Drug Design, Development and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Glucagon-Like Peptide-1 Receptor Agonist Protects Against Diabetic Cardiomyopathy by Modulating microRNA-29b-3p/SLMAP

Ping Fang[1], Zhengqin Ye[1], Ran Li[1], Dunmin She[2], Guannan Zong[1], Liya Zhang[1], Ying Xue[1], Keqin Zhang[1]

[1]Department of Endocrinology and Metabolism, Tongji Hospital, School of Medicine, Tongji University, Shanghai, 200065, People's Republic of China; [2]Department of Endocrinology, Northern Jiangsu People's Hospital Affiliated to Yangzhou University, Yangzhou, Jiangsu, 225001, People's Republic of China

Correspondence: Ying Xue; Keqin Zhang, Department of Endocrinology and Metabolism, Tongji Hospital, School of Medicine, Tongji University, No. 389, Xincun Road, Shanghai, 200065, People's Republic of China, Tel +86-21-66111061, Email doxy0622@163.com; 13621798084@126.com

Purpose: Our aims were to investigate the pathogenesis of diabetic cardiomyopathy (DCM) and to explore the protective effect of glucagon-like peptide-1 receptor agonist (GLP-1RA) on DCM.

Methods: After 12 weeks of treatment with exenatide-loaded microspheres, a long-acting GLP-1RA, in DCM mice, cardiac structure and function were evaluated by plasma B-type natriuretic peptide (BNP), echocardiography, H&E, oil red and Sirius staining. The expression of glucagon-like peptide-1 receptor in mouse heart tissue was determined by immunofluorescence staining. The label-free proteomic analysis of cardiac proteins was conducted among control, DCM and DM+GLP-1RA groups. Then, quantitative real-time PCR, Western blotting and dual-luciferase reporter assay were performed to verify the regulation of target protein by the upstream microRNA (miRNA).

Results: GLP-1RA treatment obviously improved serum BNP, myocardial fibrosis, lipid deposition of the myocardium and echocardiography parameters in DCM mice. Sarcolemmal membrane-associated protein (SLMAP) was one of 61 differentially expressed cardiac proteins found in three groups by proteomic analysis. Up-regulation of microRNA-29b-3p (miR-29b-3p) and down-regulation of SLMAP were found in the ventricular myocardium of GLP-1RA-treated DCM mice. SLMAP was a target of miR-29b-3p, while GLP-1RA regulated SLMAP expression through miR-29b-3p. Furthermore, inhibition of glucagon-like peptide-1 receptor (GLP-1R) in cardiomyocytes reversed the effects of GLP-1RA on miR-29b/SLMAP.

Conclusion: SLMAP may play roles in the pathogenesis of DCM and may be a target of GLP-1RA in protecting against DCM. After binding to myocardial GLP-1R, GLP-1RA can regulate the expression of myocardial SLMAP through miR-29b-3p.

Keywords: diabetic cardiomyopathy, microRNA-29b-3p, proteomics, sarcolemmal membrane-associated protein, glucagon-like peptide-1 receptor agonist

Introduction

It is well known that diabetes mellitus and cardiovascular disease are closely related. The prevalence and mortality of cardiovascular disease in diabetic patients are 2–4 times higher than those in non-diabetic individuals.[1,2] The mortality of cardiovascular disease accounts for 65% of diabetes-related deaths.[3] In several population-based studies, diabetes mellitus was associated with an increased risk of heart failure after adjusting for other risk factors such as obesity, dyslipidemia, hypertension, and coronary artery disease.[4]

Diabetic cardiomyopathy (DCM), a type of cardiovascular damage which increases the risk of heart failure in diabetic patients, is defined as the changes in cardiac structure and function induced by DM in the absence of coronary artery disease, ischemic heart disease, valvular heart disease, hypertension or other cardiac pathologies. It is characterized by structural changes, including myocardial hypertrophy, myocardial lipotoxicity and interstitial fibrosis, and functional changes such as diastolic and systolic dysfunction.[5] Multifactorial mechanisms such as glucotoxicity,[6] lipotoxicity,[7]

Received: 5 December 2022
Accepted: 6 March 2023
Published: 12 March 2023

inflammation,[8] mitochondrial dysfunction[9,10] oxidative stress[11] and deranged calcium homeostasis[12] are believed to be involved in the development of DCM. Despite various progress has been made, the exact pathogenesis of DCM still needs to be clearly elucidated, and specific treatment strategies for DCM remain to be explored. Glucagon-like peptide-1 receptor agonist (GLP-1RA) is an effective drug for the treatment of type 2 diabetes and obesity. A meta-analysis of seven clinical trials[13–19] using GLP-1RA have indicated that GLP-1RA treatment can reduce the major adverse cardiac events of type 2 diabetic patients by 12%, and decrease the hospitalization rate of heart failure by 9%.[20]

Previous studies have indicated that sarcolemmal membrane associated protein (SLMAP) may be associated with heart failure[21] and microvascular disease associated with type 2 diabetes.[22] However, the role of SLMAP in the myocardium of mice with DCM is still unclear. We suggested SLMAP might play a role in the pathogenesis of DCM and might be a potential target of GLP-1RA in improving DCM.

Materials and Methods

Animal Model

Eight-week-old male C57BL/6J mice were purchased from the model animal research center of Nanjing University, China. All procedures were approved by the animal ethics committee of Tongji Hospital and carried out in accordance with the guide for the care and use of laboratory animals, Eighth edition (2011). The mice were housed in an SPF environment (temperature: 21 ± 4°C, humidity: 55 ± 20%, 12/12 light–dark cycle). All mice had free access to water. After one-week adaptive feeding, mice were randomly divided into two groups: 1) control group which were fed with a normal chow diet (12% Kcal fat, 65% Kcal carbohydrate, and 23% Kcal protein with a total caloric value of 1.54 Kcal/gm); 2) DM group which were fed with a high-fat diet (consisting of 42% Kcal fat, 38% Kcal carbohydrate, and 20% Kcal protein with a total caloric value of 4.45 Kcal/gm). After 4 weeks of dietary intervention, DM group received an intraperitoneal injection of STZ (Sigma-Aldrich, USA) at a dose of 100 mg/kg. The fasting blood glucose level was monitored from the tail blood samples by a glucometer (Johnson, UltraVue, USA). Type 2 diabetes of mice were diagnosed when the fasting blood glucose level was >11.1 mmol/L (200 mg/dl) by two independent tests. After the next six weeks, the DM group was then randomly divided into two subgroups. ie, DM+GLP-1RA group and DCM group. DM +GLP-1RA group received exenatide-loaded microspheres injection intraperitoneally (10mg kg-1 w-1, LILY, USA), while DCM group underwent intraperitoneal injection of normal saline. All mice were sacrificed after 12 weeks of GLP-1RA or saline treatment. During this period, the high-fat diet was continued in DCM group and DM+GLP-1RA group.

Histological Analysis

Heart tissues of mice were fixed in 10% phosphate-buffered formalin for 24 hours, dehydrated by ethanol and xylene, and then embedded in paraffin. Cardiac tissue sections were stained with H&E to observe morphological changes and stained with Sirius red to detect collagen deposition. Lipid deposits were observed by oil red staining of frozen sections of heart tissues.

Cardiac tissue sections were stained with primary antibodies of SLMAP (Abcam, USA) and GLP-1R (Affinity, China) overnight at 4°C. After thorough washing, sections were incubated with FITC-labelled anti-rabbit IgG secondary antibodies at 37°C for 50 min. Then, the stained sections were counterstained with DAPI-staining-solution (Boster, USA). Finally, the images were captured under an inverted optical microscope (Nikon, Japan).

ELISA Measurement

After fasting for at least 12 hours, blood was collected from the mice. Serum BNP level and CK-MB level were measured using Mouse BNP ELISA Kit (TSZ Biosciences) and Mouse CK-MB ELISA Kit (TSZ Biosciences, USA) in accordance with the manufacturer's instructions.

Echocardiography

The mice were anesthetized with 2% isoflurane gas, and the Vevo 2100 imaging system (VisualSonics Inc., Canada) was performed to obtain transthoracic two-dimensional M-mode echocardiograms and pulsed Doppler spectrograms. The

heart rate of mice was controlled at 400–500 beats/min. The average of 5 consecutive cardiac cycles was taken. The percentages of ejection fraction (EF%), left ventricular end-systolic diameter (LVESd), left ventricular end-systolic volume (LVESv), left ventricular end-diastolic diameter (LVEDd), left ventricular end-diastolic volume (LVEDv), and the percentage of fractional shortening (FS%) were measured. The ratio of the peak velocity of early ventricular filling (E wave) to late filling velocity (A wave) (E/A) was also measured to determine diastolic function.

Label-Free Quantitative Proteomics Analysis

The methods of label-free quantitative proteomics analysis were showed in Supplementary Document.

