GW26-e1082

Activation of Cloned BKCa Channels by Hydrogen Peroxide Exhibited Increased Anti-oxidative Stress of HEK293 cells

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OBJECTIVES The present study was designed to clarify whether Hydrogen peroxide (H_2O_2) is an activator of BK_{Ca} channels in human embryonic kidney 293 (HEK293) cell and the function for anti-oxidative stress will change after stable expressing BK_{Ca} channel in HEK293 cell.

METHODS The cDNA encoding the α-subunit of BK_{Ca} channel, hSloα, was transfected into HEK293 cells. Stable cell line expressing BK_{Ca} channel was established by screening with G418. The cell viability in HEK-hSloα cells was detected using cell viability test (Cell Counting Kit-8,CCK8). The time-dependent (0, 6h, 12h, 24h, 48h) and concentration-dependent (0, 50μM, 100μM, 200μM, 400μM) curves of H_2O_2 effect on cell viability were also detected by CCK8. Changes in intracellular ROS levels were determined by measuring the oxidative conversion of cell permeable 2',7'-dichlorofluorescein diacetate (DCFH-DA) to fluorescent dichlorofluorescein (DCF) in fluorospectrophotometer. Single-channel patch clamp experiments were performed in HEK-hSloα cells to test the effect of H_2O_2 on BK_{Ca} channels.

RESULTS Optimized time and concentration of H_2O_2 effect were determined by time-dependent and concentration-dependent curves of H_2O_2 effect on cell viability. When the cells were cultured in different concentration of H_2O_2 for different time, treatment of HEK-hSloα cells with 100μM H_2O_2 for 24 h exhibited greater cell viability on cell survival compared to HEK293 cells. H_2O_2 (100μM) significantly increased the intracellular level of ROS. HEK-hSloα cells with 100μM H_2O_2 exhibited lower ROS level compared to HEK293 cells. Pretreatment with TEA (2mM) significantly elevated intracellular concentration of ROS by H_2O_2 in HEK-hSloα cells. Single-channel patch clamp experiments indicated that 100μM H_2O_2 would activate BK_{Ca} channels obviously with increased open probability.

CONCLUSIONS These results indicated that H_2O_2 could activate BK_{Ca} channel; HEK-hSloα cells under treatment of 100μM H_2O_2 for 24 h exhibited increased anti-oxidative stress ability; activation of cloned BK_{Ca} channels by Hydrogen peroxide was implicated in increased anti-oxidative stress of HEK293 cells.

GW26-e2238

The Contribution of Platelet MicroRNA Expression and its Interaction with CYP2C19 Allele Variants to Clopidogrel Antiplatelet Responsiveness in Acute Coronary Syndrome Patients

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OBJECTIVES To investigate relationship between platelet miRNA(miR-223, miR-221, miR-21) expression and clopidogrel antiplatelet responsiveness, as well as the contribution of platelet microRNA expression and its interaction with CYP2C19 allele variants on clopidogrel antiplatelet responsiveness in acute coronary syndrome (ACS) patients.

METHODS We enrolled consecutive 272 patients with ACS from December 2013 to December 2014 in our department of Cardiology. 39 cases were screened by the detection of the platelet aggregation. Then 39 cases were divided into two groups by the four quantiles: high platelet reactivity group of extreme values (high response group), 21 cases; and low platelet extreme value group (low response group), 18 cases. Bioinformatic analysis was used to investigate the candidate microRNA. Real time polymerase chain reaction (real time PCR)was performed to analyze the expression levels of all miRNAs which is band to platelet P2Y12 receptor, as well as to genotype candidate single nucleotide polymorphisms (SNP) including CYP2C19*2 and CYP2C19*3. Univariate and multivariate analysis and receiver operating characteristic curve (ROC curve) were utilized to assess the relationship between miRNA expression levels and clopidogrel platelet reactivity, the non-conditional Logistic regression was used to evaluate the interaction between expression of miRNA and CYP2C19 * 2 allelic variant.

