compared with model group. Masson staining revealed that no blue area and inflammatory cell infiltrate was detected in the sham group. However, normal cardiomyocytes were replaced by disorderly arranged fibers and a massive inflammatory cell infiltrated in the model group. MF after myocardial infarction was significantly reduced in model + Egb761 group compared with model group.

CONCLUSIONS Our study suggested that EGb 761 could improve HA content and MF after myocardial infarction.

GW27-e0933

MicroRNA Profile and Regulatory Roles in Post-infarction Heart Failure

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OBJECTIVES Post-infarction induced heart failure (HF) is an end stage with a poor prognosis, but the molecular mechanisms have not been fully elucidated. In the study, we tried to discuss the precise regulatory mechanisms of post-infarction hear failure and to identify key regulatory miRNAs.

METHODS We determined the differential miRNA profile in a rat model of post-infarction heart failure through high throughout sequencing and analyzed the data through bioinformatics approaches. We validated the results through qRT-PCR. Expressions patterns of 4 miRNAs in different periods after myocardial infarction were analyzed. Finally, gain- and loss-of-function experiments of rno-miR-184 were conducted in H2O2 treated H9c2 cells.

RESULTS In the HF sample, 106 miRNAs were differentially expressed compared to the controls. GO and KEGG pathway analysis was conducted to predict the likely roles of these miRNAs. Time-course analysis revealed differential expression patterns of rno-miR-122-5p, rno-miR-199a-5p, rno-miR-184 and rno-miR-208a-3p. Rno-miR-184 was proved to promote apoptosis in vitro.

CONCLUSIONS Differential profile and expression patterns of miRNAs in post-infarction heart failure were found, and the pro-apoptotic role of rno-miR-184 was discovered.

GW27-e0939

Bone marrow mesenchymal stem cells-derived exosomes improve heart function contributing to angiogenesis and anti-Inflammation

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OBJECTIVES To investigate the paracrine effects of bone marrow mesenchymal stem cells (MSCs)-derived exosomes on angiogenesis and anti-inflammatory activity to improve heart function.

METHODS MSCs were cultured in DMEM/F12 supplemented with 10% exosome-depleted fetal bovine serum and 1% penicillin-streptomycin for 48 h. MSCs-derived exosomes were isolated using ExoQuick-TC. Tube formation and T-cell proliferation assays were performed to assess the angiogenic potency of exosomes. Acute myocardial infarction was induced in Sprague-Dawley rats. Phosphate-buffered saline (PBS, control), MSCs-derived exosomes, and exosome-depleted MSCs culture medium were injected at four different sites bordering the infarcted zone.

RESULTS MSCs-derived exosomes significantly promoted tube formation of HUVECs compared with frozen MSCs-derived exosomes (15688.42 ± 8656.62 vs. 11343.64 ± 986.78, respectively, $P < 0.05$). A significant increase in new capillaries was observed in the MSCs-derived exosome group than in PBS (1267.35 ± 84.23 vs. 744.38 ± 93.88, $P < 0.05$) and exosome-depleted CM groups (1267.35 ± 84.23 vs. 647.38 ± 103.23, $P < 0.05$). Blood vessel density increased in heart injected with exosomes than with PBS (132.42 ± 11.78 vs. 46.81 ± 10.29, $P < 0.05$). There were decreased inflammatory cells in the exosome group than in PBS and exosome-depleted CM groups ($P < 0.05$).

CONCLUSIONS MSCs-derived exosomes improve heart function after ischemic injury by stimulating neovascularization and restraining the inflammation response.

GW27-e0942

Farnesyl X Receptor Activation Ameliorates Post-infarction Cardiac Remodeling and Dysfunction

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OBJECTIVES Heart failure may develop insidiously years later after an acute myocardial infarction (AMI), despite the absence of ongoing ischemia. It is a staggering clinical and public health problem, associated with significant mortality, morbidity, and healthcare expenditures. So it is of urgent necessity to develop new targets to improve the prognosis of heart failure. Farnesyl X Receptor (FXR) is an endogenous bile acid receptor, belonging to the nuclear receptor superfamily, and it is also an important nuclear transcription factor. Recent studies have found that FXR is highly expressed in cardiomyocytes. During myocardial ischemia-reperfusion injury, myocardial FXR is up-regulated and promotes cardiomyocyte apoptosis. But there is still no relevant report about the role of FXR in myocardial infarction (MI). This project aims to identify the role of FXR in post-MI heart failure.

METHODS Forty-eight adult male C57BL/6J mice were randomly subjected to sham or permanent MI and divided into the following groups: sham+vehicle, MI+vehicle, sham+GW4064 (a specific agonist of FXR), MI+GW4064. At 24h after MI, GW4064 was administered by gavage (25mg/kg/d) for 3 weeks. After 3 weeks, Myocardial FXR expression and small heterodimer partner (SHP, a downstream molecule of FXR) mRNA levels were examined by Western blot and real-time PCR respectively to determine the transcriptional activity of myocardial FXR. Echocardiography was performed to assess cardiac function. Masson-trichrome staining, myocardial ANP and BNP (remodeling marker genes) mRNA levels, myocardial MMP9 protein and mRNA levels were used to evaluate cardiac remodeling and fibrosis. In vitro study used cardiomyocytes and cardiac fibroblasts isolated from neonatal Sprague-Dawley rats. After administration of 0mM, 0.1mM, 0.5mM and 1mM GW4064 for 10 hours, MMP9 protein and mRNA levels were determined.

RESULTS At 3 weeks after MI, myocardial FXR expression was downregulated. Transcriptional activity of FXR was also remarkably impaired as evidenced by reduced SHP mRNA level. GW4064 administration restored FXR transcriptional activity and significantly