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tissue engineering as biologic scaffold in recent years. Before achieving the ultimate goal of engineering a functional heart, however, some challenges regarding the scaffold preparation and the sources of seeding cells still need to be addressed. The purpose of this study was to establish and optimized the methodology of decellularized heart scaffold. It's also expected to establish a platform for the decellularied heart tissue-based scaffold evaluation in vitro.

METHODS The decellularized heart scaffold was prepared by coronary perfusion. Histological staining, DNA content assay and scanning electron microscopy (SEM) were performed to evaluate the decellularized heart scaffold. Neonatal rat cardiac cells and rat bone marrow multipotent stromal cells (rBMSCs), which have the potential to transdifferentiate into cardiomyocytes, were adopted to serve as seed cells respectively to construct a tissue-engineered 3D cardiac model. To improve the viability of cells on 3D scaffold, rotary cell culture system (RCCS) was also applied to enhance the mass transport during the cultivation in vitro. The tissue-engineered 3D model was evaluated by histological staining, SEM as well as quantitative real-time RT-PCR analysis.

RESULTS Both histological staining and SEM detection revealed that decelluarized heart scaffold preserves the ultrastructural conformation of the heart with few nuclei. The DNA content of the scaffold was 1.64±0.03ng/mg, which is significantly lower than that of the control group (native heart tissue). Histological staining and SEM images showed their good viability during the 3D culture. Majority of the seeded neonatal rat cardiac cells or rBMSCs grow on the surface of the scaffold under the static culture condition. By contrast, a number of cells distributing within the scaffolds were observed in the dynamic cultures, indicating its good mass transport and microenvironment for cell growth. Quantitative real-time RT-PCR analysis revealed that expression level of transcript factor Gata 4 in the induced rBMSCs was improved significantly when compared to the negative control. Similarly, the expression of gene Gata4 in the rBMSCs inoculated into the decelluarized heart scaffold is much higher than that of the negative control and the induced group (5-azacytidine-treated).

CONCLUSIONS The decellularized heart scaffolds prepared in the present study were decellularized completely and preserved the full extracellular matrix. It possesses not only good biocompatibility but also naturally occurring three-dimensional structure. Moreover, our research indicates that the decellularized heart scaffold might potentially induce the differentiation of rBMSCs into cardiomyocytes. Combined with the RCCS cultivation, the tissue-engineered 3D cardiac model based on decellularized heart scaffold can better simulate the microenvironment in vivo and might potentially be utilized for cardiac tissue engineering.

GW26-e2370

Red Cell Distribution Width and Risk of Long-Term All-Cause Mortality and Cardiovascular Events Among Patients With Acute Coronary Syndrome: A Meta-Analysis of Observational Studies

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OBJECTIVES Red cell distribution width (RDW) might be a novel biomarker that reflects multiple physiological impairments related to atherosclerosis and acute coronary syndrome (ACS). We conducted this systematic review and meta-analysis to evaluate the association of RDW between risk of major adverse cardiovascular Events (MACEs), all-cause and cardiovascular mortality in ACS patients.

METHODS Relevant studies were searched and identified in the Cochrane Library, PubMed and Embase databases. English-language studies that reported risk estimates for RDW and MACEs, all-cause and cardiovascular mortality were included. Data were extracted regarding the characteristics and clinical outcomes, and a quality assessment was conducted. Results were extracted for the average RDW level, and meta-analyses were carried out using random effects models.

RESULTS We collected 14 articles. 9 investigated the association between RDW and all-cause mortality, 3 evaluated the association between RDW and risk of cardiovascular mortality, and 7 reported the association between RDW and risk of MACEs. We found that RDW was associated with a significantly increased risk of all-cause mortality (HR:3.372; 95%CI:2.014 - 5.645; $P <0.001$), cardiovascular mortality (HR: 2.342; 95%CI: 1.769 - 3.100; $P <0.001$) and MACEs (HR: 2.120; 95% CI: 1.515-2.965; $P <0.001$;).

CONCLUSIONS The meta-analysis indicates that RDW significantly increased the risk of MACEs, all-cause and cardiovascular mortality in ACS patients.

