Mesenchymal stem cells modified by FGF21 and GLP1 ameliorate lipid metabolism while reducing blood glucose in type 2 diabetic mice

CURRENT STATUS: Under Review

Binghua Xue, Xiao Xiuxiao, Tingting Yu, Xinhua Xiao, Jie Xie, Qiuhe Ji, Li Wang, Tao Na, Shufang Meng, Peiliang Geng, Lingjia Qian, Haifeng Duan

Binghua Xue
Department of Military Cognitive and Stress Medicine Institute of Military Cognitive and Brain Sciences Academy of Military Sciences

Xiao Xiuxiao
Universita degli Studi di Padova Dipartimento di Medicina

Tingting Yu
Department of Experimental Hematology Beijing Institute of Radiation Medicine Academy of Military Sciences

Xinhua Xiao
Department of Endocrinology Chinese Academy of Medical Sciences and Peking Union Medical College Peking Union Medical College Hospital

Jie Xie
Department of Experimental Hematology Beijing Institute of Radiation Medicine Academy of Military Sciences

Qiuhe Ji
Department of Endocrinology and Metabolism Xijing Hospital of Airforce Medical University

Li Wang
Department of Endocrinology and Metabolism Xijing Hospital of Airforce Medical University

Tao Na
The cell Collection and Research Center National Institutes for Food and Durg COntrol, Key Laboratory of the Ministry of Health for Research on Quality and Standardization of Biotech Products

Shufang Meng
China National Institute for Food and Drug Control

Peiliang Geng
Department of Experimental Hematology Beijing Institute of Radiation Medicine Academy of Military Sciences
Lingjia Qian  
Department of Military Cognitive and Stress Medicine Institute of Military Cognitive and Brain Sciences Academy of Military Sciences

Haifeng Duan  
Department of Experimental Hematology, Beijing Institute of Radiation Medicine, Academy of Military Sciences  
haifengduan731126@126.com  
Corresponding Author  
ORCiD: https://orcid.org/0000-0002-7740-3882

Prescreen  
10.21203/rs.3.rs-25980/v1

Subject Areas

Stem Cell & Developmental Cell Biology

Keywords

Type 2 diabetes mellitus, Mesenchymal stem cell, FGF21, GLP1
Abstract

Objective: The purpose of the study was to investigate the therapeutic effects and safety of genetic modified mesenchymal stem cells (MSCs) in type 2 diabetes mellitus (T2DM).

Methods: MSCs derived from adipose tissue were modified with over-expression of FGF21 and GLP1, which was achieved through lentiviral particles transduction. The cells were transplanted to the BKS.Cg-Dock7m+/+Leprdb/Nju mice (T2DM mice model). The injections of physiological saline (0.1 mL), Liraglutide (0.5mg/kg) were employed as negative and positive controls respectively. The protein analyses were performed using ELISA or western blot analysis, while quantitative real-time PCR was adopted for gene expression.

Results: The genetic modification had no effects on morphology, differentiation ability, or immunophenotype of MSCs. Moreover, MSC-FGF21+GLP1 cells exhibited significantly increased expression of FGF21 and GLP1. In T2DM model mice, the transplantation of MSC-FGF21+GLP1 cells ameliorated blood glucose and weight, promoted the secretion of insulin, enhanced the recovery of liver structures, and improved lipid profiles. Moreover, FGF21 and GLP1 exhibited synergistic effects in regulation of glucolipid metabolism through controlling the expression of insulin, srebp1 and srebp2.

Conclusion: The stem cell treatment based on MSCs modified by FGF21 and GLP1 genes is an effective approach for treatment of T2DM.

Introduction

Diabetes mellitus (DM) is a complex metabolic disease characterized by chronic hyperglycemia, insulin resistance, and pancreatic β cell destruction [1-3]. DM is listed as one of the top ten global diseases leading to human death by the World Health Organization (WHO). Millions of people around the world currently have diabetes or are at a high risk of developing diabetes in the future [4]. According to a report published in the lancet diabetes and endocrinology, the number of people with T2DM will increase from 406 million in 2018 to 511 million by 2030, which is caused by the sustained growth of obesity around the world [5]. Diabetes treatment is a long-term process, and the long-term use of various chemical drugs has various limitations and adverse reactions, even serious complications, such as hypoglycemia and lactic acidosis. So far, there is no medicine that can cure diabetes, and chemical medicine only controls blood glucose. The use of insulin is not only expensive, but also requires daily injection, which is not easy for patients to persist, and the time and dose of administration need to be strictly controlled, otherwise it will easily lead to hypoglycemia. In addition, the chemical drugs could not repair damaged tissues and improve insulin resistance. Therefore, it is urgently needed to develop novel therapeutic modalities.