Cell Culture

H9c2 cells, purchased from Chinese Academy of Sciences, were cultured in high-glucose DMEM (glucose 25mM) with 10% FBS, and 1% penicillin/streptomycin in an incubator (37◦C, 5% CO2). After starvation overnight, H9c2 cells were maintained in low-glucose DMEM (glucose 5.5mM) as the control group. For high glucose plus free fatty acid (FFA) group, H9c2 cells were incubated in the medium of high-glucose (30 mM) and 1mM FFA (0.33mM palmitic acid, 0.67mM oleic acid) for 48 hours.[23] High glucose plus FFA stimulated H9c2 cells were treated with exenatide (200 nM) for 48 hours as GLP-1RA group. After 48 hours of culture, all cells were collected for further analysis.

Transfection of miRNA Mimics, miRNA Inhibitors and siRNA

miRNA mimics and inhibitors and siRNA were synthesized by Genepharma (China). The sequences of miR-29b-3p mimics, miR-29b-3p mimics control, miR-29b-3p inhibitors, miR-29b-3p inhibitors control and GLP-1R siRNA were showed in Table 1. Before transfection, lipofectamine® 3000 reagent (Thermo Fisher Scientific Inc, USA) was initially mixed with 100 pmol of miR-29b-3p mimics, miR-29b-3p mimics control, miR-29b-3p inhibitors, miR-29b-3p inhibitors control, GLP-1R siRNA1, GLP-1R siRNA2, GLP-1R siRNA3 and GLP-1R siRNA control (GLP-1R siNC) respectively to form transfection reagent-vector complexes. Cells were transfected with transfection reagent-vector complexes at 37°C for 48h according to the manufacturer's instructions.

Quantitative Real-Time PCR (qPCR) for mRNA and miRNA

Total RNA was extracted from cardiac tissue or H9c2 cells using TRIzol® reagent (Invitrogen; USA). Reverse transcription of RNA was performed with PrimeScript RT kit (TAKARA, Japan) according to the kit instructions. Reverse transcription of miRNA was carried out with Poly A polymerase (TAKARA, Japan), RT primer, recombinant RNase inhibitor (TAKARA, Japan), reverse transcriptase (TAKARA, Japan), rATP and dNTPs according to the manufacturer's protocol. PCR reaction systems were prepared using SYBR® Premix Ex TaqTM (TAKARA, Japan) according to the kit instructions. The primers are listed in Table 1. MiRNA and mRNA expression levels were quantified by the 2-ΔΔCt method. GAPDH was used as an internal control for mRNAs and U6 was used for miRNA.

Western Blot Analysis

The protein extracted from Mouse ventricular myocardium or H9c2 cells were separated and transferred to polyvinylidene fluoride membranes (PVDF). Membranes were blocked and incubated overnight at 4°C with the following antibodies: anti-SLMAP antibody (Abcam, UK, dilution 1:1000), anti-GLP-1R antibody (Novus, USA, dilution 1:500) or anti-GAPDH antibody (Affinity Biosciences, USA, dilution 1:5000). AlphaView Software (FluorChem Q, ProteinSimple, USA) was used for the quantification of Western blot analysis.

Dual-Luciferase Reporter Assay

The interaction between miR-29b-3p and 3'-Untranslated Region (UTR) of *Slmap* was predicted through targetscan (http://www.targetscan.org/) which is publicly available and unrestricted re-use is permitted, and a binding site (AGGTGCTA) was found. Furthermore, a mutant sequence of binding site (CACGAAT) was designed. 3′ UTR of *Slmap* mRNA was cloned into H306 pMIR-REPORT Luciferase plasmid using Seamless Cloning Kit (OBiO, China). The corresponding *Slmap* mutant reporter gene vector was also constructed. Then, linear amplified products were

Table 1 Gene-Specific Primers for RT-qPCR Analysis

Gene		Primer Sequences
SLMAP (mouse)	Forward	TCAGGTATCAGCAAACCTGCTT
SLMAP (mouse)	Reverse	GGGGCCACTGAAATGAAATGT
GAPDH (mouse)	Forward	AGGTCGGTGTGAACGGATTTG
GAPDH (mouse)	Reverse	TGTAGACCATGTAGTTGAGGTCA
SLMAP (rat)	Forward	TTGGTGTTTCGGTCCGTTGTG
SLMAP (rat)	Reverse	CACTGTCATGGAGAAGCTCTGG
GAPDH (rat)	Forward	TGGTGGACCTCATGGCCTAC
GAPDH (rat)	Reverse	CAGCAACTGAGGGCCTCTCT
RT primer		CGAATTCTAGAGCTCGAGGCAGGCGACA TGGCTGGCTAGTTAAGCTTGGTACCGAGCTCGGA TCCACTAGTCCTTTTTTTTTTTTTTTTTTTTTTTTTVN
Uni-primer		CGAATTCTAGAGCTCGAGGCAGG
miR-29b		TAGCACCATTTGAAATCAGTGTT
U6		ATTCGTGAAGCGTTCCATAT
miR- 29b-3p mimics	Sense	UAGCACCAUUUGAAAUCAGUGUU
miR- 29b-3p mimics	Antisense	CACUGAUUUCAAAUGGUGCUAUU
miR-29b-3p mimics control	Sense	UUCUCCGAACGUGUCACGUTT
miR-29b-3p mimics control	Antisense	ACGUGACACGUUCGGAGAATT
miR-29b-3p inhibitors		AACACUGAUUUCAAAUGGUGCUA
miR-29b-3p inhibitors control		CAGUACUUUUGUGUAGUACAA
GLP-1R siNC	Sense	UUCUCCGAACGUGUCACGUTT
GLP-1R siNC	Antisense	ACGUGACACGUUCGGAGAATT
GLP-1R siRNA1	Sense	CAGGUUCCUUUGUGAAUGUTT
GLP-1R siRNA1	Antisense	ACAUUCACAAAGGAACCUGTT
GLP-1R siRNA2	Sense	GCCCUCAAGUGGAUGUAUATT
GLP-1R siRNA2	Antisense	UAUACAUCCACUUGAGGGCTT
GLP-1R siRNA3	Sense	CUUCCUUCCAGGGCUUUAUTT
GLP-1R siRNA3	Antisense	AUAAAGCCCUGGAAGGAAGTT

Abbreviations: BNP, B-type natriuretic peptide; BW, Body weight; DCM, Diabetic cardiomyopathy; EF%, The percentages of ejection fraction; FFA, Free fatty acid; FS%, The percentage of fractional shortening; GLP-1RA, Glucagon-like peptide-1 receptor agonist; GLP-1R, Glucagon-like peptide-1 receptor; HW, Heart weight; KEGG, Kyoto Encyclopedia of Genes and Genomes; LVEDd, Left ventricular end-diastolic diameter; LVEDv, Left ventricular end-diastolic volume; LVESd, Left ventricular end-systolic diameter; LVESv, Left ventricular end-systolic volume; MiR-29b, MicroRNA-29b; MiRNA, MicroRNA; SERCA, sarcoplasmic reticulum Ca2+; SLMAP, Sarcolemmal membrane-associated protein; STZ, streptozotocin; UTR, Untranslated Regions.

circularized by homologous recombination using Seamless Cloning Kit. After 24 hours, 0.2μg wild-type vector, or 0.2μg mutant vector were separately co-transfected with miR-29b-3p mimics at a final concentration of 100 nM in HEK293T cells using lipofectamine 3000 (Invitrogen, USA). After 48 hours, the cell lysates were harvested, and Firefly/Renilla

luciferase (FL/RL) ratios were detected using Dual-Glo Luciferase Assay System (Promega, USA) following the manufacturer's protocol. The ethics involved in the experiment was approved by the ethics committee of Tongji Hospital (Approval No K-KYSB-2019-015).

Statistical Analysis

Data were presented as the mean ± SD. The statistical analysis was performed using SPSS 15.0 (Chicago, IL, USA). An unpaired two-tailed Student's *t*-test was performed to determine significant differences between two groups. To compare three or more groups, one-way analysis of variance (ANOVA) followed by LSD's post hoc test or Dunnett's T3 post hoc multiple-comparison test was performed. Significant differences were set as $P < 0.05$.

Results

GLP-1RA Reduces Blood Glucose Levels, Attenuates Lipid Accumulation and Improves Myocardial Fibrosis in DCM Mice

Fasting blood glucose was remarkably increased in DCM mice compared with control group, while the increased blood glucose level was obviously reduced after treatment with GLP-1RA (Figure 1A). No major adverse events occurred in each experimental group.

The body weight (BW) and heart weight (HW) in DCM mice were significantly increased compared with the normal control group, while BW was markedly decreased after GLP-1RA treatment compared with DCM group. However, there was no significant difference in HW between DCM group and DM+GLP-1RA group (Figure 1B and C).

Compared with normal controls, H&E staining of heart tissue sections in DCM group showed abnormal morphology of cardiomyocytes, including cardiomyocyte hypertrophy, irregular morphology and muscle fiber derangement or fracture. All the above abnormal morphologies were improved after GLP-1RA treatment (Figure 1D). Moreover, GLP-1RA treatment significantly reduced the accumulation of lipid droplets in heart tissues of DCM mice and inhibited myocardial fibrosis in DCM group (Figure 1E and F).

