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Apopotis of THP-1 macrophase-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 macrophase-derived foam cells (FC) and the activation of mitochondria pathway and ER stress induced by ALA-SDT.

Methods: FC were incubated with 1 μM 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 C/EBP-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%) and the maximum apoptosis/necrosis ratio (21.7%) 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 Bad and caspase 9 into mitochondria, cleaved caspase 9, cleaved caspase 3, 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 deacetylation 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 transduction was used to overexpress or down-regulate 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 presence or absence 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

Pharmacogenetics of clopidogrel responsiveness in Chinese patients with acute coronary syndrome

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Objectives: Cytochrome P450 (CYP), ATP-binding cassette transporters (ABC1), 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, and ABCB1C3435T to the on-treatment platelet reactivity (OTPR) after 5 days maintenance dose of clopidogrel administration, and the risk for high on-treatment platelet reactivity (HPR, defined as 20μmol/L ADP-induced platelet aggregation>50%). In addition, clopidogrel responsiveness, measured by RI (platelet aggregation-posttreatment aggregation at 5 days) and pretreatment aggregation x100%, was assessed in relation to the genotypes in a subgroup of 180 patients. RI values were stratified into four quartiles, with patients in quartile 1 defined as individuals of clopidogrel non-responsiveness.

Results: Along with CYP2C19*2 and *3 were significantly associated with higher OTPR (P<10⁻⁵ 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<10⁻⁵). The carriers of at least one CYP2C19 loss-of-function allele could predict greater risk of HPR (adjusted OR: 1.79, 95% CI: 1.33-2.4, P=0.003). Patients with CYP2C19*2 alone, instead of CYP2C19*,3, had a higher risk for HPR (adjusted OR: 1.56; 95% CI: 1.04-2.33, P=0.030). No significant relation of CYP2C19*17, PON1Q192R and ABCB1C3435T to OTPR and HPR was found in the cohort. In the subgroup of 180 patients, RI values were significantly lower in patients with PON1 Q192 R 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 RI values was shown in 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 RI 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*2. 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.

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Effect of intracoronary or intravenous tirofiban bolus administration on platelet activity and short time clinic 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 clinic 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 assessed before tirofiban administration at 10 min and 24 hours after tirofiban administration, 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 (3.66±2.39%) and intravenous group (2.31±0.85%) than in control group (7.44±2.2%).