Insufficient secretion of endogenous hormones, cytokines or enzymes, reduced activity or functional defects are closely related to the occurrence of metabolic diseases. For example, insulin resistance and relative secretion deficiency of β-islet cell are the core causes of diabetes [6]. Therefore, these key molecules that balance the glucose and lipid metabolism are also the key targets of drug development for the treatment of metabolic syndrome, in which glucagon-like polypeptide 1 (GLP1) and FGF21 have become important diabetes and obesity treatment drugs at present [6-10]. GLP1 secreted by L cells of the ileum and colon plays an important role in maintaining glucose homeostasis and other physiological processes [10]. GLP1 receptor agonist can be used to treat T2DM by promoting the secretion of insulin through glucose dependence [11]. GLP1 receptor agonist promotes insulin release mainly by activating GLP1 receptor, which enhances the calcium ion influx of the calcium ion channel and the calcium ion release of the endoplasmic reticulum through the cAMP/PKA pathway, activates calmodulin, and ultimately enhances insulin exocytosis [12]. Studies have shown that GLP1 will not secrete insulin when blood glucose is lower than 4.5mmol/L. Therefore, GLP1 receptor agonist can reduce the risk of hypoglycemia that is more conducive to achieving stable hypoglycemia [13]. In addition to hypoglycemic effects, GLP1R agonists also protect and repair beta cells by inhibiting glucagon secretion and stimulating beta cell proliferation and regeneration [12, 14]. The hormone fibroblast growth factor 21 (FGF21), produced in
tissues involved in metabolism such as the liver, adipose tissues, skeletal muscle, and pancreas, is shown to improve metabolic diseases and induce weight loss in human and mice [15-17]. Recently, a synthetic FGF21 variant, LY2405319, is proved to reduce low-density lipoprotein (LDL) cholesterol and triglycerides, increase adiponectin levels, improve fasting insulin, and induce weight loss in obese patients with type 2 diabetes [15]. FGF21 administration has been associated with decreased sterol regulatory element binding protein (SREBP) levels, which are required for FGF21-induced thermogenesis [18]. Chronic treatment with recombinant FGF21 reduces serum and hepatic triglyceride levels and ameliorates fatty liver in obese mice, through suppressing lipogenic gene, Srebp-1 [7]. In addition to Srebp-1, Srebp-2 is also confirmed as a target of FGF21, which is a master regulator of cholesterol biosynthesis by preferentially activating the transcription of key cholesterologenic genes in the liver [19]. All the evidences demonstrat that FGF21 is a promising cytokine for the treatment of metabolic disorders. Interestingly, GLP1 therapy also can activate the iNKT-FGF21 axis in vivo, which contributes to weight loss [20]. In other words, GLP1 can regulate the expression of FGF21 or play a synergistic role with FGF21 in regulating glucose and lipid metabolism.

Another attractive treatment for diabetes is stem cell therapy. The stem cells have self-renewal ability, and they can also differentiate into different types of cell. In T2DM, the injection of stem cells could differentiate into β-islet cell, thus improving diabetic symptoms. All present, the focus of stem cell therapy for T2DM is on the induction of stem cells differentiation into islet like cells, that the tissue repair and insulin resistance are not taken in consideration. Mesenchymal stem cells (MSCs) derived from different adult tissues have long-term self-renewal abilities as well as the capacity to differentiate into a variety of cell types in certain conditions [21]. In different physiological and pathological conditions, MSCs could maintain homeostasis by multi-directional differentiation. They secrete large amounts of cytokines, which carry chemical signals between cells, and are widely used in studies of regenerative medicine [22-24]. Novel therapeutic approaches based on MSCs have shown satisfactory therapeutic effects in clinical applications. In clinical management of diabetes, preliminary animal experiments and clinical evidences prove that MSCs infusion can effectively decrease blood sugar level, improve muscle, fat and liver tissues of insulin sensitivity, and alleviate diabetic nephropathy, diabetic foot and the lower limb vascular lesions such as complications. Moreover, no serious adverse reactions have been reported, exhibiting good security [25-29]. However, there is a problem in the clinical application of MSCs in diabetes, in which cell characteristics and therapeutic effects may be significantly different due to the different sources of MSCs. Therefore, new complementary therapies are needed to solve the above problems.