GLP-1RA Improves the Impaired Cardiac Functions of DCM Mice

As shown in Figure 2A, DCM mice displayed significantly increased serum BNP level compared with normal group, while elevated BNP level was markedly reduced in DM+GLP-1RA group after GLP-1RA treatment. There was no significant difference in serum CK-MB levels among these three groups (Figure 2B).

In addition, as indicated in Figure 2C–L, left ventricular end diastolic diameter (LVEDd), left ventricular end systolic diameter (LVESd), left ventricular end diastolic volume (LVEDv), and left ventricular end systolic volume (LVESv) were all obviously reduced in DCM mice compared with controls, whereas those decreased parameters were significantly elevated after GLP-1RA treatment. Furthermore, E/A value was also markedly decreased in DCM group compared with normal controls, while reduced E/A value in DCM mice was reversed after GLP-1RA treatment. There was no significant different in ventricular septal thickness (IVSD), left ventricular posterior wall thickness (LVPWD), ejection fraction (EF), and left ventricular short axis shortening rate (FS) among the three groups. Our results indicated that GLP-1RA treatment ameliorated structural abnormalities and cardiac dysfunction in DCM mice.

Identification of Significant Differentially Expressed Proteins (DEPs) in Myocardium of DCM Mice After GLP-1RA Treatment

LC-MS/MS analysis identified 2162 proteins in the myocardial samples among the three groups of mice. After applying a p value ≤0.05, 61 differentially expressed proteins (DEPs), including 33 upregulated and 28 downregulated proteins, were identified in the myocardial samples of three groups. The significance and magnitude of expression changes of DEPs among the three groups were demonstrated using K-means clustering heatmaps (Figure 3).

Gene Ontology analysis and KEGG pathway of differentially expressed proteins were presented in Supplementary Figure 1.

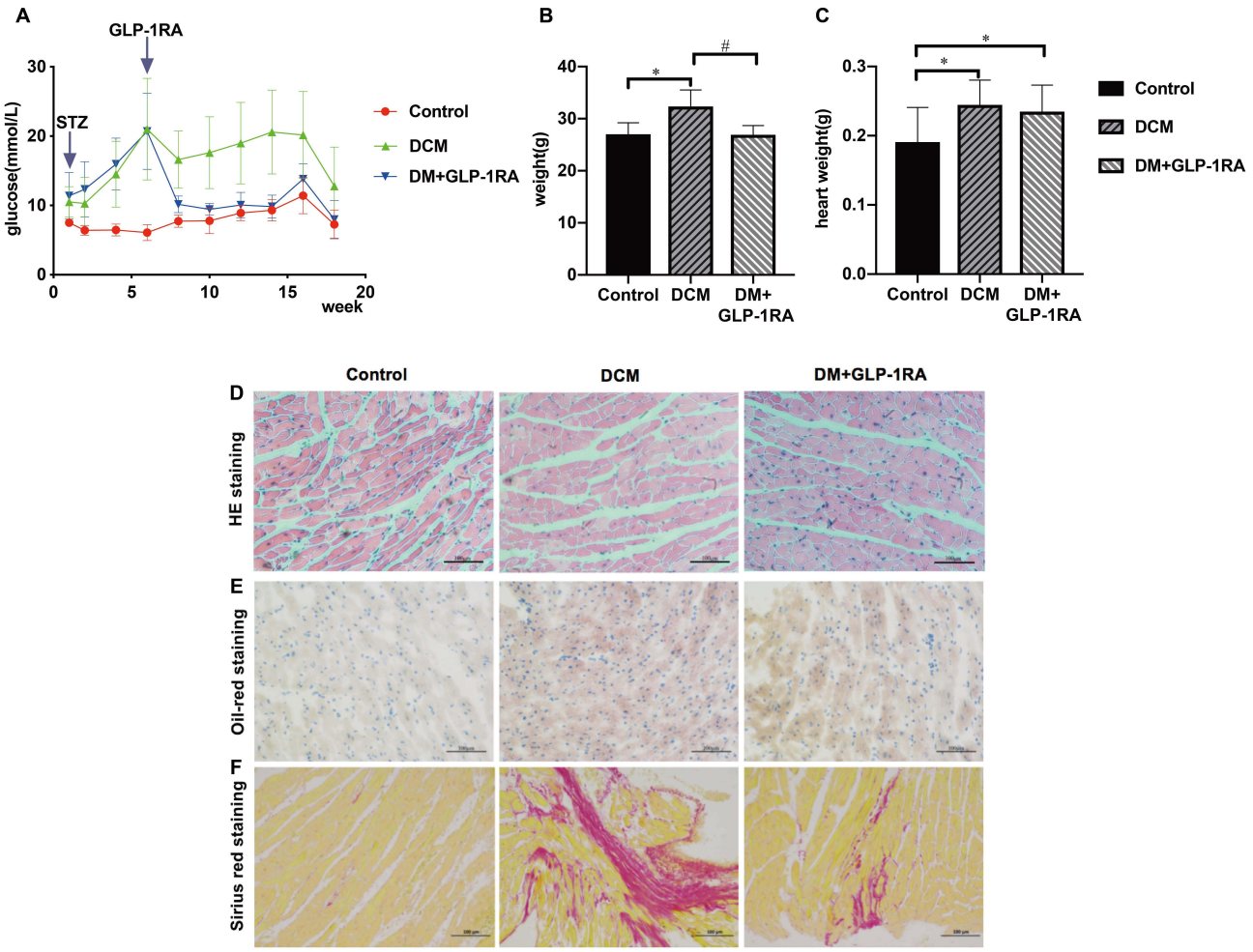

Figure 1 GLP-1RA reduces blood glucose levels, attenuates lipid accumulation and improves myocardial fibrosis in DCM mice. Eight-week-old male C57BL/6J mice were randomly divided into two groups: control group fed with a normal chow diet (n = 12) and DM group fed with a high-fat diet (n = 24). After 4 weeks of dietary intervention, DM group received an intraperitoneal injection of STZ at a dose of 100 mg/kg. T2DM of mice was diagnosed when the fasting blood glucose level was >11.1 mmol/L by two independent tests. After the next six weeks, the DM group was then randomly divided into two subgroups. GLP-1RA group (n = 12) received exenatide-loaded microspheres injection intraperitoneally once per week, while DCM group (n = 12) underwent intraperitoneal injection of normal saline. The high-fat diet was continued in DCM group and GLP-1RA group for the next 12 weeks. (**A**) Blood glucose of mice; (n = 12). (**B**) Body weight (n = 12). (**C**) Heart weight of mice (n = 12). (**D**) H&E staining on the heart sections of mice (n = 3). (**E**) Oil red staining on the heart sections of mice (n = 3). (**F**) Sirius red staining on the heart sections of mice (n = 3). Data are expressed as mean ± SD, *P <0.05 compared with control group; #P <0.05 compared with DCM group.

The Effect of GLP-1RA on SLMAP Expression in Myocardium of Mice with DCM

LC-MS/MS analysis demonstrated that SLMAP expression in myocardium of mice with DCM group was 2.70 times higher than that in control group, and it was significantly reduced after GLP-1RA treatment (Figure 4A). Then, we verified the expressions of *Slmap* mRNA and SLMAP protein in myocardium of mice among the three groups. Our data indicated that the expressions of *Slmap* mRNA and SLMAP isoforms in myocardium of mice with DCM were dramatically higher than those in control group, whereas they were significantly reduced after GLP-1RA treatment (Figure 4B and C).

We also detected levels of *Slmap* mRNA and SLMAP isoforms in H9c2 cardiomyocytes in vitro. The expression of *Slmap* mRNA and SLMAP isoforms were all significantly increased in H9c2 cells activated by high glucose plus FFA (Figure 4D and E), whereas elevated levels of three SLMAP isoforms were dramatically decreased after GLP-1RA treatment in H9c2 cells (Figure 4E). However, there was no significant difference in *Slmap* mRNA in H9c2 cells stimulated by high glucose plus FFA between GLP-1RA treatment group and GLP-1RA-free group (Figure 4D). We found that the change in *Slmap* mRNA expression among the three groups was smaller than that in SLMAP protein expression in vivo and in vitro. Therefore, we speculated that a post-transcriptional regulatory mechanism may play a role in the regulation of SLMAP protein.