RESULTS ① According to bioinformatic analysis and forecast, we finally confirmed three candidates microRNA, including miR-223, miR-221 and miR-21. ② Compared with the low response group, the levels of three miRNAs expression were up regulated with statistical differences (miR-223: $p = 0.002$; miR- 221: $p = 0.004$; miR-21: $p = 0.007$) in the high response group. ③ ROC curve analysis showed that the relative expression levels of the three miRNAs can effectively identify the high platelet response (miR-223: AUC = 0.704, miR-221: AUC=0.762, miR-21: AUC =0.796). ④ After multivariate adjustment, we found that the three microRNAs were independent predictors of high platelet reactivity. ⑤ Interaction analysis revealed significantly higher expression levels of miRNAs in CYP2C19 * 2 allele carriers who were high platelet responders (miR-223: $p= 0.037$; miR-221: $p = 0.001$; miR-21: $p < 0.001$),but not in CYP2C19 * 2 non-carriers ($p > 0.05$).

CONCLUSIONS Our study finds that up-regulation of three kinds of microRNAs (miR-223, miR-221 and miR-21) expression is associated with clopidogrel high platelet reactivity. What's more, we find that the interaction between the expression levels of miRNAs and the CYP2C19 * 2 variant carriers may have an impact on clopidogrel platelet reactivity. Our results suggest that miR-223, miR-221 and miR-21 may serve as the novel biomarkers for assessment clopidogrel antiplatelet responsiveness in ACS patients.

GW26-e0716

Forestalling SIRT1 Carbonyl Stress by ALDH2 Restores Autophagy in Aged Heart

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OBJECTIVES Cardiac aging is an intrinsic process that results in impaired cardiomyocyte proteostasis. We previously demonstrated that ALDH2 activation is sufficient to protect the heart against extravagant aging-related myocardial 'aldehydic load' induced proteoxicity. However, it is not known whether ALDH2 can regulate the clearance of damaged proteins in aged heart. Here, we determined the benefits of activation of ALDH2 on the autophagy by forestalling SIRT1carbonyl stress in aged heart.

METHODS Young adult, aged (21-22 mo.) male C57BL/6 mice and ALDH2 KO mice were perfused in the presence or absence of selective ALDH2 activator (Alda-1,6 mg/kg/d).

RESULTS Aging impairs cardiac ALDH2 activity. However, accumulation of 4-HNE-protein adducts and protein carbonyls concomitant with impairment in the autophagy flux seen in aged heart was ameliorated in Alda-1 treated aged mice, suggesting that increasing the ALDH2 activation contributes to the reduction of proteotoxicity and restoration of autophagy. Furthermore, ALDH2 KO mice progressively developed age-related heart dysfunction and increased cardiomyocyte ER stress. ALDH2 KO mice showed reduction in the life span, as compared with that of WT controls, strongly supporting that ALDH2 ablation leads cardiac aging. In addition, ALDH2 activation by Alda-1 led to a significant reduction of SIRT1 carbonylation and acetylation of FoxO1 as well as increased Rab7 expression in aged hearts, whereas SIRT1 was required for Alda-1-induced increases in autophagic flux. Although cardiac function was improved in sustained Alda-1treated aged mice after 48 hours of food starvation, it was significantly deteriorated in $Sirt1^{+/-}$ hearts, in which ALDH2 activation induced autophagy is inhibited.

CONCLUSIONS These results highlight that both accumulation of proteotoxic carbonyl stress along with autophagy decline contribute to heart senescence. ALDH2 activation is sufficient to improve the autophagy by reducing the carbonyl modification on SIRT1, which in turn plays an important role in maintaining cardiac health during aging.

GW26-e1051

MicroRNA-185 Aggravates Congestive Heart Failure by Targeting AKT1

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OBJECTIVES Mounting evidence has revealed the master regulatory role of microRNAs (miRNAs) in a variety of cardiac processes, including growth, development, cardiac function and response to diverse stress. However, the underlying mechanism of miRNA in congestive heart failure (CHF) is still less well studied. In the present study, we sought to investigate the biological role of miR-185 in CHF.

