GW25-e0820

Apoptosis of THP-1 macrophage-derived foam cells induced by 5-aminolevulinic acid-mediated sonodynamic therapy is mitochondria-caspase pathway predominant despite the participation of endoplasmic reticulum stress

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Objectives: In advanced atherosclerosis, chronic endoplasmic reticulum (ER) stress induces foam cells apoptosis and generates inflammatory reactions. Sonodynamic therapy (SDT) is a non-thermal synergistic method for cancer treatment utilizing low-intensity ultrasound and sonosensitizers. 5-Aminolevulinic acid (ALA) is the biological precursor of sonosensitizer PpIX in the heme biosynthesis pathway in mitochondria. In this study, we investigated the sub-cellular location of ALA-PpIX in THP-1 macrophage-derived foam cells (FC) and the activation of mitochondria pathway and ER stress induced by ALA-SDT.

Methods: FC were incubated with 1 mM ALA. Fluorescence spectrometer was used to detect the location and metabolism of ALA-PpIX in mitochondria and endoplasmic reticulum (ER) of FC. Annexin V-FITC staining was used to optimize ALA-SDT treatment parameters by detecting the apoptotic and necrotic rates of FC induced by ALA-SDT with different ALA incubation time and ultrasound irradiation intensities. Intracellular reactive oxygen species (ROS) level after ALA-SDT was detected by staining with CellROX Green Reagent. Mitochondrial membrane potential after ALA-SDT was detected by staining with JC-1. Pretreated with ROS inhibitor N-acetylcysteine (NAC), pan-caspase inhibitor Z-VAD-FMK and ER stress inhibitor 4-phenylbutyrate (4-PBA), expressions of mitochondria apoptosis associated proteins cytochrome c, cleaved caspase3, cleaved caspase9, Bcl-2, BAX and ER stress associated protein CEBP-homologous protein (CHOP) in FC after ALA-SDT were detected by Western blotting.

Results: Accumulation of ALA-PpIX in mitochondria and ER reached peak at 6-hour, and the fluorescence intensity in mitochondria was triple of that in ER. The highest percentage of apoptotic cells (63.6%-9.8%) and the maximum apoptosis/necrosis ratio (21.7-16.3) was observed at 5-hour after ALA-SDT with 6-hour incubation of ALA and 0.4 W/cm² ultrasound intensity. After ALA-SDT, intracellular ROS level increased and the mitochondrial membrane potential collapsed. The translocations of cytochrome c from mitochondria into cytosol and Bax into cytosol were observed after cleaved caspase9, cleaved caspase3, downregulation of Bcl-2, as well as upregulation of CHOP were detected at 5-hour after ALA-SDT, which could be suppressed by NAC. Activation of mitochondria apoptosis pathway could not be inhibited by 4-PBA. Apoptosis induced by ALA-SDT could be inhibited by Z-VAD-FMK. 4-PBA reduced FC apoptosis by one third.

Conclusions: Mitochondria-caspase pathway is predominant in the apoptosis of FC induced by ALA-SDT though ER stress participates in.

GW25-e1534

SIRT1 protects against oxidative stress-induced endothelial progenitor cells apoptosis by inhibiting FOXO3a via FOXO3a ubiquitination and degradation

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Objectives: Endothelial progenitor cells (EPCs) -based therapy holds tremendous promise for the treatment of ischemic diseases. However, the function and survival of EPCs are dramatically impaired by oxidative stress. SIRT1 plays important roles in many pathophysiological processes such as apoptosis by deacetylating various substrates, including FOXO. However, little is known about the roles of SIRT1 in the regulation of EPCs apoptosis induced by H2O2. Our previous work showed that FOXO3a could promote apoptosis of EPCs by transcriptional regulation of Bim. In the present study, we investigated whether SIRT1 exerted a protective effect against H2O2-induced EPCs apoptosis and whether SIRT1 deacetylation of FOXO3a could facilitate FOXO3a ubiquitination and subsequent degradation.

Methods: EPCs were isolated and obtained from human umbilical cord blood by density gradient centrifugation. Incubation of EPCs with H2O2 was used to induce apoptosis. Apoptosis was determined by flow cytometry and DNA fragmentation. Western Blot analysis was used to examine the expression of SIRT1, FOXO3a, Bax and cleaved caspase 3. Adenoviral-mediated transfection was used to overexpress or downregulate SIRT1. Co-immunoprecipitation (co-IP) assay was performed to test the interaction between SIRT1 and FOXO3a, FOXO3a acetylation level and FOXO3a ubiquitination level.

