Effect of homocysteine on the cultured rat vascular smooth muscle cell phenotype transformation

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OBJECTIVES To verify whether homocysteine (Hcy) could induce the dedifferentiation of vascular smooth muscle cells (VSMCs).

METHODS The primary culture and identification of rat VSMCs was conducted, using VSMCs in passage-4 for the following experiments. The VSMCs were divided into 4 groups: control group, Hcy (100 umol/L) modulation group, Hcy (500 umol/L) modulation group, Hcy (1000 umol/L) modulation group. MTT were used to investigate the proliferation of VSMCs. Transwell chambers and wound healing were employed to test the migratory ability of VSMCs. ICC were used to detect the VSMCs’ morphology structure. Western blotting used to investigate the expressions of SM-actin, SM-MHC, Calponin, OPN in VSMCs of every group.

RESULTS Compared with control group, the proliferation and migration ability of VSMCs were significantly increased in the Hcy modulation group, P<0.01. The expression of SM-actin had no significant difference between each group. The expression of SM-MCH and Calponin increased and OPN decreased in the Hcy group compared with control group, P<0.01. This effect of Hcy were positively correlated with its concentrations.

CONCLUSIONS Hcy could induce the dedifferentiation of VSMCs, this maybe the mechanism by which Hcy increased the proliferation and migration ability of VSMCs.

GW26-e2181 Association of LPA genetic polymorphisms with coronary artery disease in the Xinjiang Han and Uygur population of China

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OBJECTIVES Lipoprotein (a) [Lp(a)] is well known as an independent risk factor for coronary artery disease (CAD) and primarily determined by variation in the LPA gene coding for the apolipoprotein (a) moiety of lipoprotein (a). Our study was purposed to evaluate the association between the human LPA gene polymorphisms and CAD in Xinjiang Han and Uygur population of China.

METHODS 831 Han people (392 CAD patients and 439 control subjects) and 324 Uygur people (312 CAD patients and 317 control subjects) were selected for the present case-control study. Both patients and participants were genotyped for the same three single nucleotide polymorphisms (SNPs) (rs1801693, rs6931877 and rs9364559) of LPA gene by a Real-time PCR instrument.

RESULTS The rs1801693, rs6931877, and rs9364559 polymorphisms were found to be associated with CAD in Han population. For male, the distribution of SNPs (rs1801693) in genotypes, alleles and the recessive model (CC vs CT+TT) showed a significant difference (all P<0.05), the significant difference in the recessive models (CC vs CT+TT) was retained after adjustment for covariates just for the male (OR:0.52, 95% confidence interval [CI]:0.31-0.86, P<0.01). For total, the distribution of SNP2 (rs6932377) in genotypes and the dominant model (GG vs AG +AA) showed a significant difference for the recessive model (GG vs AG +AA) was retained after adjustment for covariates (OR:1.473, 95% confidence interval [CI]:0.002-3.02, P=0.049). For female, the distribution of SNP3 (rs9364559) in the alleles, the dominant model (AA vs AG +GG) and retained after adjustment for covariates also showed the significant difference(for the alleles: P=0.021, for the dominant model: P<0.029). For total, the distribution of SNPs (rs1801693) in the alleles, the dominant model (AA vs AG +GG) and retained after adjustment for covariates: OR:0.995, 95% confidence interval [CI]:0.345-2.56, P=0.016).

CONCLUSIONS Polymorphisms rs1801693, rs6932377 and rs9364559 of LPA gene are associated with CAD in Han population of China.

GW26-e2197 Ethnic disparities in recommended anthropometric cut-off values to identify the clustering of cardiovascular risk factors effectively in ostensibly healthy Chinese population in Xinjiang

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OBJECTIVES To investigate validity of widely recommended anthropometric cut-off points in screening for the clustering of cardiovascular risk factors in Chinese population of different ethnic.

METHODS 13090 participants (Uygur, Han and Kazakh, respectively) without hospital admission for cardiovascular disease were selected from the Cardiovascular Risk Survey (CRS). Sensitivity, specificity of body mass index (BMI; <30), waist circumference (WC; <88 cm), waist-hip ratio (WHR; >0.85), and waist-height ratio (WHHR; >0.5) cut-off points for the clustering of cardiovascular risk factors (dyslipidemias, hypertension and hyperglycemia) were calculated for each ethnic. Cut-off points yielding high sensitivity together with modest specificity were considered valid.

RESULTS The sensitivity of WC >88 cm for one or more risk factors was 57% in Han participants, and 81%, 83% in Uygur and Kazakh participants. The specificity of WC >88 cm for one or more risk factors was 72%, 53% and 49% at the three ethnics (Han, Uygur and Kazakh, p<0.05). WC >88 cm yielded >80% sensitivity for two or more risk factors across all ethnics. For BMI, specificity decreased in Uygur and Kazakh participants compared with Han participants (p<0.0001), being 33% and 35%. BMI, WHR and WHTR cut-off points were not better than WC.

