are newly discovered regulatory factors. MiRNAs in PVAT, however, whether to participate in the regulation of vascular inflammation is still not clear. In this study, we try to seek miRNAs in PVAT associated with inflammation in the DOCA-salt mice.
Methods: (1) Establish animal model. DOCA-salt hypertensive mice with uninephrectomy were buried with DOCA pump (50mg) subcutaneously. Blood pressure were measured before and after DOCA-salt treatment by tail cuff method. The structure and inflammation phenotype were stained by H&E or immunohistochemical staining method. (2) The miRNA expression profile were measured by chip and then we validated miRNAs of interest by LNA-qRT-PCR. (3) The interaction between miRNA and its targeted genes were examined by dual luciferase report gene experiment and verified the relationship between miRNA and the target gene by gain or loss of miRNA. (4) The expression of target genes detected by western blot and immunofluorescence staining and study its effect on inflammatory factor at cellular level.
Results: (1) DOCA-salt caused a significant increase in blood pressure and mice in this group had thickened thoracic aorta vascular wall, smaller cell size in PVAT, increased proinflammatory cytokines OPN protein and macrophage marker F4/80. These suggests that inflammation occured in perivascular adipose tissue which structural change. (2) Compared with control group, microRNAs expression profile of DOCA-salt mice among PVAT, mesenteric adipose tissue (MAT) and aorta were different. After Venn diagram and signal value analysis, we selected let-7b as candidates and validated by LNA-qRT-PCR. let-7b decreased significantly up to 1.7 fold in DOCA-salt PVAT. (3) Through target genes predicting for let-7b and related analysis, ADRB3 may be involved in vascular inflammation process. (4) ADRB3 was a target of let-7b which was validated by dual luciferase reporter gene system and by gain or loss of let-7b expression. Furthermore, compared with control group, let-7b in DOCA-salt PVAT was significantly reduced while ADRB3 protein level increased significantly. (5) Angiotensin II caused increasing of ADRB3 protein and OPN in adipocytes while let-7b decreased slightly. The ADRB3 selective agonist CL316243 had similar effect. However, let-7b antagonists significantly increased ADRB3 protein levels, but the expression of OPN was not increased obviously. These suggested that activating ADRB3 can increase OPN level.
Conclusions: let-7b in PVAT can regulate ADRB3 protein expression, and activating ADRB3 caused the expression of OPN. These suggested that let-7b may indirectly involved in vascular adventitial inflammation process by adjusting ADRB3, providing a theoretical basis for new therapeutic targets in cardiovascular disease research.

GW25-e5231

Activin receptor-like kinase 7 silencing alleviates cardiomyocyte apoptosis, cardiac fibrosis and left ventricular dysfunction in type 2 diabetic rat

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Objectives: To investigate whether ALK7 plays an important role in modulating diabetic cardiomyopathy (DCM) and the mechanisms involved.
Methods: The model of diabetes was induced in male Sprague-Dawley rats (120-140g) by high-fat diet and intraperitoneal injections of low-dose streptozotocin (30 mg/kg). Animals were separated into 4 groups: control, DM, DM with ALK7 silencing (ALK7-siRNA group), and DM with vehicle control (vehicle group). The cardiac function was assessed by catheterization. Histopathologic analyses of collagen content and apoptosis rate, and protein analyses of ALK7, Smad2/3, Akt, Caspase3 and Bax/Bcl2 were performed.
Results: The results showed a rat model of type 2 DCM with hyperglycemia, severe insulin resistance, metabolism abnormalities, left ventricular dysfunction and structural remodeling. The insulin resistance and metabolism abnormalities in diabetic rats were ameliorated by ALK7 silencing. The impaired cardiac function in diabetic rats was partially restored by ALK7-siRNA treatment. Rats in ALK7-siRNA group showed significantly lower LVEDP compared with the vehicle group (12.43±1.62 vs. 22.85±2.91 mmHg, respectively; $P<0.001$). With ALK7 silencing, the cardiomyocyte apoptsis rate as well as protein level of cleaved Caspase3 and Bax/Bcl2 was significantly decreased in ALK7-siRNA group compared with vehicle group ($P<0.001$, $P<0.001$, $P<0.001$, respectively). The collagen deposition was significantly ameliorated in both the interstitial and perivascular areas in ALK7-siRNA group compared with vehicle group. Both the immunohistochemistry analysis and western blotting analysis showed decreased level of collagen I-to-III ratio in ALK7-siRNA group compared with vehicle group ($P<0.001$; $P<0.01$). Furthermore, the depressed phosphorylation of Akt was restored, and the phosphorylation of Smad2 and Smad3 was abolished by 62% and 37%, respectively, after the silencing of ALK7.
Conclusions: The results suggest ALK7 silencing plays a protective role in DCM and may serve as a potential target for the treatment of human DCM.