Results: Immunofluorescence showed that SIRT1 localized in the nuclear of EPCs in the absence or presence of H2O2. SIRT1 expression in EPCs was increased by the treatment with H2O2 (500μM) for 24 hours. Incubation of EPCs with H2O2 dose dependently induced EPCs apoptosis. SIRT1 overexpression reduced H2O2-induced EPCs apoptosis, while SIRT1 downregulation and EX527, a specific SIRT1 inhibitor, exerted the opposite effect. SIRT1 overexpression decreased the total FOXO3a expression, whereas SIRT1 downregulation and EX527 increased the amount of FOXO3a. Co-IP assay showed that SIRT1 could bind to FOXO3a, reduce its acetylation level and increase its ubiquitination level.

Conclusions: The results of our work demonstrated that SIRT1 had a pivotal protective role in the regulation of EPCs apoptosis induced by H2O2 and that SIRT1 protected against apoptosis by inhibiting FOXO3a via FOXO3a ubiquitination and subsequent degradation.

GW25-e0540

Pharmacoepigenetics of clopidogrel responsiveness in Chinese patients with acute coronary syndrome

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Objectives: Cytochrome P450 (CYP), ATP-binding cassette transporters (ABC), and paraoxonase-1 (PON1) play crucial roles in clopidogrel metabolism. Genetic polymorphisms in these genes have been associated with the variability of the response to clopidogrel, however, there are controversies over the findings. The objective of the study is to elucidate the contribution of genetic polymorphisms in CYP2C19, ABCB1 and PON1 to clopidogrel responsiveness in Chinese acute coronary syndrome (ACS) patients.

Methods: Five hundred Chinese-Han patients treated with clopidogrel for ACS were consecutively recruited. We assessed the relationships of CYP2C19*2, CYP2C19*3, CYP2C19*7, PON1Q192R, PON1L114W, ABCB1C3435T and CYP2C19*2 with clopidogrel responsiveness (CYP2C19 loss-of-function allele in individuals of clopidogrel non-responsiveness.

Conclusions: The carriers with CYP2C19*2 and *3 were significantly associated with higher OTPR (P<10⁻⁶ and P=0.04, respectively). OTPR in carriers of at least one CYP2C19 loss-of-function allele (*2 or *3, accounted for 58% of the study population) was obviously higher than that in CYP2C19 wild type carriers (P<10⁻⁶). The carriers of at least one CYP2C19 loss-of-function allele could predict greater risk of HPR (adjusted OR: 1.79, 95% CI: 1.33-2.34, P=0.003). Patients with CYP2C19*2 alone, instead of CYP2C19*, had a higher risk for HPR (adjusted OR: 1.56; 95% CI 1.04-2.33, P=0.03). No significant relation of CYP2C19*17, PON1Q192R and ABCB1C3435T to OTPR and HPR was found in the cohort. In the subgroup of 180 patients, RII values were significantly lower in patients with PON1 192 QR and RR than in patients with QQ alleles (P=0.01). PON1 192 QR and RR conferred increased risk for clopidogrel non-responsiveness [adjusted OR: 3.64; 95% CI (1.21-10.92), P=0.02]. A trend for lower RII values was shown in carriers with CYP2C19*2 carriers compared to CYP2C19 wild type carriers (P=0.06). An increased risk for clopidogrel non-responsiveness was found in patients with CYP2C19*2 [adjusted OR 2.02; 95% CI (1.03-3.96), P=0.04]. No significant relation of CYP2C19*3, CYP2C19*17, and ABCB1C3435T to RII was found in the subgroup of patients.

Conclusions: In conclusion, in clopidogrel treated Chinese patients with ACS, carriers of at least one CYP2C19 loss-of-function allele could predict greater risk for HPR, with the impact mainly attributing to CYP2C19*. Both PON1 Q192R and ABCB1C3435T genotypes influence clopidogrel responsiveness, with the impact of PON1 Q192R mainly on relative platelet inhibition instead of HPR of clopidogrel.

GW25-e0542

Effect of intracoronary or intravenous tirofiban bolus administration on platelet activity and short time clinical benefit in patients with acute ST-segment elevation myocardial infarction undergoing emergency interventional treatment

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Objectives: To investigate the effect on platelet activity and short time clinical benefit of intracoronary or intravenous tirofiban bolus administration to patients with acute ST-segment elevation myocardial infarction undergoing emergency interventional treatment.