CONCLUSIONS Validity of recommended anthropometric cut-off points in screening asymptomatic population varies with ethnic. In Han population, WC >88 cm yielded the highest sensitivity and modest specificity for two or more risk factors, however, sensitivity for one or more risk factor was less than optimal. Our results support ethносpecific screening cut-off points for Chinese population.

GW26-e2203 Haplotype-Based Case-Control Study of the Human CYP4F2 Gene and Essential Hypertension in the Chinese Han population

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OBJECTIVES CYP4F2 is responsible for metabolizing arachidonic acid to 20-hydroxyeicosatetraenoic acid (20-HETE), which plays a crucial part in the regulation of blood pressure in humans. The aim of the present study was to assess the association between the human CYP4F2 gene polymorphism and essential hypertension (EH) in the western Chinese Han population.

METHODS Four CYP4F2 SNPs were genotyped (rs558139, rs3093166, rs3093194, rs2108622) by the TaqMan® SNP Genotyping Assay in Real-Time PCR system. We examined the association between the four SNPs and EH using a haplotype-based case-control study that involved 405 EH patients and 396 control subjects.

RESULTS For men, the distribution of SNP1 (rs558139) alleles and the dominant model (CC vs CT+TT) showed a significant difference between EH and control participants (for allele: P=0.029; for dominant model: P=0.009). The significant difference in dominant model was retained after adjustment for covariates (OR:1.505, P<0.003), for men, the frequency of the C-A-G-T haplotype was significantly higher for EH patients than for control subjects (P=0.039).

CONCLUSIONS The CC genotype of rs558139 in CYP4F2 gene and the C-A-G-T haplotype could be a risk genetic marker of EH in the male Chinese Han population.

GW26-e2336 Association between the NFKB1-94ATGT ins/del polymorphism (rs2362491) and coronary artery disease: A meta-analysis

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OBJECTIVES It has been reported that single nucleotide polymorphisms (SNPs) rs2362491 in NFKB1-94ATGT ins/del might be associated with the susceptibility to coronary artery disease (CAD). Owing to mixed and inconclusive results, we conducted a meta-analysis to systematically summarize and clarify the association between the SNPs and CAD risk.
METHODS A systematic search of studies on the association of SNPs with susceptibility to CAD was conducted in PubMed, Embase, Cochrane Library and CNKI. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to pool the effect size. A total of 6 case-control studies on rs28362491 in NFKB1-94ATTG were included.

RESULTS The significant association was found between rs28362491 polymorphism and CAD risk in four genetic models, (D versus I: OR = 1.00; 95% CI 0.87-1.15; PD; OR = 0.90; 95% CI 0.74-1.10; ID; OR = 0.93; 95% CI 0.78-1.11; DD: OR = 0.94; 95% CI 0.79-1.11). A significant increased risk of CAD was observed in the rs28362491 polymorphism comparison, but there was insufficient data to fully confirm the association of CAD and rs28362491 in NFKB1-94ATTG.

CONCLUSIONS NFKB1-94ATTG ins/del rs28362491 polymorphism is correlated with CAD risk. However, the results of NFKB1-94ATTG rs28362491 should be interpreted with caution due to limited sample and heterogeneity. Large-scale and well designed studies are needed to validate our findings.

GW26-e2361
Association of APOB genetic polymorphisms and Aortic valve calcification in Han populations in Xinjiang, China
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OBJECTIVES Limited information is available when it comes to the impact of genetic on valvular calcification. Apolipoprotein B (apoB) is a key component in lipid metabolism and plays an important role in the dynamic equilibrium of cholesterol. The objective of this study was to investigate the association between aortic valve calcification and apoB genetic polymorphisms in the Han, Uyghur and Kazak populations in China.

METHODS 583 participants, including 172 cases with aortic valve calcification and 411 controls were selected for the present study. Two SNPs (rs6725189 and rs693) of apoB were genotyped by using the polymerase chain reaction-restriction fragment length (PCR-RFLP) method. Independent-sample t-test, chi-square test and logistic regression were used to analyze.

RESULTS The rs6725189 was found to be associated with aortic valve calcification in the dominant model, and the difference remained statistically significant following multivariate adjustment (p = 0.036, P = 0.004, respectively). The rs693 was found to be associated with aortic valve calcification in the recessive model, and the difference remained statistically significant following multivariate adjustment (p = 0.004, p = 0.028, respectively).

CONCLUSIONS Both rs6725189 and rs693 of the apoB gene are associated with aortic valve calcification in the Han and Kazak populations of China.

GW26-e2444
MtDNA as a biomarkers in acute myocardial infarction and its effects on myocardial cell
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OBJECTIVES An increasing studies have focused on the phenomenon that mitochondrial DNA (mtDNA) activates innate immunity responses. However, the specific role of mtDNA in acute myocardial infarction remains elusive. This study was designed to examine whether exogenous mtDNA can be served as a biomarker of acute myocardial infarction (AMI) patients and try to eliminate the damage effects of mtDNA on cardiomyocyte.

