GW26-e1803

Enhanced Expression of A-Disintegrin-and-Metalloproteinase-17 Promotes Extracellular Matrix Remodeling in Rats With Myocardial Infarction

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OBJECTIVES This study aimed to investigate the effects of dynamic expression of cardiac tissue A-Disintegrin-and-Metalloproteinase-17 (ADAM-17) on myocardial remodeling after myocardial infarction (MI).

METHODS Forty adult male Wistar rats undergoing MI operation were divided into four subgroups based on survival time: MI1d (at the end of the first day), MI1w (at the end of the first week), MI4w (at the end of the fourth week) and MI12w (at the end of the 12th week). Hemodynamic characteristics were assessed by echocardiography and relative inflammation factors of left ventricular (LV) cavity were measured by ELISA. ADAM17 mRNA level was analyzed by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Protein expressions of tumor necrosis factor-α (TNF-α), ADAM17 and tissue inhibitor of metalloproteinases-3 (TIMP-3) in ischemic cardiac tissue were analyzed by Western blot.

RESULTS One week after MI, left ventricular weight index (LVWI) was increased and systolic function examined by echocardiography was sharply worsen compared with that in Con1w subgroup ($P<0.05$). A reduced cell number and increased collagen accumulation were also displayed in the healing myocardium especially followed by a deteriorated MI-induced cardiac remodeling. TNF-α level was lower in the MI1w group compared with that in MI1d subgroup ($P<0.05$). However, ΔTNF-α level, the net change of TNF-α concentration between the MI and Con groups, was significantly increased ($P<0.05$) from the MI1d to the MI1w. ADAM17 mRNA expression was significantly increased, especially at the end of the 1st week after MI ($P<0.05$). The results of Western blot also reveals that the protein expressions of ADAM17 and TNF-α were significantly up-regulated, and the expression of TIMP-3 was simultaneously sharply decreased in the MI1w subgroup ($P<0.05$).

CONCLUSIONS Enhanced ADAM17 expression may participate in myocardial remodeling, especially in the early stage after MI.

GW26-e2144

Screening of Differentially Expressed MicroRNAs via MicroRNA Array Analysis Between Han and Uyghur Coronary Artery Disease Patients in Xinjiang

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OBJECTIVES MicroRNAs (miRNAs) are approximately 22-nt-long noncoding RNAs which negatively regulate gene expression by binding to sites in the 3' untranslated regions (UTRs) of targeted messenger RNAs. Data shows that miRNAs are present in the circulation and could be used as potential prewarning biomarkers of cardiovascular diseases. In this study, we performed miRNA arrays analysis on Uyghur and Han stable angina pectoris patients and acute coronary syndrome (ACS) patients to obtain differential miRNAs expression profile between two ethnic groups under stable (stable angina pectoris) and unstable (ACS) status of coronary artery disease (CAD).

METHODS 18 CAD patients in total were selected after angiographic documentation of CAD, including 5 Han ACS (HA) patients, 5 Uyghur ACS (UA) patients, 5 Han stable angina pectoris patients (HS) and 3 Uyghur stable angina pectoris (US) patients. Patients with heart failure, leukopenia, thrombocytopenia, severe hepatic or renal dysfunction and inflammatory or malignant disease were excluded. Firstly, Patients' blood samples were drawn so as to isolate RNA from EDTA-plasma by using mirVana miRNA Isolation Kit (ABI), then we pre-amplified the products with Megaplex™ RT Primers Human Pool A v2.1 (ABI) to raise the RNA concentration. Subsequently, RNA products were reverse transcribed using the TaqMan microRNA Reverse Transcription (RT) kit (ABI) according to the instructions of the manufacturer. Finally, the RT products were used for detecting miRNA expression with TaqMan Human MicroRNA Array by quantitative PCR for the corresponding miRNAs. Comparisons were drawn between UA and HA groups as well as US and HS individuals. Significantly changed ($RQ>2.0$ or $RQ\leq 0.5$, $p<0.05$) miRNAs were assessed for correlation with different ethnic groups.