To solve these problems, MSCs were engineered with over-expressing FGF21 and GLP1 genes through lentiviral vector transduction, and transfused DM mice in our study. The results showed that the MSCs modified by FGF21 and GLP1 genes could significantly reduce insulin resistance, and promote beta cell function recovery. Moreover, the modified MSCs had significantly increased biological activities such as lowering blood glucose and blood lipid, which were caused by the decreased expressions of srebp1 and srebp2 in lipid metabolism and the increased expression of insulin. Based on these results, we developed a new modified cell for metabolic disorders.

Materials And Methods

1. Construction of fgf21-glp1-IgG4fc lentiviral expression plasmid

pGSI-fgf21-glp1-IgG4fc (synthesized by Taihe Biotechnology) was used for subclonning of fgf21 and glp1-IgG4fc gene to the lentivector pCDH-EF1 (Addgene) with EF1α promoter. The amino acid sequence and the nucleotide sequence of the fgf21+ glp1-IgG4fc gene were listed in the supplementary materials. Primers for amplifying the cDNA of the fgf21+ glp1-IgG4fc gene (forward 5’- CGCGGATCCGCCACCATGGACTCGGACGAGACC -3’, reverse 5’- ACGCGTCGACTTATTACCACCGAAGACAG -3’) were synthesized from TsingKe (Beijing).

2. Lentivirus production

Lentiviral vector plasmids and the packaging plasmids (psPAX and pMD.2G) were purchased from Addgene (Watertown). Lentiviral particles with pCDH-EF1-human FGF21, FGF21+GLP1 and GLP1produced through transfection of HEK293T packaging cells with 3rd generation plasmids system. 293T cells were transfected with
24 µg of plasmids, 48 µl of Lipofectamine LTX and 24 µl of PLUS reagents, and the proportions of pMD. 2G, psPAX, and pCDH-EF1 plasmid were 1:2:3. The supernatants were collected at 24 and 48h after transfection, filtered by 0.45 µm filters, and harvested by ultrafiltration with 100 kDa spin column (Millipore) at 4°C and 4,000g for 30 min. Lentiviral particles were dispensed in aliquots, and stored at -80 °C until use. Transfection efficiency was examined by EGFP using flow cytometry (Beckman), and viral titer was determined according to the equation: virus titer (pfu/mL) = cell number in each well × virus dilution factor × 10/ volume of added virus fluid (mL).

3. Mesenchymal stem cell culture, flow cytometry analysis, and characterization

To obtain the upper adipose tissue the healthy adult adipose tissue extracted by liposuction was transferred to a 50mL centrifuge tube, fully washed with PBS, and centrifuged at 1500rpm for 5 minutes. 0.2% mixed collagenase (Type I, II and IV collagenases=1:1:1) was prepared, and adipose tissue: collagenase =1:1 was added to the mixed collagenase digestion solution. The adipose tissue was digested in a 37 °C shaker for 30 minutes. Digested adipose tissue was immediately added to α-mem cell culture medium containing 10% FBS (Gibco). To precipitate cells and tissue clumps, the mixture was centrifuged at 1500rpm for 10 min. Cells were resuspended using α-mem complete medium and the undigested tissue was removed by nylon mesh. The cells were inoculated in a culture flask and incubated at 37 °C in a 5% CO2 incubator. Two days later, the unadhered cells were discarded and the adhered cells were washed gently with PBS. Then the stem cells were replaced with the complete culture medium.

MSCs were harvested from passage 5, washed three times with PBS, and then incubated with 0.1–10 µg/mL of conjugated antibody (BD Biosciences) for 30-min without light at room temperature. Then, the cells were examined by flow cytometer analysis (Beckman). In total, above 95% of cells expressed CD73, CD90, and CD105, while 2% or less of cells expressed CD45, CD34, and HLA-DR. Released cells were negative for pathogenic microorganisms, HBV, HCV, HIV, cytomegalovirus, syphilis, and ALT, and endotoxin levels were found within 40 IU/L and 0.5 EU/mL, respectively. The total cells were counted, and cell viability (≥ 85%) was determined by Trypan blue staining.