METHODS Plasma nuclear and mtDNA levels were measured by quantitative PCR in 50 AMI patients, 50 non-myocardial infarction (MI) with MI risk and 50 healthy control. Purified mtDNA or nuclear DNA was added to H9c2s cells, with or without pretreatment with chloroquine (an inhibitor of endosomal receptors like TLR9). The cell viability and apoptosis were tested by MTT and Flow cytometry, respectively. The levels of TLR9, p-p38 mitogen-activated protein kinase (MAPK) and caspase 3 were detected by western blot.

RESULTS The concentrations of mtDNA were significantly higher in the AMI group of hospital day 1 than that in the non-MI controls and healthy individuals (3.75±0.384 ng/μL vs. 1.85±0.3483 ng/μL, P<0.05; 3.75±0.384 ng/μL vs. 0.157±0.0924 ng/μL, P<0.05) and decreased shortly after PCI. Exogenous mtDNA reduced the viability of H9c2 cells and induced TLR9 and p-p38 MAPK and caspase 3 activation. These effects were inhibited by chloroquine. Nuclear DNA did not induce TLR9, caspase3, p-p38 MAPK activation.

CONCLUSIONS MtDNA level is increased after AMI and can used as a biomarker in AMI patients. MtDNA activates TLR9-P38MAPK and inducing cardiomyocyte cells death.

GW26-e2445
Protective effects and its mechanism of Helix B-surface peptide against cardiac microvascular endothelial cell injury induced by ischemia / reperfusion
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OBJECTIVES MI/R injury could paradoxically reduce the beneficial effects of myocardial reperfusion and cause contractile dysfunction and cellular damage, lacking of effective strategies of prevention cure. A helix-B surface peptide (HBSP), which is composed of 11 amino acids derived from the aqueous face of helix B of EPO, recently was developed and retained tissue-protective but avoiding erythropoietic property of EPO. Our previous experiment results have demonstrated that HBSP could reduce myocardial ischemia / reperfusion (MI/R) injury in rats and increased the post ischemic myocardial functions via activating the phosphatidylinositol 3-kinase (PI3-K)-Akt cascade. However, it is still unclear whether HBSP can protect the cardiac microvascular endothelial cells (CMECs) when subjected to ischemia / reperfusion injury Therefore, the aims of the present study were to investigate the protective effect of HBSP against cardiac microvascular endothelial cells (CMECs) injury induced by ischemia / reperfusion and further explored the underlying mechanisms involved.

METHODS CMECs isolated from the adult hearts of Sprague-Dawley rats were exposed to hypoxia and ischemia buffer for 2h followed by 4h reoxygenation. Then CMECs were randomized to receive different concentrations of EPO-derived peptides HBSP, EPO, HBSP plus LY294002 (specific inhibitor of PI3K), HBSP plus rapamycin (specific inhibitor of mTOR) at the start of reperfusion. The cell viability of CMECs was measured by MTT colorimetric assay and the apoptosis of CMECs was detected by Tunel method. The wound scratch assay and transwell method were performed to detect the migration of CMECs. The expression of p-AKT, p-mTOR and p-p70S6K were analyzed by western blot analysis.

RESULTS Both cell viability and migration ability of CMECs were impaired after SI/R (P <0.01 vs.control), and the apoptotic index increased in comparison with control group (P<0.01). While administration of EPO and HBSP during reperfusion dramatically attenuated the dysfunction of CMECs. Compared with the SI/R group, HBSP treatment in CMECs exerted protective effects as evidenced by the increase of cell viability (P<0.05), inhibited CMECs apoptosis (25.5±0.43% vs. 41.1±0.8%, P<0.01) and improved the migration ability of CMECs (P<0.05). Moreover, HBSP caused over Akt phosphorylation in the reperfusion the CMECs, which was abrogated by the treatment of LY294002 (P <0.05), but not by rapamycin. Furthermore, mTOR phosphorylation following HBSP treatment was prevented by either LY294002 or rapamycin (P<0.05). Similarly, the phosphorylation of the mTOR downstream molecule p70S6K were up-regulated by HBSP treatment (P<0.05). While treating with LY294002 or rapamycin prevented HBSP-induced phosphorylation of p70S6K (P <0.05). Compared with the HBSP group, the apoptotic index increased while treating with LY294002 or rapamycin (P<0.05).

CONCLUSIONS HBSP might have protective effect of CMECs against ischemia/reperfusion injury, which may be related with activation of PI3K/AKT/mTOR signaling pathway.

GW26-e2449
The Role of Calpain in Myocardial Apoptosis Induced by Oxidative Stress in Mouse cardiomyocytes Hypoxia/Reoxygenation
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OBJECTIVES In the present study, we aimed to explore the effects of calpain and its inhibitor PD169931 on oxidative stress induced myocardial apoptosis in mouse hypoxia/reoxygenation (H/R) injury.

METHODS The ventricular myocytes of adult C57BL/6 mice were isolated and cultured. The cardiomyocytes were randomly divided...