RESULTS Three up-regulated miRNAs (hsa-miR-130a, hsa-miR-221, and hsa-miR-411) were identified in UA patients compared to HA patients (miR-130a,RQ=3.3307; miR-221,RQ=2.6705; miR-411,RQ=2.5407,$p<0.05$ for all three miRNAs). Among stable angina pectoris subjects, we detected three up-regulated miRNAs(hsa-miR-219-1-3p, hsa-miR-551b, and hsa-miR-886-5p) and two down-regulated miRNAs(hsa-let-7a, hsa-miR-128) comparing US to HS subjects. (miR-219-1-3p, RQ=2.1641; miR-551b, RQ=2.2171; miR-886-5p, RQ=2.2737; let-7a,RQ=0.1546;miR-128,RQ=0.4325, $p<0.05$ for all).

CONCLUSIONS The differentially expressed miRNAs in different ethnic groups under different states of the same disease may represent different pathology processes, probably due to the heterogeneity of genetic susceptibility and miRNA regulatory system. The significance of these findings needs to be further determined.

GW26-e2206

Phosphatidylinositol 4-Kinase β (PI4Kβ) and Phosphatidylinositol 4,5-Bisphosphate (PIP2) Modulated the Expression and Trafficking of BKCa Channels

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OBJECTIVES Phosphatidylinositols (PI) play crucial roles in regulating cytoskeletal organization and trafficking of membrane protein. PI metabolism is controlled by phosphatidylinositol kinases. Phosphatidylinositol 4-kinase β (PI4Kβ)-mediated phosphatidylinositol 4,5-bisphosphate (PIP2) production has been suggested to regulate biosynthetic traffic in yeast and mammalian cells. Here, we investigated the effect of PI4Kβ and PIP2 on the expression and trafficking of large conductance calcium activated potassium (BK_{Ca}) channels, the key modulator of the tone of vascular smooth muscles, expressing in HEK293 cells.

METHODS HEK293 cells with expressing BK_{Ca} channels α subunit (h*Slo1*) were constructed with transfection. BK_{Ca} currents were recording by patch clamp technique under whole-cell and inside-out configuration. Western Blotting, flow cytometry (FCM), confocal were used to investigate the expression and trafficking of BK_{Ca} channels. The direct interaction between phosphoinositides and BK_{Ca}channels was investigated with PIP strips.

RESULTS PI4Kβ increased the total and membrane expression of BK_{Ca} channels expressing in HEK293 cells. The wild type PI4Kβ ($PI4K\beta^{WT}$) increased the expression of BK_{Ca} channels, while the dominant negative, kinase-dead PI4Kβ ($PI4K\beta^{D656A}$, $PI4K\beta^{KD}$) decreased the expression of BK_{Ca} comparing with the control group. Wortmannin and PAO, the inhibitors of PI4Kβ, decreased the current density under whole-cell configuration. PIP2 increased total open probability (*N*Po) near three-fold from 0.011±0.002 to 0.036±0.008 ($P < 0.05$, n=6) under inside-out configuration. PIP strips shown that BK_{Ca}channels protein may bind with multiple PIs including P(3, 4)P2, P(3, 5)P2, P(4, 5)P2 and P(3, 4, 5)P3 directly. Wortmannin, PAO and the short hairpin interference RNA (shRNA) of PI4Kβ significantly inhibited the trafficking of BK_{Ca} channels protein to cell membrane with confocol and FCM ($P < 0.05$ or $P < 0.01$).

CONCLUSIONS these data above suggested that PI4Kβ increased the expression and trafficking of BK_{Ca} channels to membrane. PI4Kβ may control the PI metabolism and the production of downstream PIs such as PIP2 in order to modulate the expression and trafficking of BK_{Ca} channels indirectly.

GW26-e2335

The Construction of Nano-Protein Complexes and Induce Human Bone Marrow-Mesenchymal Stem Cells (hBMSCs) Differentiate Into Cardiac Progenitor Cells (CPCs)by Direct Reprograming

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OBJECTIVES To explore the best protein modified condition and influence factors to construct nano-protein complexes, and preliminarily analyze their process of entering into cells and intracellular metabolism. Then we try to induce hBMSCs toreprogram into CPCs by using this protein transfection technology, which shows high efficiency and low cytotoxicity to cells. It opens up new way for clinical cardiac reparations and regeneration treatment after myocardial infarction.

METHODS 4 kinds of cardiac specific transcription factors (Tbx5, Hand2, Mef2c, Gata4) are expressed and purified in an improved producing way, we've try to synthesis saveral compact and tightening