4. Transduction of MSCs with lentiviral particles and detection of target gene expression

The MSCs (<3 passages) were transduced with concentrative lentivirus with a multiplicity of infection (MOIs) of 40 for 6 h in the α-MEM medium containing 8 µg/ml polybrene. To detect the expression patterns of the FGF21 and GLP1-IgG4 in MSCs, western blot analyses of cellular supernatant using anti-FGF21 and human IgG4-Fc monoclonal antibody were performed. To further measure the secreted the FGF21 and GLP1-IgG4, culture medium (CM) of MSCs and WJ-MSCs transduced with pCDH-EF1-FGF21, pCDH-EF1-FGF21+GLP1, pCDH-EF1-GLP1, or pCDH-EF1-vector lentiviral particles were collected after incubation for 48 h. The secreted FGF21 and GLP1 in the medium of MSC culture were measured by ELISA (Abcam) according to the manufacturer’s protocol. For testing the proliferation of secreted MSCs, each MSCs were seeded in 96-well plates at 5 × 10^4 cells/well and preconditioned in culture medium. After 48 h incubation, 20 µl of CCK8 was added to each well and incubated for 4 h at 37 °C and absorbance was measured at 570 nm with a Quant microplate reader. All samples were analyzed as duplicates and samples with coefficient of variation (CV) values > 15% were excluded.

5. Adipogenic and osteogenic differentiation

MSCs were cultured in a 24-well plate in complete α-MEM medium supplemented with adipogenic and osteogenic-inducing agents (Sigma Aldrich) at an initial cell density of 1×10^4 cells/well. After 2-3 weeks, cells were washed twice with PBS and fixed by 4% paraformaldehyde at room temperature for 30 min. Oil-red-O or alkaline phosphatase staining was applied to detect adipogenic and osteogenic differentiation.

6. Western blotting

The cells were washed with PBS buffer and subsequently lysed using cell lysis buffer (Tiangen) with complete protease inhibitor mix (Biotool). The cell lysate and control were run in SDS-PAGE gels (12% or 15%) respectively and transferred onto nitrocellulose membranes (Millipore). Membranes were blocked with 5% milk in Tris-buffered saline plus Tween 20 (TBST) and exposed to primary antibodies against
rabbit or mouse (1:3000, Abcam or Cell Signaling). Blots were probed with horseradish peroxidase (HRP)-conjugated goat anti-rabbit (or mouse) IgG (H+L) secondary antibodies and visualized using a Pierce ECL Western Blotting Substrate kit (Thermo Scientific) for signal detection.

7. **Relative quantitative real time polymerase chain reaction (RT-PCR)**

Total RNA was extracted from cells using RNA isolation kits (TRizol; Sigma). The isolated total RNA was reversely transcribed into the first chain of cDNA using BioScript All-in-One cDNA Synthesis SuperMix (Biotool).

Quantitative real-time PCR reactions were performed using the SYBR Premix Ex Taq (Tli RNaseH Plus) (Takara) in 7500 Real-Time PCR System (Applied Biosystems). For normalization, threshold cycles (Ct-values) were normalized to β-actin/GAPDH within each sample to obtain sample-specific ΔCt values (ΔCt ¼ Ct gene of interest - Ct β-actin/GAPDH). The values of 2^ΔΔCt were calculated to obtain fold expression levels. Primers for quantitative analyses of FGF21 gene (forward 5′- ATCGCTCCACTTTGACCCTG -3′, reverse 5′- GGGCTTGGACTGGATAACA -3′), GLP1-IgG4Fc gene (forward 5′- CCCCCAAAACCCAAGGACACT -3′, reverse 5′- GCCATCCACGTACCAGTTGA -3′), srebplc gene (forward 5′- CACTGTGACCTCGCAGATCC -3′, reverse 5′- ATAGGCACTTCCGATCC -3′), insulin gene (forward 5′- GGTGAATGGAGGAGACATG -3′, reverse 5′- GGAGCGAGATCCCTCCAAAAT -3′), GAPDH gene (forward 5′- GGCTGTTGTCATACTTCTCATGG -3′) were synthesized by TsingKe Company (Beijing).

