Inhibition of the NF-kB pathway by the R65 ribonucleic gene via adeno-associated virus serotype 9 in human umbilical vein endothelial cells

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Objectives: NF-kB signaling plays a central role in the regulation of inflammatory responses in atherosclerosis. To our knowledge, it is less intensively studied whether R65 ribonucleic gene can decrease the expression of NF-kB pathway. Therefore, we used a recombinant adeno-associated virus serotype 9 vector to deliver the R65 ribonucleic gene (rAVV9-R65) into oxidized LDL (ox-LDL) induced human umbilical vein endothelial cells (HUVECs). Third, over-expression of rAVV9-R65 gene can be expressed for a longterm and inhibit the activity of NF-kB.

Methods: rAVV9-R65 was transfected into HUVECs at different multiplicities of infection, which was measured by flow cytometry. The activity of NF-kB pathway was analyzed by Western blot and Immunofluorescence staining. NF-kB inflammatory factors and endothelial cell function markers were measured by ELISA.

Results: First, rAVV9-R65 was transfected into HUVECs at different multiplicities of infection and it could be stably and efficiently expressed. Second, ox-LDL led to increased NF-kB expression in a dose- and time-dependent manner, and it increased inflammatory factors (TNF-α, IL-6) and cell apoptosis in HUVECs. Third, over-expression of rAVV9-R65 gene could inhibit the NF-kB pathway and protect HUVECs against ox-LDL induced cell apoptosis.

Conclusions: These results demonstrate that rAVV9-mediated R65 ribonucleic gene transfection may inhibit the expression of NF-kB and consequently down-regulate the expression of downstream components of NF-kB and its transcription factors.

Association of coronary artery disease and Metabolic Receptors C5aR and C5L2 gene

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Objectives: Adipose tissue receptors C5aR and C5L2 and their heterodimerization/functionality and interaction with ligands C5a and acylation stimulating protein (ASP) have been evaluated in cell and rodent studies. Their contribution to coronary artery disease (CAD) factors in humans remains unclear. We hypothesized that C5a receptors, classically required for host defense, are also associated with CAD.

Methods: Anthropometry and fasting blood parameters were measured in 492 CAD patients and 577 control subjects.

Results: C5L2 gene Heterozygote carriers of the CT genotype were more frequent among CAD patients than among controls (7.3% versus 1.7%). The odds ratio (OR) for carriers of the CT genotype for CAD was 4.484 (95% confidence interval (CI) : 2.197-9.174). C5AR gene Heterozygote carriers of the CT genotype were more frequent among CAD patients than among controls (4.7% versus 1.6%). The odds ratio (OR) for carriers of the 69CT genotype for CAD was 4.484 (95% confidence interval (CI) : 2.197-9.174). C5L2 gene C5L2/C5L2 carriers increased with BMI (P<0.001) with correlations between C5L2/C5L2 and waist circumference, HDL-C, and adiponectin.

Conclusions: C5L2 gene polymorphisms may be important risk markers for CAD. C5AR gene polymorphisms may be a genetic maker of CAD and highlight adipose-immune interactions.

DISTRIBUTIONAL CHARACTERISTICS OF β-ADRENERGIC RECEPTOR GENE PHENOTYPING AND ASSOCIATION WITH SERUM LIPIDIN HAN AND UIGHUR POPULATIONS IN XINJIANG

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Objectives: To investigate the distributional characteristics of β-adrenergic receptors gene polymorphisms (rs2298423 and rs6986132) and association with serum lipid in Han and Uighur populations in Xinjiang.

Methods: The genotypes of the ADRB3 gene were detected in 362 Uighur and 653 Han healthy individuals by real-time PCR (TagMan).

