evaluate the therapeutic effect after shPHD2-EGFP gene transfer by UTMD, a flow cytometric analysis was used to demonstrate that silencing of PHD2 reduced the H9C2 cell apoptosis induced by oxygen glucose deprivation (OGD), and molecular studies revealed a significant inhibition of PHD2 and activation of HIF-1α and its downstream genes in the shPHD2-EGFP group (P \(< 0.05"). In vivo, we also studied the transfection efficiency of naked plasmid DNA using ultrasound with CMb transfection into the myocardium. Three days after transfection, EGFP in myocardium transfected with CMbs using ultrasound was significantly increased compared to that in the other treatment groups (P \(< 0.05). The impact of UTMD-mediated shPHD2 transfection on the protective effects was evaluated with a TUNEL assay. The number of TUNEL+ cardiomyocyte nuclei per mm² was significantly reduced in shPHD2-EGFP-treated hearts at 48 hours post-MI compared with EGFP-treated hearts. Finally, we examined the feasibility of therapeutic angiogenesis using naked shPHD2-EGFP plasmid in a rat ischemia model using the UTMD method.

RESULTS: Four weeks after transfection, compared with the MI-EGFP group, left ventricular function at the infarct area had improved and scar size was reduced in the MI-shPHD2-EGFP group (P \(< 0.05). PHD2 expression was lower and HIF-1α and its downstream expression were higher in the MI-shPHD2-EGFP group than in the sham, MI-EGFP and MI-shPHD2-EGFP groups (P \(< 0.05). The numbers of HIF-1α and VEGF-positive cells/mm² in the MI-shPHD2-EGFP group were greater than those in the MI-EGFP group (P \(< 0.05). Capillary densities in the MI-shPHD2-EGFP group were increased 2.95-fold (P \(< 0.05) over those in the MI-EGFP group.

CONCLUSIONS: In conclusion, ultrasound with CLMs successfully promoted the transfection efficiency of plasmid DNA in vitro as well as in vivo, thus improving the efficacy of therapeutic angiogenesis and myocardial function. The UTMD technique may serve as an ideal method for noninvasive, repetitive gene delivery after an MI.

GW29-e1780 Acacetin inhibits the apoptosis induced by lysophosphatidylcholine via activating Nr2f2 in cultured rat aortic vascular smooth muscle cells
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OBJECTIVES: Our recent study demonstrated that the major ox-LDL component lysophosphatidylcholine (lysoPC) induced a remarkable intracellular Ca²⁺ increase mediated by Ca²⁺ influx via TRPC1/TRPC3 channels and apoptosis in primary cultured human coronary vascular artery smooth muscles cells (VSMCs). In the present study, we investigate whether the natural flavone acacetin would inhibit the intracellular Ca²⁺ overload and apoptosis induced by lysoPC in primary cultured rat aortic VSMCs.

METHODS: Rat aortic VSMCs were primarily cultured for determining the effects of acacetin on Ca²⁺ influx, apoptosis, and related changes in signal molecules induced by lysoPC with multiple biochemical and molecular biological approaches.

RESULTS: It was found that acacetin had no effect on intracellular Ca²⁺ overload, but remarkably inhibited cell apoptosis induced by lysoPC via increasing the anti-apoptotic protein Bcl-2, and decreasing the pro-apoptotic proteins Bax and cleaved caspase-3 expression. Additional experiments revealed that these effects were associated with upregulating the Nr2f2 nuclear translocation, followed by increasing HO-1, NQO-1 and catalase, thereby reversing SOD-1 reduction. Moreover, we observed that acacetin could inhibit oxygen species (ROS) production induced by lysoPC. Silencing Nr2f2 with siRNA abolished the anti-apoptotic action of acacetin in primary cultured rat aortic VSMCs.

CONCLUSIONS: Our results demonstrate for the first time that acacetin can suppress lysoPC-induced VSMCs apoptosis via reducing ROS production by activating Nr2f2 anti-oxidation signaling pathways. Thus, acacetin may play an important role in preventing the pathophysiological process of disorders associated with apoptosis of VSMCs, such as atherosclerosis and restenosis.

