Results: 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 intimal thickening 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 immuno-fluorescence 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 the thoracic aortic wall. The DOCA-salt mice aorta had thicker aortic wall, smaller cell size in PVAT, increased proinflammatory cytokines OPN protein and macrophage marker F4/80. These suggests that inflammation occurred 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 candidate 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 in DOCA-salt PVAT. (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. Therefore, let-7b may be a target gene of diabetes-related aortic wall remodeling. (5) Let-7b overexpression could cause significant upregulation of inflammation in PVAT and aorta of diabetic mice. Let-7b antagonists significantly decrease inflammatory response of let-7b in aorta with high glucose. Furthermore, let-7b antagonists significantly decrease inflammatory response of let-7b in aorta with high glucose. Let-7b significantly increased inflammatory cytokines OPN protein and macrophage marker F4/80. These suggested that activating ADRB3 could cause OPN expression. Let-7b could indirectly involved in vascular inflammatory process by adjusting ADRB3, providing a theoretical basis for new therapeutic targets in cardiovascular disease research.

GW25-e0231 Activin receptor-like kinase 7 silencing alleviates cardiomyocyte apoptosis, cardiac fibrils and left ventricular dysfunction in type 2 diabetic rat Liu Lin, Ming Zhong, Li Li, Yuanuang Shang, Zhihao Wang, Yun Zhang, Yuguao Chen, Wei Zhang, Mengxiang Tang Qin Hospital of Shandong University Objectives: To investigate whether AKL7 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 AKL7 silencing (AKL7-siRNA group), and DM with vehicle control (vehicle group). The cardiac function was assessed by catheterization. Histopathologic analyses of collagen deposition content and apoptosis rate, and protein analyses of AKL7, Smad2/3, Act, Caspase, and Bax/Bcl2 were performed. Results: The results showed a rat model of type 2 DMC with hyperglycemia, severe insulin resistance, metabolic abnormalities, left ventricular dysfunction and structural remodeling. The insulin resistance and metabolism abnormalities in diabetic rats were ameliorated by AKL7 silencing. The impaired cardiac function in diabetic rats was partially restored by AKL7-siRNA treatment. Rats in AKL7-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 AKL7 silencing, the cardiomyocyte apoptosis rate as well as protein level of cleaved Caspase3 and Bax/Bcl2 was significantly decreased in AKL7-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 AKL7-siRNA group compared with vehicle group. Both the immunohistochemistry analysis and western blotting analysis showed decreased level of collagen I-III ratio in AKL7-siRNA group compared with vehicle group (P<0.001; P=0.064). Furthermore, the expression of collagen I and III was significantly decreased in AKL7-siRNA group compared with vehicle group (P=0.001; P=0.001). The results suggested that AKL7 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 Huaizhao Zhang1,2, Jiao Liu, Yu Huang2 1National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 2Institute of Vascular Medicine and Li Ka Shing Institute of Health Sciences, Chinese University of Hong Kong 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 adenoviral vector, respectively. Oxygen- and nitric oxide-induced apoptosis were determined by flow cytometry. Cell death rate was determined by Annexin V-FITC/PI double-staining and Western blot (PI). PI double-staining and Western blot) were performed.

Results: miR-200c was highly expressed in normal vascular walls, and increased in db/db mouse aorta and diabetic patient renal arteries; whereas ZEB1 expression was significantly decreased in db/db aorta. Overexpression of miR-200c dramatically suppressed diabetes-induced ZEB1 expression in mouse primary endothelial cells, and vice versa. miR-200c severely impaired endothelial-dependent relaxations (EDRs) in the aorta 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, which was significantly stronger than ZEB1. Furthermore, overexpression of ZEB1 or anti-miR-200c inhibited the expression of miR-200c as well as COX-2 in db/db aorta.

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.

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-linked, 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 on ER-stress 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, oxenim 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, AT F4 and CHOP were significantly up-regulated 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 1) the formation of atherosclerotic plaques. The fluorescence CML, a major active AGE, has significantly stronger than ZEB1. Furthermore, overexpression of ZEB1 or anti-miR-200c inhibited the expression of miR-200c as well as COX-2 in db/db aorta.

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 cell layers.