Results: (1) The frequencies of TT, CT and GG genotypes of the rs2298423 locus (rs6986132) locus were not different between the two populations (P>0.05). (2) The frequencies of GG,AG and AA genotypes of the rs6986132 locus were 81.5%,16.6% and 1.9% in Uighurs, and 59.6%,33.7% and 6.7% in Hans. There was no significant difference in distribution of genotypes between the two populations (P>0.001). (3) Total cholesterol and low density lipoprotein cholesterol level were significantly higher in GT or GT genotype than in TT genotype carrier of rs2298423 in Uighur individuals, but not in Han populations. The serum lipid level which include TC, LDL-C, TG, HDL-C were no significant difference between the two populations (P>0.001). (4) The mutational frequencies of the tagging SNPs in rs2298423 between the two populations. After adjusting for gender, age, height, weight, waist circumference, blood urea nitrogen, creatinine, glucose, triglyceride, smoking, drinking, logistic regression analyses revealed that individuals carrying GG or GT genotype of rs2298423 face an increased risk for total cholesterol and low density lipoprotein cholesterol level in Uighur populations in Xinjiang.

GENETIC POLYMORPHISMS OF SAA1 AND CORONARY ARTERY DISEASE RISK AND CONCENTRATION OF TOTAL CHOLESTEROL-LDL-C AND SAA IN THE UIGHUR AND HAN POPULATIONS

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Objectives: Serum amyloid A protein (SAA) is not only an inflammatory factor but also an apolipoprotein that can replace apolipoprotein A1 (apoA1) as the major apolipoprotein of HDL. However, the relationship between genetic polymorphisms of SAA1 and coronary artery disease (CAD) remains to be investigated.

Methods: 4 Single Nucleotide Polymorphisms (rs12218, rs4638289, rs7131332, and rs1603089) of SAA gene were genotyped by use of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method in two independent case-control studies: one was in the Han population (1 416 CAD patients and 1 373 healthy individuals) and the other was in the Uygur population (588 CAD patients and 529 control subjects).

Results: We found rs12218 CC genotype were more frequent among CAD patients than in the controls not only in the Han population (22.8% versus 17.0%, OR=1.38, 95% CI: 1.09-1.75) but also in the Uygur population (15.5% versus 11.3%, OR=1.05). After adjustment of confounding factors such as sex, age, smoking, drinking, hypertension, diabetes, serum levels of triglyceride, total cholesterol, high density lipoprotein, the difference remained significant in the Han group (P<0.001, OR=5.906, 95% CI: 2.877-12.124) and in the Uygur group (P=0.026, OR=3.288, 95% CI: 1.015-6.671).

Conclusions: Genetic polymorphisms of SAA1 may be a genetic maker of CAD in the Han and Uygur population in western China.

THE EFFECTS OF RESVERATROL ON CARDIOPROTECTION OF ISCHEMIC POSTCONDITIONING IN DIABETIC AND NON-DIABETIC RATS

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Objectives: This study aimed to investigate whether acute or chronic administration of Resveratrol (3, 5, 4’-trihydroxystilbene) affect the cardioprotective effects of ischemic postconditioning (Ipost) in diabetic and non-diabetic rat hearts.

Methods: Isolated rat hearts were subjected to 30 min of global ischemia, followed by 120 min of reperfusion. The hearts were subjected to the following treatments: non-conditioned (Ipost), post (six cycles of 10 s-reperfusion-ischemia after ischemia), acute Resveratrol (10 μmol/L Resveratrol during reperfusion), acute Resveratrol with Ipost, chronic Resveratrol (10 mg/kg/day Resveratrol for 2 weeks), and chronic Resveratrol with Ipost.

Results: Our results show that Ipost did not limit infarct size and recover contractile dysfunction in the hearts of diabetic rats (infarct size: 58.8% and 59.2%, P<0.05). Acute Resveratrol treatment with Ipost resulted in infarct size-limiting and contractile dysfunction-recovering effects in both diabetic and non-diabetic hearts (infarct size: 38.8% and 59.2% in diabetic ones and 24.3% and 43.7% in non-diabetic ones, P<0.05, respectively), and produced a further activation of Akt and eNOS signaling pathways to...
enhance these protective effects in the hearts of diabetic rats. Chronic Resveratrol treatment with the lowest neither reduced infract size nor increased myocardial dysfunction recovery in both diabetic and non-diabetic rats (infract size: 59.5% and 59.2% in diabetic ones and 45.0% and 43.7% in non-diabetic ones, P>0.05, respectively), and this might be associated with an inhibition of Akt and eNOS phosphorylation.

