GW25-e0805

Insulin promotes the degradation of HDL Generation-Related Functional Protein ABCA1 through IRS/PI3K/Akt signaling pathway in 3T3-L1 adipocytes

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Objectives: Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of human death. Dyslipidemia is one of the most important risk factors for atherosclerosis. Although the early intensive statin therapy is closely associated with the improved survival in patients with coronary heart disease (CHD), cardiovascular residual risk still exists. Hyperinsulinemia/insulin resistance is the major risk factor for ASCVD. And decreased high-density lipoprotein (HDL) levels are usually the main changes of blood lipid spectrum of the hyperinsulinemia /insulin resistance. But the exact mechanism is not fully clearly understood. This study is aimed to discuss effects of high insulin environment on HDL generation-related functional protein ATP binding cassette transporter A1 (ABCA1), and explore mechanisms of its specific signaling pathways so as to provide a new basic medical evidence for the intervention of residual cardiovascular risks.
Methods: In this experiment, 3T3-L1 preadipocytes were induced to differentiation and maturation. Mature 3T3-L1 adipocytes were taken as the objects and stimulated by different concentrations of insulin (0 nmol/L, 10 nmol/L, 10^2 nmol/L, 10^3 nmol/L) for 12 hours. The efflux rates of [^{3}H]- cholesterol in cells were detected by liquid scintillation counter; Effects of different concentrations of insulin on ABCA1 mRNA expression in mature 3T3-L1 adipocytes were detected via real-time PCR; Effects of insulin on ABCA1 protein expression in mature 3T3-L1 adipocytes and related mechanisms were determined by Western blot.
Results: (1) Insulin could inhibit cholesterol efflux from 3T3-L1 adipocytes in a dose and time-dependent manner (both $P<0.05$); (2) Different concentrations of insulin had no effect on the regulation of ABCA1 mRNA ($P>0.05$); (3) Insulin could down-regulate ABCA1 protein expression in a dose and time-dependent manner (both $P<0.05$); (4) Insulin promoted ABCA1 protein degradation by calpain and proteasome pathway; (5) Insulin could promote insulin receptor phosphorylation in a concentration-dependent manner ($P<0.01$) and selective inhibitor LY294002 of PI3K/Akt signaling pathway could inhibit the role of insulin in promoting ABCA1 protein degradation, whereas the selective inhibitor Raf inhib I of MAPK signaling pathway could not.
Conclusions: We demonstrate for the first time that insulin promotes the ABCA1 protein degradation by IRS/PI3K/Akt signaling pathway, which is not conducive to ABCA1-mediated cholesterol efflux and nascent HDL generation in 3T3-L1 adipocytes.

GW25-e0862

Overexpression or Silencing of FOXO3a Affects Proliferation of Endothelial Progenitor Cells and Expression of Cell Cycle Regulatory Proteins

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Objectives: Endothelial dysfunction is involved in the pathogenesis of many cardiovascular diseases such as atherosclerosis. Endothelial progenitor cells (EPCs) have been considered to be of great significance in therapeutic angiogenesis. Furthermore, the Forkhead box O (FOXO) transcription factors are known to be important regulators of cell cycle. Therefore, we investigated the effects of changes in FOXO3a activity on cell proliferation and cell cycle regulatory proteins in EPCs.
Methods: The constructed recombinant adenovirus vectors Ad-TM (triple mutant) -FOXO3a, AdshRNA-FOXO3a and the control Ad-GFP were transfected into EPCs derived from human umbilical cord blood. Assessment of transfection efficiency using an inverted fluorescence microscope and flow cytometry indicated a successful transfection. Additionally; the expression of FOXO3a was markedly increased in the Ad-TMFOXO3a group but was inhibited in the Ad-shRNA-FOXO3a group as seen by western blotting. Overexpression of FOXO3a suppressed EPC proliferation and modulated expression of the cell cycle regulatory proteins including upregulation of the cell cycle inhibitor p27kip1 and downregulation of cyclin-dependent kinase 2 (CDK2), cyclin D1 and proliferating cell nuclear antigen (PCNA).
Results: In the Ad-shRNA-FOXO3a group, the results were counter-productive. Furthermore, flow cytometry for cell cycle analysis suggested that the active mutant of FOXO3a caused a noticeable increase in G1- and S-phase frequencies, while a decrease was observed after FOXO3a silencing.
Conclusions: In conclusion, these data demonstrated that FOXO3a could possibly inhibit EPC proliferation via cell cycle arrest involving upregulation of p27kip1 and downregulation of CDK2, cyclin D1 and PCNA.