GW26-e5367

SIRT3 Deficiency Induces Endothelial Insulin Resistance and Vascular Dysfunction in Obese Mice and Human Subjects

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OBJECTIVES Recent evidence implicates SIRT3 as a central regulator of mitochondrial redox balance and metabolic homeostasis but the contribution of SIRT3 to vascular function remains unknown. The aim of this study was to investigate the role of SIRT3 in obesity-induced endothelial insulin resistance and subsequent vascular dysfunction.

METHODS Both vascular response to insulin and SIRT3 expression were detected in morbid obese human subjects undergoing bariatric surgery and non-obese controls. Male SIRT3 knockout mice and wide type littermates were fed with a standard chow diet or a high fat diet (HFD) for 24 weeks.

RESULTS We found an impaired insulin-induced mesenteric vasorelaxation (82.46±8.5% vs. 54.93±6.46%, n=8-12, $P<0.05$) and concomitant a 50% reduced vascular SIRT3 expression in morbid obese human subjects compared with non-obese controls. Downregulation of SIRT3 either by siRNA or by palmitate excess treatment in cultured human endothelial cells resulted in overproduction of mitochondrial reactive oxygen species (mtROS) and impaired insulin signaling as indicated by decreased phosphorylation of Akt and eNOS and subsequent reduced NO release. Additionally, obese mice induced by 24-week HFD displayed an impaired endothelium-dependent vasorelaxation to both insulin (40.68 ± 3.68% vs. 64.98 ± 2.85%, n = 8, $P < 0.01$) and acetylcholine (46.45 ± 4.93% vs. 100.59 ± 2.35%, n = 6, $P < 0.01$), which was further exacerbated by gene deletion of SIRT3 ($P < 0.05$). Moreover, lentivirus-mediated restoration of vascular SIRT3 rescued HFD-induced endothelial dysfunction in SIRT3 knockout mice (46.84 ± 3.29% vs. 29.56 ± 2.99%, n = 6, $P < 0.01$ for response to insulin; 75.59 ± 4.93% vs. 57.25 ± 3.81%, n = 6, $P < 0.01$ for response to acetylcholine). Elimination of mtROS with MitoTEMPO not only restored insulin-stimulated NO production in SIRT3 knockdown cells but also improved insulin-induced vasorelaxation in SIRT3 knockout mice fed with HFD.

CONCLUSIONS Our findings suggest that SIRT3 positively regulates endothelial insulin sensitivity and show that SIRT3 deficiency and resultant mtROS overproduction contribute to vascular dysfunction in obesity.

GW26-e1025

Fenofibrate Prevention of Diabetic Cardiomyopathy Is Mediated by FGF21 Via Sirt1-Dependent Autophagy Modulation

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OBJECTIVES Diabetes mellitus (DM) is one of the greatest public health threats in modern societies. And among all the diabetic complications, diabetic cardiomyopathy (DCM) has been recognized as the leading cause of morbidity and mortality. Thus, the elucidation of pathological mechanisms in DCM is urgently needed, so as to develop novel therapies. Fenofibrate (FF) is a peroxisome proliferator-activated receptor-α (PPARα) agonist. It has long been used clinically to lower lipid levels for about three decades. Here we tested whether FF can be repurposed to prevent diabetic cardiomyopathy (DCM) for type 1 diabetes, and delineated the mechanism of its action.

METHODS Streptozotocin (STZ) was used to induce type 1 diabetes in wild type C57BL/6J mice and fibroblast growth factor 21 knock-out (FGF21KO) mice, diabetic and age-matched control mice were then treated with vehicle or FF by gavage every other day for 3 or 6 months. After that we sacrificed the animal models and obtained heart tissue for further study including western blot assay, real-time PCR, pathology staining and so on. The H9c2 cardiac myoblast cells were used for the in vitro study.