GW29-e1785 Cardiac Fibroblast Specific Deletion of Thioredoxin Interacting Protein (TXNIP) Exacerbates Ventricular Remodeling and Dysfunction After Myocardial Infarction.
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OBJECTIVES: Fibrosis is a hallmark of adverse ventricular remodeling after myocardial infarction (MI). Metabolic reprogramming of cardiac fibroblasts (CFs) provides essential energy and permits them to rapidly differentiate, proliferate, and produce and degrade extracellular matrix in anoxic and nutrient-deficient microenvironments. However, the underlying mechanisms are still unclear. Thioredoxin interacts with non-atherosclerotic antioxidant proteins and is an inhibitor of the antioxidant thioredoxin (Trx), but very little is known about its roles in the CF metabolism and myocardial fibrosis progression. Here we sought to determine if TXNIP affects CF metabolism and myocardial fibrotic progression after MI.

METHODS: To obtain inducible, fibroblast-specific TXNIP deficient mice, collagen12-CreER (T) mice were crossed with TXNIPfl/fl mice. MI models were induced by permanent coronary artery ligation and ventricular fibrosis progression was evaluated in wild-type (WT) and fibroblast-specific TXNIP knockout (Col-KO) mice.

RESULTS: Herein we report that TXNIP is obviously downregulated in myocardial fibroblasts and fibrotic tissues isolated from post-MI mouse hearts. Next, we show that deletion of TXNIP in CFs leads to fibrogenesis, left ventricular dysfunction and excessive scarring in the post-MI heart. Deletion of TXNIP induces a profibrotic myofibroblast phenotype in both human and mouse CFs and in post-MI mouse hearts. Mechanistically, TXNIP crucially reduces glycolysis of CF during their transformation to myofibroblasts independent of its binding to Trx and redox regulatory effects. Notably, TXNIP transcriptionally inhibits the expression of multiple glycolytic enzymes in CFs, which is intrinsic to its conserved arrestin domain. Moreover, a small molecule inhibitor of glycolysis largely prevented post-MI fibrosis and limited LV remodeling in both WT and Col-KO mice.

CONCLUSIONS: These studies support TXNIP is a key regulator of CF metabolic reprogramming and cardiac fibrotic progression following MI as an arrestin protein. Targeting TXNIP in CFs might be a novel therapeutic strategy for post-MI ventricular fibrosis and dysfunction.

GW29-e1787 Cig/TNF-Related Protein 5 Promotes Atherogenesis by Enhancing Transcytosis and Oxidative Modification of Low-density Lipoprotein through Increasing 12/15-Lipoxygenase
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OBJECTIVES: Increased transcytosis of low-density lipoprotein (LDL) across the endothelium and oxidation of LDL deposited within the subendothelial space are crucial early events in atherosclerosis. Cig/TNF-related protein (CTRP5) is a novel secreted glycoprotein and its biological functions are largely undefined.

METHODS: In this study, we analyzed CTRP5 levels in sera of patients with coronary artery disease (CAD, n = 288) and non-CAD controls (n = 264). In this study, we analyzed CTRP5 levels in sera of patients with coronary artery disease (CAD, n = 288) and non-CAD controls (n = 264). The role of CTRP5 in LDL transcytosis and oxidative modification of LDL in human atherosclerotic lesions. In vivo and in vitro experiments demonstrated that CTRP5 was predominantly localized in endothelium and macrophages in human atherosclerotic lesions. In vivo and in vitro experiments demonstrated that CTRP5 is a novel secreted glycoprotein and its biological functions are largely undefined.

RESULTS: We found CTRP5 serum levels were higher in patients with than without CAD (247.26 ± 61.71 vs. 167.81 ± 68.08 ng/mL, P < 0.001), and were positively correlated to the number of diseased vessels (Spearman’s r = 0.611, P < 0.001). Increased expression of CTRP5 was detected in human coronary endarterectomy specimens as compared to non-atherosclerotic arterial tissues. Immunohistochemistry showed that CTRP5 was predominantly localized in endothelium and macrophages in human atherosclerotic lesions. In vivo and in vitro experiments demonstrated that CTRP5 promoted transcytosis of LDL across endothelial monolayers, as well as the oxidative modification of LDL in human atherosclerotic arterial tissues. Immunohistochemistry showed that CTRP5 was predominantly localized in endothelium and macrophages in human atherosclerotic lesions. In vivo and in vitro experiments demonstrated that CTRP5 was predominantly localized in endothelium and macrophages in human atherosclerotic lesions. In vivo and in vitro experiments demonstrated that CTRP5 is a novel secreted glycoprotein and its biological functions are largely undefined.

CONCLUSIONS: These data indicate that CTRP5 is a novel proatherogenic cytokine and promotes transcytosis and oxidation of LDL in endothelium through up-regulating 12/15-LOX.