GW25-e1437

Protective Effect of Tanshinone IIA through the Rho/Rho kinase system on oxidative stress injured human umbilical vein endothelial cell

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Objectives: To observe the intermittent high glucose induced human umbilical vein endothelial cells (HUVEC) injury in vitro, and to explore the protective effect of tanshinone IIA through the Rho/Rho kinase system on the injury of vascular endothelial cells.
Methods: The experiment was divided into: A. Intermittent high glucose injury group (5.5 mmol/L/20 mmol/L Glu intermittent high glucose group); B. Tanshinone II A. protection group (On the basis of the concentration of intermittent high glucose injury group tanshinone IIA 10, 30, 50 ug/ml); C. Positive control group (vitamin C in intermittent high glucose injury group based on the adding concentration of 100 mg/L). HUVEC was incubated with 48 h, the supernatant was measured in each groups of cells MDA, SOD, NO, NOS content and ROCK1mRNA content.
Results: Tanshinone IIA (10, 30, 50 ug/mL) drug protection group cell culture medium SOD, MDA, NO, NOS were relatively high 5.5 mmol/L/20 mmol/L Glu wave group increased, with 50 ug/ml of tanshinone II was more pronounced in the A group ($P<0.01$), the content of ROCK1mRNA in intermittent high glucose injury group compared to the normal control group and positive control group ($P<0.05$), tanshinone IIA protection group than in injury group decreased.
Conclusions: Tanshinone IIA may protect against oxidative stress injury in human umbilical vein endothelial cells through the Rho/Rho kinase system.

GW25-e1621

Cholesterol efflux regulating gene expression in peripheral blood leukocytes and associated factors for subjects with low plasma HDL Cholesterol Levels

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Objectives: Low high-density lipoprotein cholesterol (HDL-C) is a significant risk factor for atherosclerotic cardiovascular disease (ASCVD). as a cholesterol receptor, HDL improves cholesterol efflux from macrophage, and plays a important role in reverse cholesterol transport (RCT). However, Many studies have suggested that the formation of HDL is adjusted by related genes. Several cell cholesterol transport genes are played close attention. To investigate ATP binding cassette transporters (ABCA1 and ABCG1) gene expression in peripheral blood leukocytes from subjects with low plasma HDL cholesterol levels.
Methods: Thirty-eight cases were enrolled, and divided into two groups (control or low HDL-c group). Peripheral blood leukocytes ABCA1 and ABCG1 mRNA expression were measured by real-time quantitative PCR.
Results: ABCA1 mRNA expression in subjects with low plasma HDL Cholesterol Levels was obviously less than that in controls (0.23 ± 0.09vs1.18 ± 0.49, P=0.042; 0.18 ± 0.07vs0.39 ± 0.17, P=0.011), there was not different between two groups for ABCG1 mRNA expression (3.09 ± 1.08vs3.94 ± 1.48, P=0.355). The serum hscrp concentration in subjects with low plasma HDL Cholesterol Levels was statistically greater than that in controls (2.34 ± 1.68 vs 1.15 ± 0.48, P=0.008), and negatively associated with ABCA1 mRNA expression (r=-0.330, P=0.043).
Conclusions: ABCA1 mRNA expression in the peripheral blood leukocytes from subjects with low plasma HDL cholesterol levels was reduced, inflammation maybe a a mechanism, it is one probably factor to result in higher cardialvascular disease risk for these patients.

GW25-e1666

The knockdown of the HDAC1 gene promotes the directed differentiation of bone mesenchymal stem cells into cardiomyocytes

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Objectives: The objective of this study was to conduct a preliminary exploration of the role of the histone deacetylase 1 (HDAC1) gene in the phenotypic differentiation of rat bone mesenchymal stem cells (BMSCs) into cardiomyocytes by transfecting BMSCs with an HDAC1-RNAi lentiviral vector and thereby specifically inhibiting the expression of HDAC1 in BMSCs.
Methods: BMSCs from Sprague-Dawley (SD) rats were subjected to in vitro isolation, culture, and identification. In addition, HDAC1-RNAi lentiviral vectors were constructed, and screening procedures were implemented to identify the optimal vector and MOI for the infection of BMSCs. Real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) was utilised to detect the messenger RNA (mRNA) expression levels of various genes in infected BMSCs. In particular, these genes included not only GATA-binding protein 4 (GATA-4) and Nirenberg and Kim gene 2 homeobox 5 (Nkx2.5), which relate to myocardial development, but also