METHODS Quantitative real-time PCR (Q-PCR) was carried out to measure the circulating miR-185 in the serum of congestive heart failure patients (n=80). Meanwhile, a CHF rat model was established by intraperitoneal injection of doxorubicin. Echocardiography and pressure analyses were performed on all animals. Furthermore, Primary cardiomyocytes were cultured and transfected with miR-185

mimics or inhibitors to assess the in vitro effect of miR-211 on CHF. The target gene of miR-185 was predicted by using bioinformatic algorithm. A further luciferase reporter assay was performed to confirm the specific binding between miR-185 and the 3'-UTR of its target. Western-blots assays were used to check relevant gene expression.

RESULTS We found that circulating miR-185 was significantly upregulated in CHF patients as well as in the heart tissues from CHF rat model compared with respective controls. Silencing of miR-185 exhibited a cardiac protective role in doxorubicin induced mice model. Using Targetscan and Miranda, we identified AKT1 as the target of miR-185, which was confirmed by dual luciferase reporter assays. Ectopic expression of miR-185 enhanced the apoptosis of primary cardiomyocytes induced by doxorubicin by directly targeting AKT1. Downregulation of AKT1 phenocopied the pro-apoptotic effect of miR-185. Furthermore, administration of constitutively active AKT1 promoted primary cardiomyocytes proliferation, and reversed pro-apoptotic effect of miR-185 overexpression.

CONCLUSIONS MiR-185 exacerbates CHF and reduces cardiomyocyte proliferation through downregulation of AKT1, suggesting that miR-185 might be a target to manage cardiac dysfunction.

GW26-e4759

The Anti-atherosclerotic Effect of Pseudolaric acid B in High-Fat Fed ApoE Knockout Mice: Alterations in Circulating and Atherosclerotic Plaque Monocytes/Macrophages and The Underlying Mechanisms

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OBJECTIVES Atherosclerosis (AS) is the pathological basis of cardiovascular and cerebrovascular diseases. The chronic inflammation and lipid infiltration theory are thought to be the pathogenesis of AS. Mononuclear phagocyte (MNP) were key players involved in the lipid metabolism of plaque and chronic inflammation. Pseudolaric acid B (PB) is an extract of pseudolaricis. Recently, we show that PB could activate peroxisome proliferator activated receptor γ, while is an important molecular target involved in the process of inflammation reaction and lipid metabolism. Accordingly we hypothesis that PB has therapeutic potential for AS.

METHODS In vivo study, *ApoE*$^{-/-}$mice (8 weeks) were consumed high fat diet for 8 weeks, then randomly grouped into the baseline group, high fat diet group, PB treated with high fat diet group, normal diet group and PB treated with normal diet group (n=7 each group). We did experiments as followings, the proportions of monocyte subsets were analyzed. Confocal microscopy was used to investigate the density of the proliferation macrophages in plaque of aortic root. Oil Red O staining was used to explore the plaque size of aortic, common carotid artery and the aortic root. plasma triglycerides and total cholesterol concentration were measured by ELISA. In vitro study. Flow cytometry was used to explore the effluence and uptake of lipid in RAW264.7 cells by Dil-ox-LDL. RAW264.7 cells were intervened with oxidized low density lipoprotein for 24 hours, which would form foam cells, then divided into the model group, PB intervention group and PB with PPARγ antagonist block group. The Foam cells were subject to the following experiments, Oil Red O staining was used to investigate foam cell. The gene expression of PPARγ, LXRα, ABC1 and CD36 was measured by real-time PCR.

RESULTS In vivo study showed that, the proportion of Ly6C^{hi} monocyte subsets are lower in PB treated with normal diet (33.01%± 3.66% *vs.* 47.20%±3.43%, $P<0.05$). The density of the proliferation macrophages are lower in PB treated with normal diet ($P<0.05$). The levels of plasma triglycerides and total cholesterol are lower in the groups of PB treated with normal diet (all $P<0.05$). The load of plaque in the root of aorta, common carotid arteries and aorta tended to be lower in the groups of PB (12.02%±1.69% vs. 5.70%±1.38%, 7.14%± 1.57% *vs.* 3.71%±0.95% and 24.72%±3.80% *vs.* 15.3%±1.88%, respectively all $P<0.05$). In vitro study showed that PB can decrease the level of lipid in macrophage ($P<0.05$). PB can increase the gene expression of PPARγ (1.07±0.36 *vs.* 3.15±0.42, $P<0.05$), LXRα (0.99±0.06 *vs.* 2.05±0.22, $P<0.05$) and ABC1 (1.88±0.16 *vs.*0.96±0.06, $P<0.05$).