Conclusions: The combination of acute Resveratrol treatment with Ipost shows a stronger protective effect on the hearts of diabetic rats, but chronic Resveratrol treatment with Ipost fails to protect hearts against reperfusion injury in either diabetic or non-diabetic rat hearts. These findings will be important for the design of future clinical investigations.

GW25-e3108
Deletion of TRPV1 Accelerates DOCA-salt Hypertension-induced Renal Injury via Activation of CCR2
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Objectives: Our studies demonstrate that deletion of the transient receptor potential vanilloid type 1 (TRPV1) gene aggravates deoxycorticosterone acetate (DOCA) -salt hypertension-induced renal injury, which is associated with increased intrarenal monocyte/macrophage infiltration and inflammation. The results suggest that TRPV1 may act as a potential regulator of monocyte/macrophage infiltration to reduce renal injury in DOCA-salt hypertension. Therefore, this study was designed to test the hypothesis that deletion of TRPV1 exagerates salt-sensitive hypertension-induced renal injury via activation of chemokine receptor 2 (CCR2).

Methods: Male Sprague-Dawley rats (n=20) were treated with conventional saline and the MCP-1/CCR2 signaling pathway. [This work was supported by a grant from the National Natural Science Foundation of China (No. 81100243)].

GW25-e3129
β1-adrenergic receptor-mediated HO-1/IMGBl axis via PI3K and p38 MAPK attenuates rat myocardial ischemia/reperfusion injury in vivo
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Objectives: It has been reported that the induction of heme oxygenase-1 (HO-1) mediated by β1-adrenergic receptor inhibits high mobility group box 1 protein (HMGB1) release and increases the survival rate in cecal ligation and puncture-induced septic mice. The present study was aimed to investigate whether dobutamine, a selective β1-adrenergic receptor agonist, could inhibit HMGB1 release via β1-adrenergic receptor-mediated HO-1 induction and attenuate myocardial ischemia reperfusion (IR) injury in rats.

Methods: Anesthetized male rats were pretreated with dobutamine (5 or 10 microg/Kg, i. min-1, intravenous) before ischemia in the absence and/or presence of L-NAME (10 microg/Kg, intravenous). The myocardial I/R injury and oxidative stress were assessed. Likewise, the expressions of HO-1 protein, nuclear factor kappa B (NF-kappa B) p65, and HMGB1 were measured by Western blot analysis.

Results: After 4 h reperfusion, compared with I/R group, the pretreatment of dobutamine (5 or 10 microg/Kg, i. min-1) significantly reduced the infract size in a dose-dependent manner, and the increase of LDH and CK levels were significantly inhibited by dobutamine (both P<0.05 versus I/R group). Meanwhile, dobutamine dose-dependently reduced oxidative stress by inhibiting the increase of the MDA level and the decrease of the SOD level (both P<0.05 versus I/R group). Otherwise, dobutamine produced a statistically significant reduction in the production of TNF-α and IL-6 compared with the I/R group (both P<0.05 versus I/R group). Furthermore, compared with I/R group, dobutamine significantly and dose-dependently mediated HO-1 induction and NF-kappa B p65 and HMGB1 inhibition (all P<0.05 versus I/R group). However, all the effects caused by dobutamine were significantly reversely by the presence of LY294002, SB203580, and ZnPPix, respectively.

Conclusions: The present study demonstrated that dobutamine mediated the induction of HO-1 by selectively stimulating β1-adrenergic receptor via PI3K and p38 MAPK, which inhibited HMGB1 release and attenuated rat myocardial IR injury in vivo.