Methods: Selected 90 patients with acute ST-segment elevation myocardial infarction undergoing emergency interventional treatment, randomly divided into the intracoronary group (intracoronary tirofiban 10, 30 cases), intravenous group (intravenous tirofiban 10 μg/kg bolus then 0.15 μg/kg-1.min-1 intravenous continuous infusion for 36 h, 30 cases) and control group (without tirofiban, 30 cases). The level of PMPs was detected at 1, 5, 10 and 24 hours before tirofiban infusion, at 10 min and 24 hours after tirofiban infusion, and at 12 hours after stopping tirofiban infusion by the flow cytometry. Clinical and angiographic features were recorded and analyzed.

Results: There was no significant difference in baseline of PMPs between intracoronary group, intravenous group and control group (P>0.05). The level of PMPs were significantly lower in intracoronary group (3.66±2.3%) and intravenous group.
GW25-e0832
TNF-receptor-associated factor 6 (TRAF6) mediates the angiotensin-induced non-canonical TGFβ pathway activation and differentiation of c-kit+ cardiac stem cells
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Objectives: TNF-receptor-associated factor 6 (TRAF6) acts as a multifunctional regulator of the transforming growth factor (TGF)-β signaling pathway, and mediates the intrinsic platelet activation downstream of TNF receptor stimulation. This study was performed to test the hypothesis that TGF-β/TRA6 is essential for angiotensin-II (Ang II) -induced differentiation of rat c-kit+ cardiac stem cells (CSCs).

Methods: c-kit+ CSCs were isolated from neonatal Sprague Dawley (SD) rats, and TRAF6 were studied by coimmunoprecipitation. A TRAF6 dominant-negative construct was conducted to rule out the off target effects of the siRNA.

Results: Rescue of TRAF6 siRNA transfected cells with a 3′ UTR deleted siRNA activation was conducted to rule out the off target effects of the siRNA. TRAF6 dominant negative (TRA6D) vector was constructed and used to infect c-kit+ CSCs, and western blotting was used to assess the expression of TRAF6, JNK, p38, cardiac-specific proteins, and Wnt signaling proteins. Physical interactions between TRAF6 and JNK/TGFβ receptors were studied by coimmunoprecipitation.

Conclusions: Cardiac differentiation was suppressed in the absence of TRAF6. Forced expression of TRAF6 enhanced the expression of TGF-β-activated kinase1 (TAK1), and inhibited Wnt signaling. Furthermore, TRAF6 increased the expression of cardiac-specific proteins (cTnT and Cx-43) but inhibited the expression of Wnt signaling proteins.

GW25-e1647
Effects of Herbal medicine Aconite Compound on Heart failure
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Objectives: Currently, cardiosphere (CSP) and cardiosphere-derived cells (CDCs) are mainly obtained through myocardial biopsy and surgical, but there is limited access to tissue size, technical requirements and faced with infection, trauma and other problems, we assume that there still a large number of functional cSPs and CDCs through cadaveric cardiac, we can get a sufficient amount of functional CSPs and CDCs through cadaver autopsies.

Methods: Mouse (C57BL/6) were sacrificed and placed in a refrigerator at 4°C for 0 to 48h after death, the heart tissue was removed at different time points (D0, D1, D2, D3). No significant differences in proliferation ability were tested by cck-8, c-ELISA and vWF were stained by immunofluorescence. The proliferation ability was tested by cck-8, c-ELISA and vWF were stained by immunofluorescence. The proliferation ability was tested by cck-8, c-ELISA and vWF were stained by immunofluorescence.

Conclusions: After cardiac transplantation in mice, the number of CSPs and CDCs is greatly increased and has been verified in the current study. It is possible to get sufficient functional CSPs and CDCs through cadaver autopsies.

GW25-e4537
Statin effects on UA patients microRNA expression profile and regulatory network analysis
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Objectives: Unstable angina (UA), an acute coronary syndrome caused by disruption of atherosclerotic plaque triggered thrombosis. The blood vessel narrow and reduction of blood flow induce the symptom. Statin therapy benefits UA patients by cholesterol independent effect. Yet the mechanism of statin pleiotropic effect remained to be study. MicroRNAs (miRNAs), small non-coding RNAs, are post-transcriptional regulators of gene expression. In this study we aim to investigate statins’ novel mechanism mediated by miRNAs. Moreover we carry out systematic analysis of the miRNAs functional networks in atherosclerotic lesions.