CONCLUSIONS PB with normal diet not only delay the progression of atherosclerosis but also reduce plaque burden. PB could activate the signal pathway of PPARγ-LXRα-ABC1 and PPARγ-CD36 to regulate the lipid droplet and efflux of foam cells, and inhibit the formation of foam cells.

GW26-e0194

Apelin Ameliorates Myocardial Insulin Resistance and Improves Myocardial Injury in Diabetes

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OBJECTIVES Apelin as a beneficial adipokine has been linked to insulin resistance and cardiovascular protection in diabetes, however, the underlying molecular mechanisms of how apelin affects myocardial insulin resistance and myocardial injury remain poorly understood.

METHODS We collected 131 T2DM patients serum, myocardial tissues of 8 patients with T2DM and 6 accidental deaths without T2DM at Beijing Hospital. After the TNF-α administration, C57BL/6J male mice were randomized to receive either vehicle, apelin-13(10μg/kg), or F13A (a competitive antagonist for apelin receptor 20μg/kg) by i.p. injection.

RESULTS Plasma samples of 131 patients with type 2 diabetes mellitus (T2DM) and 54 healthy controls, and myocardial tissues of 8 T2DM patients and 6 accidental deaths without T2DM were collected. Increased levels of plasma apelin, ROS and TNF-α were accompanied by insulin resistance and myocardial injury in patients with T2DM, similar results were confirmed in diabetic ob/ob mice, suggesting that apelin, TNF-α and ROS might be correlated with the states of insulin resistance. In vitro, TNF-α could stimulate myocardial insulin resistance, ROS generation, and apelin expression in H9c2 cells. However, exposure apelin-13 to TNF-α-treated H9c2 cells and intraperitoneal injection of apelin-13 to TNF-α-induced insulin-resistant C57BL/6J mice rescued TNF-α-induced higher intracellular glucose content and impaired insulin signaling pathway. Moreover, the results demonstrated that an intraperitoneal injection of apelin-13 improved MI-induced increased glucose content and cardiac injury as evidenced by attenuated myocardial infarct size and decreased myocardial apoptosis in both ob/ob mice and C57BL/6J mice. Further studies indicated that apelin-13 suppressed ROS generation through down-regulation of iNOS expression. Finally, F13A, a competitive antagonist for apelin receptor APJ, suppressed the effects of apelin-13 on insulin signaling pathway in both insulin-resistant H9c2 cells and insulin-resistant mice. Interestingly, F13A also impaired the effects of apelin-13 on iNOS expression, demonstrating that apelin ameliorated TNF-α-induced myocardial insulin resistance by its receptor APJ.

CONCLUSIONS We provide novel experimental evidence that apelin could ameliorate myocardial insulin resistance and improve myocardial injury though suppressing ROS generation via down-regulation of iNOS expression, in term, elevating insulin signaling transduction.

GW26-e0456

Exosomes Derived From Dendritic Cells Activate CD4+ T Lymphocytes After Myocardial Infarction

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OBJECTIVES Activation of $CD4^+$ T cells plays key roles in facilitating wound healing after myocardial infarction (MI). However, the mechanism of how $CD4^+$ T cells are activated post-MI remains largely unknown. This study was aimed to clarify whether exosomes derived from dendritic cells (DCs) mediate activation of $CD4^+$ T cells post-MI.

METHODS Bone marrow-derived DCs (BMDCs) of mouse were incubated with the supernatant of necrotic (Necrosis group) or normal HL-1 myocardial cells (Control group), or without stimulation (Negative group). DEXs were then isolated from the BMDCs and identified by electron micrograph and Western Blotting using the exosomal marker proteins. For DEXs tracker and dynamics experiments, the labeled DEXs (DiR) were injected into mice through tail vein and monitored by fluorescence imaging. For DEXs uptake experiments, the labeled DEXs (PKH67) were added to $CD4^+$ T cells suspensions or injected into mice tail vein, the $CD4^+$ T cells and frozen splenic sections were fixed for