GW25-e0266

The ZEB1/miR-200c feedback loop regulates endothelial function in diabetic mice:a critical role of COX-2

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Objectives: The impaired vasodilation under diabetes is the main reason contributing to diabetes-associated vasculopathy. However, the role and the relevant mechanism of ZEB1/miR-200c feedback loop in diabetes-associated vascular dysfunction are currently unknown. Here we investigated the expression and modulation of miR-200c and ZEB1 and their downstream target, COX-2, in vascular dysfunction under diabetes.
Methods: qPCR and Western blotting were carried out to detected miRNA and protein expression. Adenoviruses were constructed to mediate ZEB1, miR-200c or anti-miR-200c ectopic over-expression or knockdown in primary endothelial cells and/or mouse aortae. Wire myograph system and pressure myograph system were utilized to analysis the endothelium-dependent relaxation in mouse conduit arteries and resistant arteries. Luciferase reporter gene assay was used to dissect the molecules involved in the functional variation.
Results: miR-200c was highly expressed in normal vascular walls, and increased in db/db mouse aortae and diabetic patient renal arteries; whereas ZEB1 expression was significantly decreased in *db/db* aortae. Overexpression of miR-200c dramatically suppressed ZEB1 expression in mouse primary endothelial cells, and vice versa. miR-200c severely impaired endothelial-dependent relaxations (EDRs) in the aortae of wild type but not COX-2 knockout mice. Consistent with this functional result, miR-200c-reduced vasodilation was rescued by cyclooxygenase-2 (COX-2) inhibitors (Celecoxib and NS398), but unaffected by cyclooxygenase-1 (COX-1) inhibitors (SC560) and ROS scavenger (Tempol). More importantly, overexpression of anti-miR-200c or ZEB1 greatly improved endothelium-dependent relaxations impaired by miR-200c or in diabetic conditions. Further examination indicated that miR-200c up-regulated COX-2 expression in endothelial cells via suppression of ZEB1, whereas over-expression of ZEB1 or anti-miR-200c inhibited the expression of miR-200c as well as COX-2 in *db/db* aortae.
Conclusions: Our data indicate for the first time that miR-200c and ZEB1 are new modulators for diabetes-related vascular dysfunction. These novel findings may have extensive implications for the diagnosis and drug intervention in the treatment of diabetic vascular complications.

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Mechanism of CML/RAGE inducing ERS-mediated apoptosis in atherosclerotic apoE-/- mice

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Objectives: The aim of our current study is to investigate whether and how non-cross-link and non-fluorescent CML, a major active AGE, affects the progression of atherosclerotic apoptosis in diabetes through ER-stress pathway.
Methods: The present study consisted of an in vivo investigation and three in vitro investigations. In study 1, male apoE$^{-/-}$ mice were first rendered diabetic by the administration of 5 daily intraperitoneal injections of streptozotocin (STZ, 40 mg/kg), and then given a semi-synthetic high-fat diet (HFD) plus daily injections of CML (10 mg/kg/day). The mice were euthanized and analyzed at 0 month (group 0M, n=10), 2 months (group 2M, n=10), and 4 months (group 4M, n=10) after the triple administrations of STZ-CML-HFD. In study 2, effects of CML in different concentrations on the apoptosis and lipid-accumulation of RAW264.7 under high-lipid conditions. In study 3, effects of CML on ER-stress of RAW264.7-derived foam cells under high-lipid conditions. In study 4, mechanisms of CML-inducing ER-stress apoptosis of RAW264.7-derived foam cells under high-lipid conditions. Related analyses (i.e., analysis of plasma lipid and glucose, immunohistochemical / immunocytochemical staining, oil red O staining, measurement of intracellular cholesterol, annexin V-FITC/PI double-staining and Western blot) were performed.
Results: Morphological analysis showed that the ERS-molecular chaperones GRP78 and CHOP were mainly restricted in the lipid pools of atherosclerotic plaques. Similarly, the expression of activated cleaved caspase-3 appeared in the aortic walls of all three groups, while its distribution was more enriched in the plaques. Dynamic analysis of plasma lipid and glucose indicated that hyperglycemia and hyperlipidemia in apoE$^{-/-}$ mice could be successfully induced by the triple administrations of STZ-CML-HFD for 2 months, and that the reverse cholesterol transport was severely impaired by the triple administrations for 4 months. Furthermore, the expression of CD36 and the ERS-related indexes including GRP78, Phospho-PERK, Phospho-eIF2, ATF4 and CHOP were significantly upregulated with the extension of the experimental time, while the expression of ABCA1 in aortic wall increased first and decreased afterwards. Consistent with the data of animal experiment, the results of oil red O staining, cholesterol oxidase method and western blot analysis showed that 10umol/L CML promoted cholesterol accumulation in RAW264.7 macrophages, but inhibited the reverse cholesterol transport. In addition, the upregulated extent of ERS-related indexes and apoptosis rate by 10 umol/L CML was significantly stronger than by 50 ug/mL oxLDL. Subsequent cell study with the use of anti-RAGE blocking antibody, anti-CHOP blocking antibody, caspase-12 inhibitor Z-ATAD-FMK and JNK inhibitor SP600125, respectively, suggested that CML plays the key role in ERS-apoptosis mainly through CML/RAGE -Lipid accumulation-PERK-eIF2 -ATF4-CHOP - Caspase-3-Apoptosis pathway. In addition, caspase-12 may account for a little part of apoptosis induced by CML, but this part of apoptosis isn't transmitted by RAGE.
Conclusions: The CML/RAGE axis may play an important role in the development of diabetic atherosclerosis through the mechanism that induces the lipid-accumulation followed by ERS - apoptosis of aortic foam cells.