8. **Animal experiments**

In our study, BKS.Cg-Dock7m+/+Leprdb/Nju mice (T2DM model mice) model was used, and they were purchased from the Model Animal Research Center of Nanjing University. Thirty-six male BKS mice aged 6-8 weeks (>20 g body weight) were randomly divided into six groups. Each group contained six mice kept in two cages. Mice in the six groups were administered physiological saline (0.1 mL), Liraglutide (Selleck, 0.5mg/kg), MSC, MSC-FGF21, MSC-FGF21+GLP1 and MSC-GLP1 (1×10^6 cells suspended in 0.1 mL of physiological saline) thrice on day 0, day 7 and day 14 of the treatment by intravenous injection. Tail blood glucose was measured every week during the experiments. On day 28, peripheral blood was collected from the retro-orbital sinus of each mouse.

9. **Glucose stimulated insulin secretion (GSIS)**

Rat INS-1 pancreatic β cell line was purchased from CCTCC (China Center for Type Culture Collection). The cells were cultured at 37 °C in a humidified atmosphere containing 5% CO₂. The culture medium was RPMI medium 1640 containing 11 mM glucose and supplemented with 10% FBS, 10 mM HEPES, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate and 50 µM mercaptoethanol. The culture medium was replaced every second day, and cells were passaged once a week following trypsinization.

To determine the effect of transduced-MSCs on GSIS, INS-1 cells were seeded onto 12-well plates and cultured for 24 hours. Then, the cells were washed two times with Krebs-Ringer bicarbonate buffer (KRBB, 129 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 2.5 mM CaCl₂, 5 mM NaHCO₃, 0.1% BSA, 10 mM HEPES, (pH 7.4) and 2.8 mM glucose) and starved for 2 hours in KRBB. The cells were incubated in fresh KRBB containing different MSCs conditioned medium for 1 hour in the presence of glucose. The supernatants were collected to measure insulin concentration.

10. **Fasting glucose and glucose tolerance tests**

For the weekly fasting glucose test, the mice were starved overnight to assess glycemia. At the end of the experiment, after overnight fasting, the mice were administered glucose (1 g/kg) by oral gavage, and blood samples were collected from the tail vein for glucose determination. Glycemia was assessed using an Accu-Chek glucometer (Roche, Basel, Switzerland, http://www.roche.com), and the area under the curve was calculated.

11. **Statistical analysis**

All statistical analyses were conducted using the software of SPSS. Data were analyzed using one-way ANOVA followed with the Tukey’s post-doc test or two-way ANOVA followed with the Bonferroni’s post-hoc test for the
Results

1. Morphology and immunophenotypic characterization of adipose derived MSCs

MSCs isolated from human adipose tissue display a uniform spindleshaped morphology like fibroblast cells under phase-contrast microscopy (Figure 1A). In vitro differentiation analysis confirmed that the isolated MSCs could differentiate into osteoblasts and adipocytes (Figure 1B). For further characterization of MSCs, a panel of surface markers were tested using flow cytometric analysis. MSCs were negative for CD34, CD45 and HLA-DR, whereas they were positive for CD73, CD90 and CD105 (Figure 1C).

2. FGF21 and GLP1-IgG4Fc (GLP1-fc) expression in transduced MSCs

MSCs were transduced with the lentiviral vectors pCDH-EF1 expressing EGFP as a marker. Transduced cells were examined for EGFP using a fluorescence microscope (Figure 2A). Flow cytometry analysis of EGFP was performed with the cells at 48 h after transduction, and EGFP-positive cells ranged from 79 to 98% (Figure 2B). Flow cytometry analysis showed that EGFP-positive cells were over 95% when multiplicities of infection (MOI) was 40, and transfection efficiency was not significantly raised between MOI 40 and 55 (Figure 2B). Consequently, the optimal MOI for transduction protocol was 40. Then, the differentiation ability and MSC surface markers of the transduced MSCs were detected. The results showed that lentiviral particles transduction did not affect the biological characteristics of MSCs (Figure 2C, D).

To further demonstrate the expression profile of FGF21 and GLP1-IgG4Fc, quantitative RT-PCR analysis was performed. The results confirmed that the expression of FGF21 and GLP1-fc mRNA was significantly higher in FGF21 or/and GLP1-fc transduced MSCs compare to non-transduced or vector-transduced MSCs (P<0.05 for all) (Figure 3A). The concentration of secreted FGF21 and GLP1 proteins in the culture medium of MSCs transduced with FGF21 or/and GLP1-IgG4Fc was much higher than that in the MSCs or MSCs transduced with vector (Figure 3B). Furthermore, western blot analyses demonstrated the expression of FGF21 and GLP1-IgG4Fc in cell supernatant from f21 or/and glp1-fc -transduced MSCs was significantly enhanced, compare to the MSCs and vector-transduced MSCs (Figure 3C).