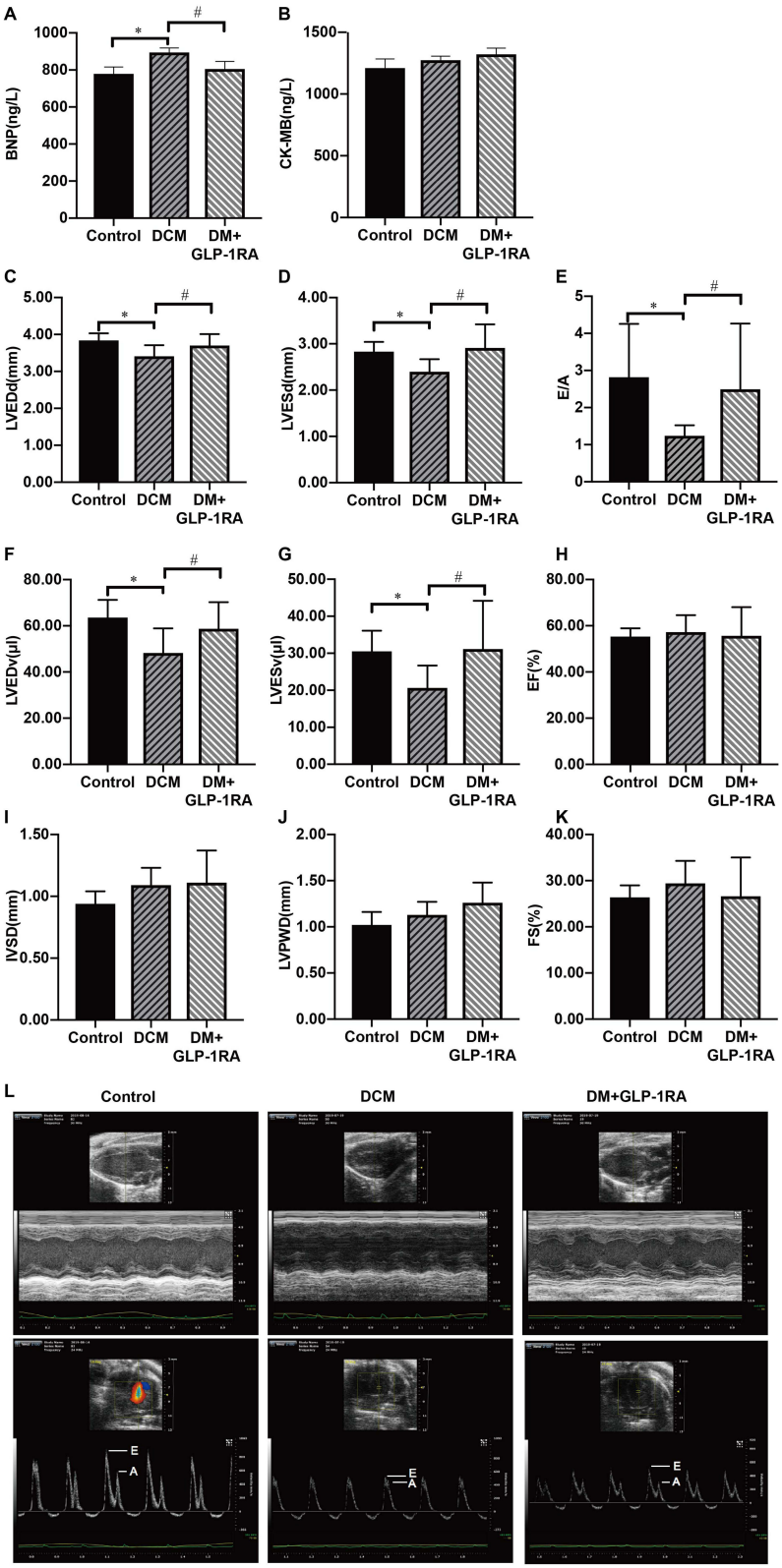

Figure 2 GLP-1RA improves impaired cardiac functions of DCM mice. (**A**) Plasma BNP levels of mice (n = 12). (**B**) CK-MB levels of mice (n = 12). (**C–L**) Echocardiography functional assessment (n = 6). (**C–K**) left ventricular end-diastolic diameter (LVEDd), left ventricular end-systolic diameter (LVESd), The ratio of the peak velocity of early ventricular filling to late filling velocity (E/A), left ventricular end-diastolic volume (LVEDv), left ventricular end-systolic volume (LVESv), The percentages of ejection fraction (EF%), ventricular septal thickness (IVSD), left ventricular posterior wall thickness (LVPWD) and left ventricular short axis shortening rate (FS) were evaluated. The image of Echocardiography (**L**) was shown. Values are expressed as mean ± SD, *P <0.05 compared with control group; #P <0.05 compared with DCM group.

https://doi.org/10.2147/DDDT.S400249 DovePress

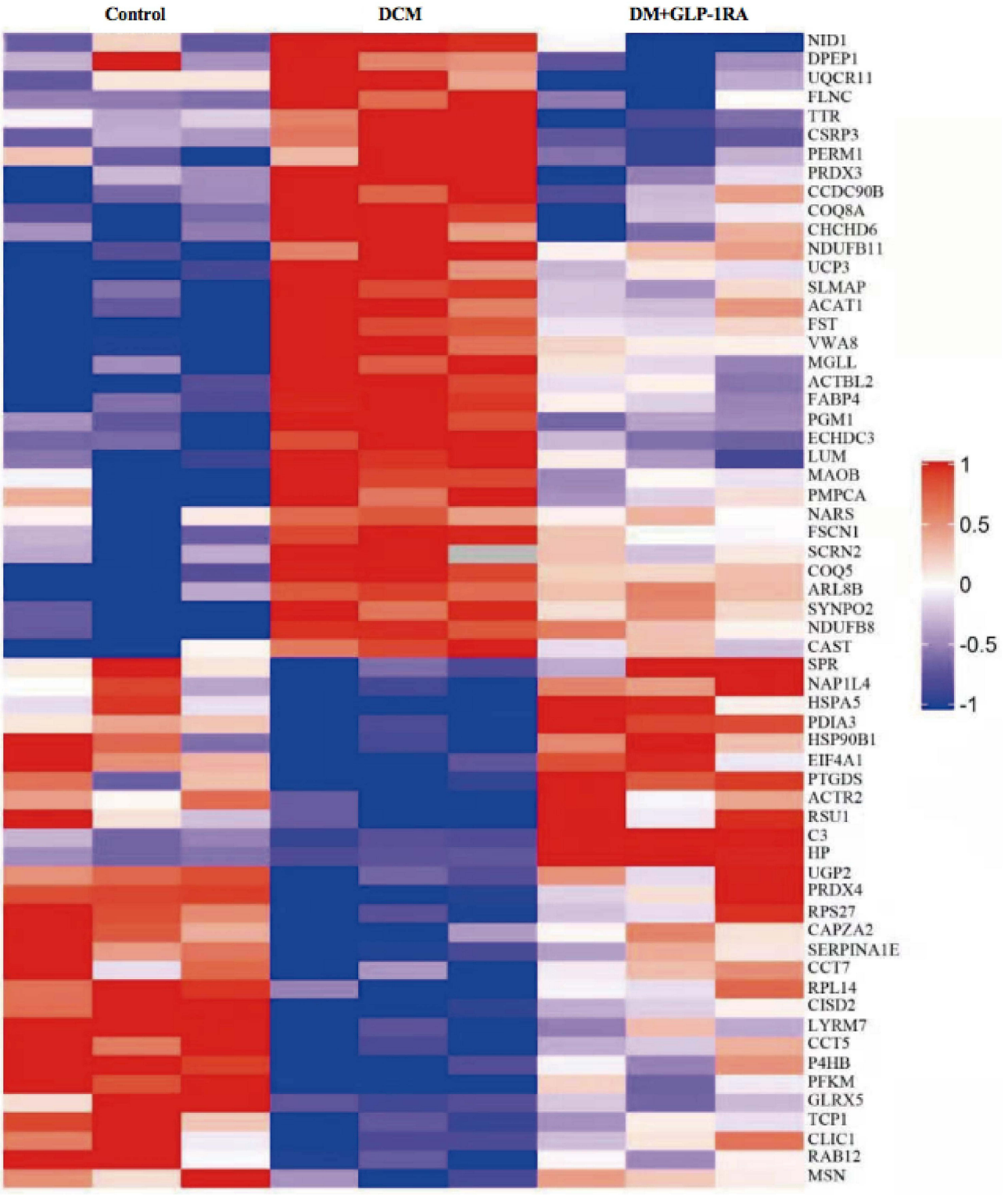

Figure 3 Identification of significant differentially expressed proteins (DEPs) in myocardium of DCM mice after GLP-1RA treatment. Heat map representation of the profiles of 61 differentially expressed proteins in the myocardium among three groups. The percentage variation is represented by a colour scale (top right) from low (blue) to high (red) (n = 3).