RESULTS FF prevented diabetes-induced cardiac dysfunction as well as cardiac inflammation, oxidative stress and remodeling (PAI-1, TNFα, 3NT, 4HNE, CTGF, TGFβ), along with up-regulated cardiac expression of FGF21 and sirtuin1 (Sirt1). In contrast to wild-type diabetic mice, FGF21-KO mice showed worse biochemical, pathological, and functional changes of the heart following diabetes induction, and failed to respond to FF treatment even though FF similarly lowered systemic lipid profile in both WT and FGF21-KO diabetic mice.

CONCLUSIONS FF treatment restored autophagy in the WT diabetic mice but not in the FGF21-KO diabetic mice. Mechanistic study with H9C2 cells in vitro showed that autophagy, measured by cytoplasmic form microtubule-associated protein 1A/1B-light chain 3 (LC3) expression, was significantly inhibited by high glucose (HG, 30 mM) that also significantly increased inflammation, oxidative stress, and fibrosis. These HG effects were prevented by FF treatment. Inhibition of autophagy by 3-methyladenine (3MA) or inhibition of Sirt1 by sirtinol abolished FF protection against HG-induced effects. Together, these results suggested that FF could prevent DCM by inducing FGF21, which in turn enhances the Sirt1-mediated autophagy.

GW26-e1079

Comparative Proteomics Reveals Abnormal Binding of ATGL and Dysferlin on Dysfunctional Cardiac Lipid Droplets

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OBJECTIVES Excessive retention of neutral lipids in cardiac lipid droplets (LDs) is a common observation in cardiomyopathy. We anticipate that the systematic investigation of the cardiac LD proteome will help to dissect the underlying mechanisms linking cardiac steatosis and myocardial dysfunction.

METHODS LDs isolated from Sprague-Dawley rat hearts were analyzed using morphological and biochemical approaches and then subjected to iTRAQ quantitative proteomic analysis. The LD localization of the identified LD proteins was verified by immunofluorescence assays. The set of dysferlin truncation mutants were used to determine the LD-binding structure.

RESULTS 771 heart LD proteins were identified and categorized into 10 functional groups, including 467 proteins previously unreported on LDs. The LD localization of these proteins was verified by immunofluorescence assays. The most noteworthy finding was the identification of the membrane resealing protein, dysferlin. An analysis of dysferlin truncation mutants indicated that its C2 domain was responsible for its LD localization. Using the iTRAQ quantitative proteomic method we determined that the quantity of 30 proteins was increased and 16 proteins was decreased in LDs from pressure overload-induced dysfunctional heart, compared with normal hearts. Notably, adipose triacylglycerol lipase (ATGL) was dramatically decreased and dysferlin was substantially increased on dysfunctional cardiac LDs, a finding that was confirmed using immunoblotting.

CONCLUSIONS This study for the first time reveals the dataset of the heart LD proteome in healthy tissue and the variation of it under cardiac dysfunction. These findings highlight an association between the altered LD proteinlocalization of dysferlin and ATGL and myocardial dysfunction.

GW26-e1360

Effects of Sirt1 on Protection Against Atherosclerosis Plaque via Anti-angiogenesis in Mice

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OBJECTIVES Atherosclerosis (AS) is an age-related disease attributed to vascular endothelial cell injury Angiogenesis might contribute to the progression of the atherosclerotic plaque SIRT1, has been reported to have the function of anti-angiogenesis and prevent senescence In this report, the present study aims to investigate whether resveratrol, an activator of SIRT1, is capable of protecting against atherosclerosis progress via anti-angiogenesis.

METHODS 25 Apolipoprotein E-knockout mice were divided into three groups: control group (n =5) with normal diet, high-fat diet (HFD) group (n=10) for 16 weeks, high-fat diet(16 weeks)+ resveratrol (2 months) group (HFD+RES) (n=10) following with resveratrol intraperitoneal injection (100 mg/kg/day, i.p) for two months Body weight, serum total cholesterol(TC), triglyceride(TG), LDL-C, HDL-C level and oil red O stain were performed to determine the establishment of atherosclerosis model. Immunofluorescence staining of CD31 was used to detect angiogenesis in atherosclerotic plaque. In vitro HUVECs study included six groups: control group, H2O2 treated group (HO), H2O2+resveratrol group (HO+ RES), sh-SIRT1 group, sh-SIRT1+ H2O2 group (sh-SIRT1+HO) and sh-SIRT1+ H2O2+ resveratrol group (sh-SIRT1+ HO+ RES) Oxidative stress damage was induced by H2O2. HUVECs proliferation was evaluated with wound assay and transwell migration assay. HUVECs apoptosis was assessed with TUNEL. Intracellular production of reactive oxygen species (ROS) was detected by a fluorescence probe-2′,7′-dichlorofluorescin diacetate (DCFH-DA). Subsequently, Western Blot was performed to determine relative changes of proteins such as SIRT1, HIF-1α and VEGF. We also explored the dose-dependent effect of resveratrol on HUVECs subjected to oxidative stress.

