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 uninn- ephrectomy were buried with DOCA pump (50mg) subcutaneously. Blood pressure were measured before and after DOCA-salt treatment by tail cuff method. The structure and inflammatory cytokine profile 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-q-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 immu- noluciferase 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 DOCA-salt had bigger aortic aorta vascular 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 can- didates and validated by LNA-qRT-PCR. (3) The interaction between miRNA and its target 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.

Conclusions: (1) Establish animal model. DOCA-salt hypertensive mice with unin- ephrectomy were buried with DOCA pump (50mg) subcutaneously. Blood pressure were measured before and after DOCA-salt treatment by tail cuff method. The structure and inflammatory cytokine profile 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-q-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.

activin receptor-like kinase 7 silencing alleviates cardiomyocyte apoptosis, cardiac fibrillation 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 intraperterional 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 echocardiography. Histopathologic analyses of collagen content and apoptosis rate, and protein analyses of ALK7, Smad2/3, Akt, Caspase-3, and Bax/Bcl2 were performed.

Results: The results showed a rat model of type 2 DCM with hyperglycemia, severe inflammatory response, metabolic abnormalities, cardiac fibrillation and structural and re- 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 LVDFP compared with the vehicle group (12.4±3.6 vs. 22.8±5.91 mmHg, respectively; *P<0.001). With ALK7 silencing, the card- iomyocyte apoptosis 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 signifi- cantly 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-III ratio in ALK7- siRNA group compared with vehicle group (P<0.001; P<0.001). 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.

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 adenovirus for regulate over-expression or knockdown in primary endothelial cells and/or mouse aorta. 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 aorta and diabetic patient renal arteries; whereas ZEB1 expression was significantly decreased in db/db aorta. 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 (Cele- cotib and NS398), but unaffected by cyclooxygenase-1 (COX-1) inhibitors (SC-560) 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 endotelial cells, which directly increased inflammatory factor at cellular level.

Conclusion: Our data indicate for the first time that miR-200c and ZEB1 are new targets for treating vascular dysfunction in atherosclerosis. 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 intraperterional 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 adminis- tration of STZ+CML+HFD. In study 2, we examined the effects of CML 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 up-regulated with the extension of the experimen- tal period, 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 CML treatment promoted the progression of atherosclerotic plaque.

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 foam.