GLP-1RA Affects the Expression of SLMAP Through miR-29b-3p in Cardiomyocytes

To investigate the regulatory mechanism of SLMAP, the miRNAs, important factors involved in post-transcriptional regulation, were analyzed. A network bioinformatics tool was used to predict the miRNA, which could regulate the

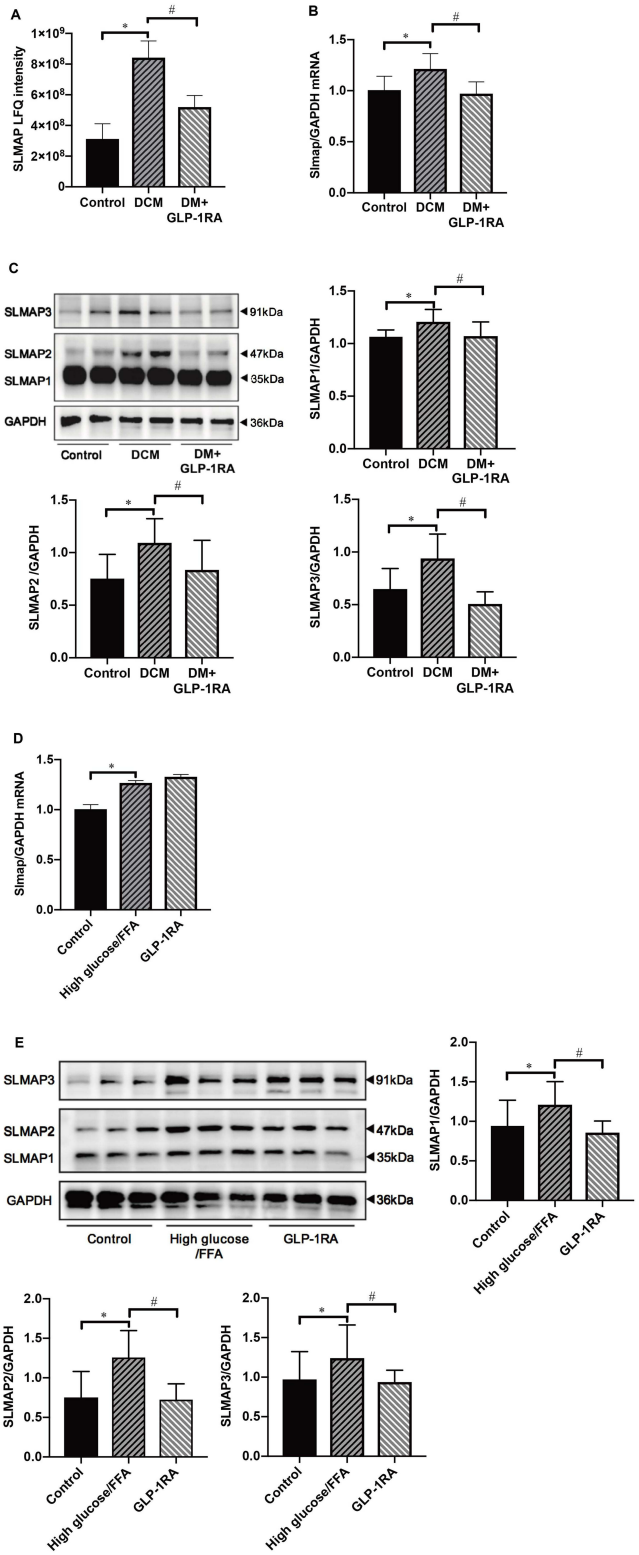

Figure 4 The effect of GLP-1RA on SLMAP expression in myocardium of mice with DCM and H9c2 cells. (**A**) The expression of myocardial SLMAP among three groups was detected by Label-free quantitative proteomics analysis (n = 3/group). (**B**) The expression of Slmap mRNA in the myocardium of mice among three groups was measured by qPCR (n = 6/group). (**C**) Representative Western blot and quantification of three isoforms of SLMAP (SLMAP1-3) protein levels in the myocardium of mice (n = 6/group). (**D**) The expression of *Slmap* mRNA in H9c2 cardiomyocytes was detected by qPCR (n = 6). (**E**) Representative Western blot and quantification of SLMAP1-3 protein levels in H9c2 cells (n = 6/group). Data are expressed as mean ± SD. *P <0.05 compared with control group; #P <0.05 compared with DCM group or high glucose/FFA group.

https://doi.org/10.2147/DDDT.S400249

expression of SLMAP (http://www.targetscan.org/). We found that miR-29b-3p could match with the 3'UTR region of *Slmap* and may regulate the expression of SLMAP. Compared with the control group, the expression of miR-29b-3p in myocardium of DCM mice was significantly decreased. After GLP-1RA treatment, the expression of miR-29b-3p was obviously higher than that of DCM group (Figure 5A). The changes of miR-29b-3p expression in H9c2 cardiomyocytes were similar to those in the myocardium of mice (Figure 5B). Furthermore, miR-29b-3p mimics and miR-29b-3p inhibitors were used to investigate the effect of miR-29b-3p on SLMAP expression in H9c2 cells. It was confirmed that miR-29b-3p mimics could significantly increase the miR-29b-3p level (Figure 5C), while miR-29b-3p level was remarkably decreased after miR-29b-3p inhibitors treatment (Figure 5D) in H9c2 cells. Three isoforms of SLMAP (SLMAP1, SLMAP2 and SLMAP3) expressions were all significantly downregulated in H9c2 treated with miR-29b-3p mimics than those in control group (Figure 5E), whereas the expressions of SLMAP1, SLMAP2 and SLMAP3 were remarkably increased in H9c2 cells activated by miR-29b-3p inhibitors compared with the control group (Figure 5F). The direct interaction of miR-29b-3p with wide type (WT) and mutant (MUT) *Slmap* 3'UTR was studied using a dual-luciferase reporter assay. The binding sites of miR-29b-3p to *Slmap* 3'UTR and mutant (MUT) *Slmap* 3'UTR are shown in Figure 5G. The results indicated that miR-29b-3p mimics could significantly inhibit the expression of Slmap 3'UTR, and the inhibitory effect of miR-29b-3p on *Slmap* 3'UTR was dramatically attenuated by the mutation of the binding site (Figure 5H). These results suggested SLMAP might be a target of miR-29b-3p.

To investigate whether GLP-1RA could regulate SLMAP expression through miR-29b, we assessed the expression of SLMAP protein in H9c2 cells stimulated by GLP-1RA plus miR-29b-3p inhibitors. After GLP-1RA administration, the expression of SLMAP protein was dramatically decreased in H9c2 cells stimulated by high glucose plus FFA (Figure 6D). The effect of GLP-1RA on reducing SLMAP protein expression was completely eliminated after treatment with miR-29b-3p inhibitors in H9c2 cells (Figure 6D). The above results suggested that the regulation of SLMAP expression in cardiomyocytes by GLP-1RA was mediated by miR-29b.

Furthermore, to determine whether GLP-1RA can directly act on cardiomyocytes, we detected the expression of GLP-1R in heart tissues by immunofluorescence staining. As shown in Figure 6A, GLP-1R was widely expressed in the myocardium of mice in each group. And GLP-1R expression in H9c2 cells was inhibited by GLP-1R siRNA3 (Figure 6B). As shown in Figure 5B, compared with the control group, the expression of miR-29b-3p was obviously decreased in H9c2 cells stimulated with high glucose plus FFA. After GLP-1RA treatment, the expression of miR-29b-3p in H9c2 cells was significantly higher than that in the high glucose plus FFA group (Figure 5B). Our study also indicated that the effects of GLP-1RA on increasing miR-29b expression and decreasing SLMAP protein expression were dramatically abolished after the inhibition of GLP-1R in H9c2 cells by GLP-1R siRNA3 (Figure 6C and D). We speculated that GLP-1RA regulated the expression of miR-29b/SLMAP by binding to GLP-1R in cardiomyocytes.

Discussion

Our study found that GLP-1RA could significantly improve serum BNP level, cardiac ultrasound changes, myocardial fibrosis and myocardial lipid deposition in DCM mice. SLMAP was significantly up-regulated in the myocardium of mice with DCM compared with controls, whereas it was remarkably down-regulated after GLP-1RA treatment. We also found SLMAP was a target of miR-29b-3p, and GLP-1RA could modulate the expression of SLMAP through miR-29b-3p. We speculated that miR-29b-3p/SLMAP may play a crucial role in the pathogenesis of DCM and may be a potential target for GLP-1RA in improving DCM.

Previous study has described DCM as a unique pathological subgroup caused by abnormal myocardial metabolism, which seems to be independent of traditional risk factors such as coronary artery disease and hypertension.[24] The Framingham heart research report[4] suggested that 19% of patients with heart failure were suffered from type 2 diabetes. After adjusting for obesity, dyslipidemia, hypertension or coronary artery disease, the incidence of heart failure in diabetic patients was still increased significantly. The manifestations of DCM include cardiomyocyte hypertrophy, interstitial fibrosis, cardiomyocyte apoptosis, and decreased diastolic and systolic functions.[25,26] In our study, our data confirmed the main manifestations of DCM in mice. We found that compared with control group, the lipid deposition in the heart and myocardial fibrosis of mice were dramatically increased in DCM group, the plasma BNP level was remarkably elevated, and the echocardiography of DCM mice was also significantly changed.