RESULTS As compared with control group mice, HFD mice showed increased body weight, serum TC, TG, LDL-C level and reduced HDL-C. The size of tissues positive for oil red O expression was higher in the HFD mice ($P<0.05$), indicating that the model of AS was established successfully. Subsequently, immunofluorescence staining of CD31 showed that angiogenesis in HFD+RES group significantly decreased compared with the HFD only group, indicating that resveratrol was capable of inhibiting angiogenesis in AS plaque. In vitro results revealed that H2O2 induced oxidative stress damage on HUVECs, evidenced by increased ROS generation. Furthermore, HIF-1α and VEGF expression were increased whereas SIRT1 expression was reduced ($P<0.05$). Resveratrol reversed these changes in HO group, however this effect was diminished in sh-SIRT1+ HO group. Moreover, high-concentration resveratrol inhibited tube formation and cell migration in HUVECs by contributing to apoptosis ($P<0.05$).

CONCLUSIONS High-concentration resveratrol protects against atherosclerosis and improves vascular function in HFD mice through anti-angiogenesis in plaque. SIRT1 plays an important role in the resveratrol beneficial effects against atherosclerosis.

GW26-e1553

Long-Term Treatment With Ivabradine in Transgenic Atrial Fibrillation Mice Counteracts HCN Channel Overexpression and Reduces Atrial Fibrillation Incidence

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OBJECTIVES Current studies show that ivabradine, a specific heart rate lowering drug, it selective inhibitor of funny (I_f), performing antiarrhythmic effect of some disease condition, such as heart failure, myocardial ischemia, and so on. But little is known regarding the long-term ivabradine treatment on I_f current and prevention of atrial fibrillation (AF).

METHODS We firstly investigated the I_f current and the HCN channels expression between the wild-type (WT) mice and the transgenic mice overexpressing heart-specific (pro) renin receptor (TG), a useful mouse model of AF, and then examined the effects of ivabradine on the I_f current and HCN channels expression, and the prevention of AF episode.

RESULTS Compared with WT mice, the enhanced I_f current density (at -170mV: TG, -39.6±4.6pA/pF; WT, -26.9±3.0pA/pF, $p<0.001$) and the faster activation kinetic ($V_{1/2}$: TG, -109.45±1.35 mV; WT, -128.20±1.65 mV), as well as the mRNA of HCN2 and HCN4, accompanying the HCN4 protein were significantly increased in atrial myocytes from TG mice. After treatment with ivabradine for 4 months (7mg/kg per day orally), it partially reverses the electrophysiological remodeling occurring in TG mice, and I_f density was reduced in the atrial myocytes (at -170mV: TG, -17.7±3.0pA/pF; WT, -18.0±3.1pA/pF, $p=0.89$). The effects of ivabradine on electrophysiological remodeling were accompanied by an inhibition of upregulation of HCN2 and HCN4 protein in atrium tissue. Furthermore, we found that ivabradine significantly reduce the incidence of AF among TG mice (41.2% in TG mice, 16.7% in TG+ivabradine mice, $p<0.01$), most likely by suppressing an increase in automaticity.

CONCLUSIONS Thus, our findings provide the first evidence that ivabradine reduced the incidence of AF in mice, and the antiarrhythmic of ivabradine was beyond the hate rate reduction, it partially due to counteract the HCN overexpression and reverse electrophysiological cardiac remodeling by reducing I_f gain-of-function.