3. FGF21+GLP1 transduced MSCs transplantation ameliorated blood glucose and weight in T2DM mice

The effects of MSC-FGF21+GLP1 cells in system metabolic disturbance were investigated in our study. BKS.Cg-Dock7m+/+leprdb/Nju mice (BKS mice) with leptin receptor deficient is characterized by obesity, insulin resistance, hyperglycemia, and dyslipidemia. The nontransduced MSCs and GLP1 analogues (liraglutide) were employed as the control. 3 weeks after the administration of transduced MSCs, the increased tendency of body weight was significantly inhibited in the MSC-FGF21+GLP1 group (Figure 4A), and adipose tissue weight was also decreased in the BKS mice treated by MSC-FGF21+GLP1 in comparison with the notreatment groups. Intriguingly, MSC-FGF21+GLP1 had similar effects with liraglutide (Figure 4B). Meanwhile, the fasting serum glucose level of BKS mice was determined once a week for 4 weeks. As shown in Figure 4C, MSC-FGF21+GLP1 cells administration remarkably decreased the fasting blood glucose levels of BKS mice. It was noted that the glucose-lowering effect of MSC-FGF21+GLP1 was slightly greater than that of other cell treatment groups. The same phenomenon was observed from the results of the oral glucose tolerance test (Figure 4D). In addition, the plasma insulin level of the BKS mice was slightly increased after MSC-FGF21+GLP1 treatment (Figure 4E). These observations suggested that MSC-FGF21+GLP1 could enhance insulin secretion.

4. FGF21+GLP1 transduced MSCs transplantation could improve lipid disorders in T2DM mice

The histopathological analysis was performed to determine the potential target tissues or organs of MSC-FGF21+GLP1 in T2DM mice. We found that MSC-FGF21+GLP1 cells could improve the tissue structures of the liver and adipose in BKS mice. Microscopic observation showed that administration of MSCs resulted in the
recovery of liver structures of the BKS mice (Figure 5A). However, histological observation of adipose tissues showed that the size of abdominal adipocytes in the MSC-FGF21+GLP1 treated mice had decreased substantially, but this was not remarkably observed in the other groups (Figure 5A, B). This is interesting, because this response of adipose tissues to MSC-FGF21+GLP1 treatment may partially explain the antiobesity and lipid-lowering effects. Interestingly, and in accordance with our initial expectations, the plasma lipid profiles of FGF21+GLP1 treated mice were significantly improved, as manifested by the markedly reduced triglyceride (TG), cholesterol (TC), and low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL) levels (Figure 5C).

5. Over-expression of FGF21+GLP1-Fc in MSCs (MSC-FGF21-GLP1) significantly suppressed srebpt1c transcription and promoted insulin expression

To preliminarily verify the reasons why MSC-FGF21-GLP1 can correct glucose and lipid metabolism, we primarily examined the expression of key genes, srebpt and insulin, that influence glycolipid metabolism. In order to detect the effects of MSC cells on the expression of srebpt1 gene and insulin gene, we used conditional media of different gene-modified MSC cells (FGF21 or/and GLP1 modified) to treat human HepG2 cells and rat INS-1 cells, respectively. As shown in Figure 6A and 6B, the supernatant isolated from MSC-FGF21+GLP1 treatment group could significantly inhibit the mRNA level of srebpt1 and increase insulin mRNA level, and the activity of MSC-FGF21+GLP1 was significantly higher than that of MSC-FGF21, MSC-GLP1, and even the positive control drug Liraglutide. The promoting effects on insulin secretion was also confirmed by GSIS experiment. The results suggested that MSC-FGF21+GLP1 could significantly stimulate the secretion of insulin in rat INS-1 cells (Figure 6C). Therefore, FGF21 and GLP1-IgG4Fc double gene modified MSCs showed a significant synergistic effect in regulating glucolipid metabolism, especially in regulating srebpt1c and insulin genes expression.