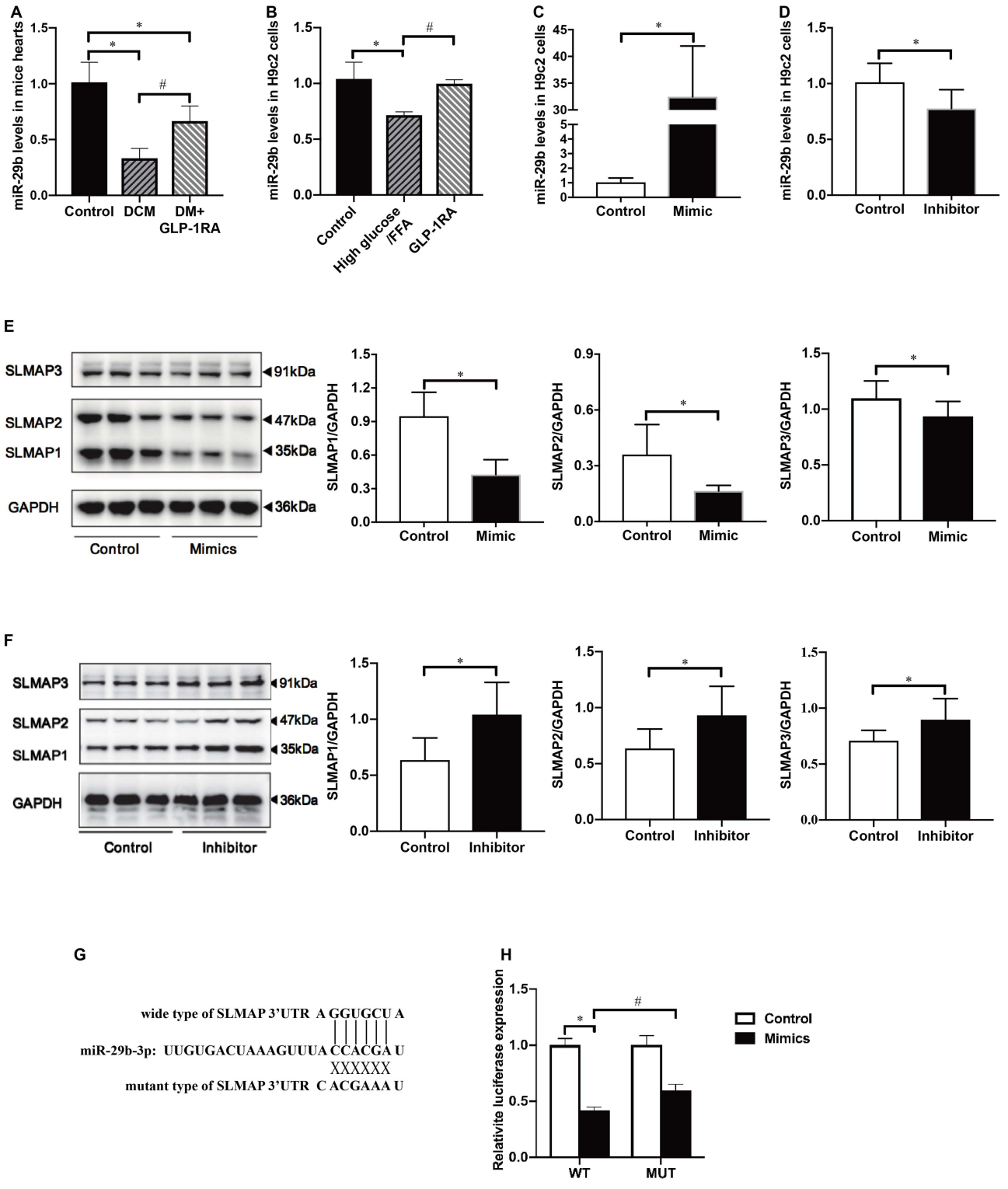

Figure 5 GLP-1RA affects the expression of SLMAP through miR-29b-3p in cardiomyocytes. (**A**) The expression of miR-29b-3p in the myocardium of mice among three groups was measured by qPCR (n = 6/group). *P <0.05 compared with control group; #P <0.05 compared with DCM group. (**B**) The expression of miR-29b-3p in H9c2 cardiomyocytes was detected by qPCR (n = 6). *P <0.05 compared with control group; #P <0.05 compared with high glucose/FFA group. (**C** and **D**) The expression of miR-29b-3p in H9c2 cardiomyocytes activated by miR-29b-3p mimics (**C**) or miR-29b-3p inhibitors was measured by qPCR (**D**) (n = 6). *P <0.05 compared with control group. (**E**) Representative Western blot and quantification of SLMAP1-3 protein levels in H9c2 stimulated by miR-29b-3p mimics (n = 3). (**F**) Representative Western blot and quantification of SLMAP1-3 protein levels in H9c2 stimulated by miR-29b-3p inhibitors (n = 3). (**G**) The binding sites of miR-29b-3p to *Slmap* 3'UTR and mutant (MUT) *Slmap* 3'UTR. (**H**) Dual-Luciferase reporter assay verified that SLMAP was a target of miR-29b-3p (n = 6). *P <0.05 compared with control group; #P <0.05 compared with wide type (WT) *Slmap* 3'UTR treated with miR-29b-3p mimics.

https://doi.org/10.2147/DDDT.S400249 DovePress

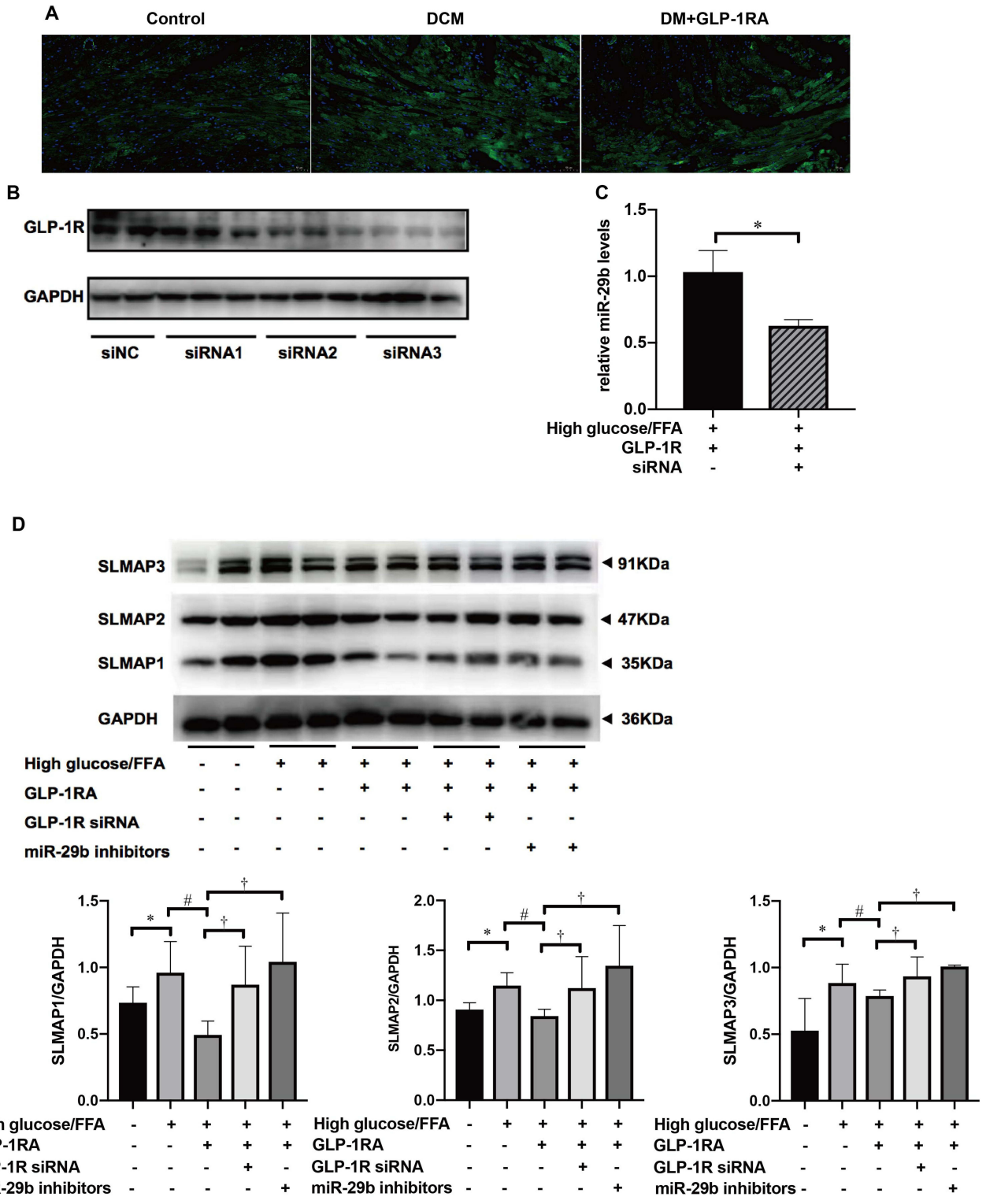

Figure 6 GLP-1RA affects the expression of SLMAP through miR-29b-3p. (**A**) Immunofluorescence staining of GLP-1R was performed on the heart sections of mice (n = 3). (**B**) GLP-1R expression in the H9c2 cells was inhibited by GLP-1R siRNA (n=3). (**C**) GLP-1R siRNA inhibited the expression of miR-29b-3p in H9c2 cells after H9c2 cells were treated with high glucose plus FFA combined with GLP-1RA (n = 3). *P <0.05 compared with GLP-1R siRNA control group (GLP-1R siNC). (**D**) Representative Western blot and quantification of SLMAP1-3 protein levels in H9c2 cells after treatment with high glucose/FFA, GLP-1RA, miR-29b-3p inhibitors or GLP-1R siRNA (n = 4). *P <0.05 compared with control group; #P <0.05 compared with high glucose/FFA group. †P <0.05 compared with high glucose/FFA+GLP-1RA group. Data are expressed as mean ± SD.