GW25-e3132
Single nucleotide Polymorphism of the CYP2J2 Gene is Associated with Essential Hypertension in Uygur Population in China
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Objectives: Human Cytochrome P450 2J2 (CYP2J2) is the major arachidonic acid epoxygenase, which can metabolizes arachidonic acid (AA) to bioactive epoxyeicosatrienoic acids (EETs). The EETs are potent endogenous vasodilators and inhibitors of vascular inflammation. Recently, much evidence from models and human studies has shown that variability in the CYP2J2 gene plays a mechanistic role in the development of hypertension. The aim of the present study was to assess the association between the CYP2J2 gene polymorphism and Essential Hypertension (EH) in a Han and Uygur population in China.

Methods: We used two independent case-control studies: A Han population (302 EH patients and 300 control subjects) and a Uygur population (567 EH patients and 215 control subjects). All EH patients and controls were genotyped for the same three single nucleotide polymorphisms (SNPs) (rs900293, rs11572223 and rs2280275) of CYP2J2 gene by a Real-time PCR instrument.

Results: In the Uygur population, the distribution of SNP3 (rs2280275) genotypes, alleles and the dominant model (CC vs CT + TT) showed a significant difference between EH and control participants (for genotype: P=0.007; for allele: P=0.001; for dominant model: P=0.002). The significant difference in dominant model was retained after adjustment for covariates (OR: 3.500, 95% confidence interval [CI]: 1.680-7.300, P=0.001). However, all the above differences were not shown in the Han population.

Conclusions: The CC genotype of rs2280275 in CYP2J2 gene could be a risk genetic marker of EH and T allele may be a protective genetic marker of EH in Uygur population in China.

GW25-e3140
Endothelial cells induced by inflammation release multiple angiogenesis-associated microRNAs into circulation through microparticles
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Objectives: Recent studies demonstrated that endothelial-derived microRNAs (miRNAs) can be detected in clinical plasma/serum samples, and there are also evidences indicated that inflammation disease states (e.g., atherosclerosis, cancer, etc.) may affect the levels of circulating miRNAs, but so far there is no direct proof about whether inflammation could induce endothelial cells to release miRNAs into circulation. This study aimed to explore whether inflammation could induce endothelial cells to release miRNAs into circulation and to investigate whether these released miRNAs derived from endothelial cells were transported in microparticles.

Methods: Microparticles were isolated from human atherosclerotic plaques with an active inflammatory phenotype and normal vascular tissue. Endarterectomy specimens constituting the intima and inner media of carotid atherosclerotic regions were obtained from the patients undergone carotid endarteretomy (CEA) (n=9). Patients undergoing coronary artery bypass surgery (CABG) procedure (n=9) were included as controls and the whole thickness vascular rings of normal vascular tissue (LIMA) were obtained. Flow cytometry and real time PCR were used to detect the levels of microparticles and microRNAs. To investigate whether these upregulated ECs-enriched miRNAs levels in the MPs isolated from atherothclerotic plaques were caused by inflammatory stimuli, human umbilical vein endothelial cells (HUVEC) were treated with TNF-α (TNF-α group, n=3) or DMEM (control group, n=3) for 24 hours, and then HUVEC and the culture medium were respectively collected.

Results: By comparing microparticles isolated from human atherosclerotic plaques with those derived from normal vascular tissue (LIMA, n=9), we found levels of annexin V+ microparticles and annexin V+ CD144+ microparticles were significantly increased in plaques (P <0.001) and angiogenesis-associated miRNAs (100b, 25, 92a and 21) were also significantly increased in microparticles from plaques (P <0.05). After exposure to TNF-α at a concentration of 10 ng/ml (TNF-α group, n=3) or DMEM (control group, n=3) for 24 hours, counts of microparticles and expressions of miRNAs-106b, 25, 92a and 21 in microparticles