Recently studies have shown that the risk of heart failure is increased even in the pre-diabetic status.[27] In those patients with heart failure, pre-diabetes also increased the mortality.[28] These data emphasized the importance of interventions for DCM. Although the meta-analysis of clinical trials of GLP-1RA confirmed that GLP-1RA can dramatically reduce the cardiovascular mortality and hospitalization rate of heart failure in diabetic patients,[13] human RCTs handling GLP-1RA on heart failure were controversial. Moreover, most of these clinical trials are aimed at adult T2DM patients with confirmed CVD or high risk of CVD, rather than patients with DCM. Therefore, the effect and mechanism of GLP-1RA on diabetes cardiomyopathy need further study. GLP-1RA also has a beneficial effect on the survival of rodents after myocardial infarction and the improvement of cardiac structure/function, and this protective effect was independent of its metabolic improvement.[29] In addition, GLP-1RA can significantly protect against myocardial remodeling after myocardial infarction in euglycemic mice, suggesting that the protective effect of GLP-1RA on the heart may be independent of its hypoglycemic effect.[30] The above studies all support that in addition to its hypoglycemic effect, GLP-1RA can also treat DCM through other mechanisms. Although several studies have found that GLP-1RA has cardiovascular benefits for diabetic patients, the protective effect of GLP-1RA on DCM is still unclear. Our results suggested that GLP-1RA may have an effective therapeutic effect on the impaired myocardial structure and function in DCM. Our results demonstrated that GLP-1RA could significantly improve the main manifestations of DCM mice, including serum BNP level, cardiac ultrasound changes, myocardial fibrosis and myocardial lipid deposition. Therefore, our research provides a theoretical basis for the clinical application of GLP-1RA in the prevention and treatment of diabetes cardiomyopathy.

One of the challenges in understanding the effects of GLP-1RA on myocardial SLMAP is to clarify whether GLP-1RA acts directly or indirectly on cardiomyocytes. Previous studies have detected GLP-1R mRNA transcripts in rat and mouse hearts by RT-PCR,[31,32] and GLP-1R protein expression in all chambers of mouse hearts by Western blotting.[32] In addition, GLP-1R expression was confirmed in human ventricular tissue by RT-PCR[33] and monoclonal antibody-binding method.[34] In our study, the expression of GLP-1R in mouse ventricle was confirmed by immunofluorescence staining, and GLP-1R expression in H9c2 rat cardiomyocytes was detected by Western blotting. After GLP-1R inhibition in H9c2 cells by GLP-1R siRNA, we found that the effect of GLP-1RA on miR-29b up-regulation and SLMAP down-regulation in cardiomyocytes was abolished. Therefore, we speculate that GLP-1RA directly acts on the myocardium, and the effect of GLP-1RA on miR-29b/SLMAP expression is mediated by GLP-1R.

SLMAP belongs to the tail anchor membrane protein family, and three isoforms of SLMAP (SLMAP1, SLMAP2 and SLMAP3) are widely expressed in the myocardium. Previous studies have shown that SLMAP plays a regulatory role of cardiac excitation-contraction coupling in cardiomyocyte contraction and cardiac electrophysiology.[35] In the pathological model of congestive heart failure, the expression of SLMAP protein was significantly increased.[21] Transgenic mice with cardiac overexpression of SLMAP1 bearing the transmembrane domain 2 (TM2) displayed impaired contractility and relaxation, prolonged QT interval of the sarcoplasmic reticulum/endoplasmic reticulum, and abnormal cardiac electrophysiology and performance.[36] In addition, the expression of SLMAP was downregulated in the ventricles of dilated cardiomyopathy. Knockdown of SLMAP in cardiomyocytes could also reduce the spontaneous contraction rate of cardiomyocytes by 50%–60%, lead to defective adrenergic response, resulting in manifestations similar to heart failure.[37] The above studies have suggested that abnormal expressions of SLMAP (whether the increase or decrease of expression) may be involved in the pathogenesis of different type of heart failure, which further proves the important regulatory role of SLMAP in heart diseases. Our study first proposed the expression changes and potential roles of myocardial SLMAP in DCM And SLMAP may become a potential target of DCM treatment.

The effect of SLMAP on cardiac function may be related to its effect on cardiac ion channel such as calcium channel and sodium channel. Myocardial electrophysiological alterations and cardiac dysfunctions in the transgenic mice with cardiac SLMAP1-TM2 overexpression were related to the decreased expression of calcium cycling proteins of the sarcoplasmic reticulum, including ryanodine receptor, sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA ATPase), calsequestrin and triadin, and reduced calcium uptake in microsome.[36] A higher intracellular calcium transient peak was shown in H9C2 cardiomyocytes overexpressing SLMAP3 treating with isoproterenol.[37] In addition, a previous report found that missense mutations of SLMAP3 in human were related to Brugada syndrome and were associated with defects in voltage gated sodium channel (Nav1.5). It also demonstrated that SLMAP3 was a modifier of Nav1.5 and regulated the

trafficking and function of Nav1.5 at the sarcolemmal.[38] Transgenic mice with gain of SLMAP3 protein in myocardium were present with myocardial dysfunction, and the protein and transcription levels of Nav1.5, SERCA2a, and Phospholamban in the heart were significantly decreased.[39] This result implied that SLMAP might impact cardiac electrophysiology/function through regulating cardiac calcium channel and sodium channel.

Recent evidence has suggested that miRNA is an important post-transcriptional regulatory factor and plays an important role in the pathogenesis of DCM.[40] We speculate that miR-29b-3p may play a role in post-transcriptional regulation of SLMAP in DCM mice. The expression of miR-29b-3p was significantly decreased in the myocardium of DCM mice, whereas the reduced level of miR-29b-3p could be reversed after GLP-1RA treatment. miR-29b-3p mimics inhibited the level of SLMAP protein in H9c2 cells, while miR-29b-3p inhibitors increased the expression of SLMAP protein. Meanwhile, after treatment with miR-29b-3p inhibitors in H9c2 cells, the effect of GLP-1RA on reducing SLMAP protein expression was completely abolished. Therefore, the effect of GLP-1RA on regulating SLMAP expression was mediated by miR-29b. Luciferase reporter genes technology suggested that the expression of 3'UTR of *Slmap* could be regulated by miR-29b-3p. Previous studies have shown that the level of miR-29b-3p in left ventricular of patients with dilated cardiomyopathy was significantly reduced.[41] In the heart of diabetic rats, down-regulation of miR-29b-3p was related to the significant disturbance of cardiac myocytes and myofibrils.[42] We speculate that miR-29b-3p may play a critical role in the pathogenesis of DCM through targeting SLMAP. Further research on the role of miR-29b-3p/SLMAP in the pathogenesis and treatment of DCM remains to be done.

Conclusion

Our study indicated that GLP-1RA treatment had a significant protective effect on serum BNP, myocardial diastolic dysfunction demonstrated by cardiac ultrasound, myocardial fibrosis and lipid deposition in DCM mice. SLMAP was one of the most differentially expressed proteins among DCM, DM+GLP-1RA and control group. We found that the effect of GLP-1RA on the expression of SLMAP could be regulated by miR-29b-3p. We speculate that the therapeutic effect of GLP-1RA on DCM may be achieved by binding to GLP-1R in cardiomyocytes and further regulating miR-29b-3p/SLMAP expression.

Funding

This study was sponsored by the National Natural Science Foundation of China (Grant No. 81974105, 8227032471, 82200970), Scientific research project of Shanghai Municipal Health Commission (Grant No. 20194Y0460), and Doctoral Fund of Northern Jiangsu Hospital (Grant No. BSQDJ0142).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Benjamin S, Geiss L, Pan L, Engelgau M, Greenland K. Self-reported heart disease and stroke among adults with and without diabetes – United States, 1999–2001: a report of the Center for Disease Control and Prevention. *MMWR Morb Mortal Wkly Rep*. 2003;52:1065–1070.
2. Grundy SM, Benjamin IJ, Burke GL, et al. Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. *Circulation*. 1999;100(10):1134–1146. doi:10.1161/01.CIR.100.10.1134
3. Lee WS, Kim J. Diabetic cardiomyopathy: where we are and where we are going. *Korean J Intern Med*. 2017;32(3):404–421. doi:10.3904/kjim.2016.208
4. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. *Am J Cardiol*. 1974;34(1):29–34. doi:10.1016/0002-9149(74)90089-7
5. Boudina S, Abel ED. Diabetic cardiomyopathy, causes and effects. *Rev Endocr Metab Disord*. 2010;11(1):31–39. doi:10.1007/s11154-010-9131-7
6. Aronson D. Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. *J Hypertens*. 2003;21(1):3–12. doi:10.1097/00004872-200301000-00002
7. Wu LJ, Wang K, Wang W, et al. Glucagon-like peptide-1 ameliorates cardiac lipotoxicity in diabetic cardiomyopathy via the PPAR alpha pathway. *Aging Cell*. 2018;17(4):e12763. doi:10.1111/acel.12763
8. Wang M, Zhang WB, Zhou BQ, Zhu JH, Fu GS. The signal transduction pathway of PKC/NF-κB/c-fos may be involved in the influence of high glucose on the cardiomyocytes of neonatal rats. *Cardiovasc Diabetol*. 2009;8(1):1–14. doi:10.1186/1475-2840-8-1
9. Zheng H, Zhu H, Liu X, Huang X, Huang A, Huang Y. Mitophagy in diabetic cardiomyopathy: roles and mechanisms. *Front Cell Dev Biol*. 2021;9:750382. doi:10.3389/fcell.2021.750382
10. Lin J, Duan J, Wang Q, Xu S, Zhou S, Yao K. Mitochondrial dynamics and mitophagy in cardiometabolic disease. *Front Cardiovasc Med*. 2022;9:917135. doi:10.3389/fcvm.2022.917135

11. De Geest B, Mishra M. Role of oxidative stress in diabetic cardiomyopathy. *Antioxidants*. 2022;11(4). doi:10.3390/antiox11040784
12. Pierce GN, Ramjiawan B, Dhalla NS, Ferrari R. Na(+)-H+ exchange in cardiac sarcolemmal vesicles isolated from diabetic rats. *Am J Physiol*. 1990;258(1 Pt 2):H255–261. doi:10.1152/ajpheart.1990.258.1.H255
13. Pfeffer MA, Claggett B, Diaz R, et al. Lixisenatide in patients with type 2 diabetes and acute coronary syndrome. *N Engl J Med*. 2015;373 (23):2247–2257. doi:10.1056/NEJMoa1509225
14. Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. 2016;375(4):311–322. doi:10.1056/NEJMoa1603827
15. Marso SP, Bain SC, Consoli A, et al. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med*. 2016;375(19):1834–1844. doi:10.1056/NEJMoa1607141
16. Holman RR, Bethel MA, Mentz RJ, et al. Effects of once-weekly exenatide on cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. 2017;377 (13):1228–1239. doi:10.1056/NEJMoa1612917
17. Hernandez AF, Green JB, Janmohamed S, et al. Albiglutide and cardiovascular outcomes in patients with type 2 diabetes and cardiovascular disease (Harmony Outcomes): a double-blind, randomised placebo-controlled trial. *Lancet*. 2018;392(10157):1519–1529. doi:10.1016/S0140-6736(18)32261-X
18. Gerstein HC, Colhoun HM, Dagenais GR, et al. Dulaglutide and cardiovascular outcomes in type 2 diabetes (REWIND): a double-blind, randomised placebo-controlled trial. *Lancet*. 2019;394(10193):121–130. doi:10.1016/S0140-6736(19)31149-3
19. Husain M, Birkenfeld AL, Donsmark M, et al. Oral semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med*. 2019;381(9):841–851. doi:10.1056/NEJMoa1901118
20. Kristensen SL, Rorth R, Jhund PS. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials (vol 7, pg 776, 2019). *Lancet Diabetes Endo*. 2020;8(3):E2–E2.
21. Previlon M, Le Gall M, Chafey P, et al. Comparative differential proteomic profiles of nonfailing and failing hearts after in vivo thoracic aortic constriction in mice overexpressing FKBP12.6. *Physiol Rep*. 2013;1(3):e00039. doi:10.1002/phy2.39
22. Ding H, Howarth AG, Pannirselvam M, et al. Endothelial dysfunction in Type 2 diabetes correlates with deregulated expression of the tail-anchored membrane protein SLMAP. *Am J Physiol Heart Circ Physiol*. 2005;289(1):H206–211. doi:10.1152/ajpheart.00037.2005
23. Ma T, Huang X, Zheng H, et al. SFRP2 improves mitochondrial dynamics and mitochondrial biogenesis, oxidative stress, and apoptosis in diabetic cardiomyopathy. *Oxid Med Cell Longev*. 2021;2021:9265016. doi:10.1155/2021/9265016
24. Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Grishman A, Grishman A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am J Cardiol*. 1972;30(6):595–602. doi:10.1016/0002-9149(72)90595-4
25. Wang J, Song Y, Wang Q, Kralik PM, Epstein PN. Causes and characteristics of diabetic cardiomyopathy. *Rev Diabet Stud*. 2006;3(3):108–117. doi:10.1900/RDS.2006.3.108
26. Poornima IG, Parikh P, Shannon RP. Diabetic cardiomyopathy: the search for a unifying hypothesis. *Circ Res*. 2006;98(5):596–605. doi:10.1161/01.RES.0000207406.94146.c2
27. Cai X, Liu X, Sun L, et al. Prediabetes and the risk of heart failure: a meta-analysis. *Diabetes Obes Metab*. 2021;23(8):1746–1753. doi:10.1111/dom.14388
28. Mai L, Wen W, Qiu M, et al. Association between prediabetes and adverse outcomes in heart failure. *Diabetes Obes Metab*. 2021;23(11):2476–2483. doi:10.1111/dom.14490
29. Timmers L, Henriques JP, de Kleijn DP, et al. Exenatide reduces infarct size and improves cardiac function in a porcine model of ischemia and reperfusion injury. *J Am Coll Cardiol*. 2009;53(6):501–510. doi:10.1016/j.jacc.2008.10.033
30. Robinson E, Cassidy RS, Tate M, et al. Exendin-4 protects against post-myocardial infarction remodelling via specific actions on inflammation and the extracellular matrix. *Basic Res Cardiol*. 2015;110(2). doi:10.1007/s00395-015-0476-7
31. Bullock BP, Heller RS, Habener JF. Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. *Endocrinology*. 1996;137(7):2968–2978. doi:10.1210/endo.137.7.8770921
32. Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation*. 2008;117(18):2340–2350. doi:10.1161/CIRCULATIONAHA.107.739938
33. Baggio LL, Bernardo Y, Mulvihill EE, et al. GLP-1 receptor expression within the human heart. *Endocrinology*. 2018;2018(4):4.
34. Clarke SJ, Giblett JP, Yang LL, et al. GLPIs a coronary artery vasodilator in humans. *J Am Heart Assoc*. 2018;7(22). doi:10.1161/JAHA.118.010321
35. Nader M. The SLMAP/Striatin complex: an emerging regulator of normal and abnormal cardiac excitation-contraction coupling. *Eur J Pharmacol*. 2019;25:858.
36. Nader M, Westendorp B, Hawari O, et al. Tail-anchored membrane protein SLMAP is a novel regulator of cardiac function at the sarcoplasmic reticulum. *Am J Physiol Heart Circ Physiol*. 2012;302(5):H1138. doi:10.1152/ajpheart.00872.2011
37. Nader M, Alsolme E, Alotaibi S, Alsomali R, Bakheet D, Dzimiri N. SLMAP-3 is downregulated in human dilated ventricles and its over-expression promotes cardiomyocyte response to adrenergic stimuli by increasing intracellular calcium. *Can J Physiol Pharmacol*. 2019;97(7):623–630. doi:10.1139/cjpp-2018-0660
38. Ishikawa T, Sato A, Marcou CA, et al. A novel disease gene for Brugada syndrome: sarcolemmal membrane-associated protein gene mutations impair intracellular trafficking of hNav1.5. *Circ Arrhythm Electrophysiol*. 2012;5(6):1098–1107. doi:10.1161/CIRCEP.111.969972
39. Mlynarova J, Trentin-Sonoda M, Gaisler da Silva F, et al. SLMAP3 isoform modulates cardiac gene expression and function. *PLoS One*. 2019;14 (4):e0214669. doi:10.1371/journal.pone.0214669
40. Liu X, Liu S. Role of microRNAs in the pathogenesis of diabetic cardiomyopathy. *Biomed Rep*. 2017;6(2):140–145. doi:10.3892/br.2017.841
41. Naga Prasad SV, Gupta MK, Duan ZH, et al. A unique microRNA profile in end-stage heart failure indicates alterations in specific cardiovascular signaling networks. *PLoS One*. 2017;12(3):e0170456. doi:10.1371/journal.pone.0170456
42. Arnold N, Koppula PR, Gul R, Luck C, Pulakat L. Regulation of cardiac expression of the diabetic marker microRNA miR-29. *PLoS One*. 2014;9 (7):e103284. doi:10.1371/journal.pone.0103284