6. The mechanisms of FGF21 and GLP1 synergistically improving lipid metabolism.

To further elucidate the pathway by which MSC-FGF21+GLP1 synergistically regulates lipid metabolism, we detected the key lipid metabolism regulation genes upstream and downstream of srebpt genes. Conditional media of different gene-modified MSC cells (FGF21 or/and GLP1 modified) were added to human HepG2 cells processing 48 hours. Then the cells were collected to extract cytoplasmic and nuclear proteins to detecting the expression level of proteins. As shown in Figure 7, MSC-FGF21+GLP1 could significantly increased the phosphorylation level of AMPK, which was much higher than that of single gene modified MSCs. Next, we detected the expression of SREBP1 and SREBP2 genes in the nucleus. For SREBP1, the expression of both spliced active protein (the bottom band) and intact protein (the top band) in the nucleus was reduced. But for SREBP2, the intact protein (the top band) in the nucleus was not influenced, and the spliced active protein (the bottom band) was remarkably decreased in the MSC-FGF21+GLP1 group. Consistent with the above results, the phosphorylation level of ACC protein downstream of SREBP was reduced, and the expression level of FAS protein was also slightly reduced. However, the phosphorylation level of HSL, an enzyme associated with promoting fat decomposition, was significantly increased.

The summary mechanisms of FGF21 and GLP1 synergistically improving lipid metabolism was shown in Figure 8, the diagram illustrates three existing approaches to regulate lipid metabolism. The red box represents the known metabolism regulation aimed at PCSK9 and HMC-CoA reductase. Currently, antibodies and statins are mainly used to block the expression of PCSK9 and HMC-CoA reductase so as to reduce cholesterol and lipid synthesis. In this study, we focused on the cytokines that could regulate lipid metabolism. FGF21 and GLP1 bind to their receptor, then they synergistically enhanced AMPK phosphorylation levels. Activated AMPK further inhibits the expression of SREBP1/2 genes and the entry of mature SREBP1/2 protein into the nucleus. At last, the expression of enzymes directly involved in lipid metabolism is significantly inhibited.

Discussion

Diabetes is a chronic disease with high morbidity and mortality in the world. Traditionally, diabetes is divided into type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), among which the incidence of T2DM is more than 90% [30]. Current studies suggest that insulin resistance (IR) and secretion defects of islet beta cells
are two major pathogenesis of T2DM, and persistent inflammatory state (chronic inflammation) is an important feature of T2DM [30-33]. Therefore, diabetes is also fundamentally considered to be a combination of the other three diseases. Firstly, it is an internal secretory disease, which involves a variety of hormone level disorders, including insulin, glucocorticoid and adrenal hormone [34]. Secondly, it can be regarded as a metabolic disease with the dysfunctions of glucose, lipid metabolism, mitochondrial function, nucleic acid regulation and so on [35-37]. Thirdly, it is a kind of systemic disease, which is embodied in the reducing of insulin sensitivity in the whole body's metabolic tissues and organs, and can cause damage to the structure and function of various tissues and organs in the body, including liver, heart, kidney, peripheral micro-vessel and nervous system [38, 39]. Based on the above theory, in order to efficiently change the symptoms of diabetes, tissue repair requires the combined actions of multiple aspects, such as stem cells, regeneration factors, and nutrients, as shown in the following figure. However, the current therapeutic strategies for DM mainly focus on the control of sugar and lipid metabolism. The tissue repair and insulin resistance are not took in consideration. In order to improve the status, the double-gene modified MSCs (FGF21 and GLP1) were designed to achieve multiple repair effects in the treatment of diabetes in this study.

Sugar, fat and protein metabolism is the most basic metabolism of the body. The main organs involved in the metabolism include brain, liver, gastrointestinal tract, pancreas, adipose tissue and muscle tissue. The mutual regulation of metabolic organs forms a complex regulatory network, in which the neuroendocrine system, growth factors and enzymes are involved. Generally speaking, there are three types of endogenous molecules involved in the regeneration factor regulation of metabolism. The first one is the hormones, including insulin, glucagon, GLP1 and glucocorticoid. The second factor is the growth factors of cells with hormone-like functions, including FGF19, FGF21 and FGF23. The last one is the enzymes involved in metabolism regulation or cell signal transduction, such as PI3K and HSL. These endogenous hormones, cytokines or enzymes are closely related to the occurrence of metabolic diseases. In this study, we selected two interrelated regulatory factors, GLP1 and FGF21, as the combined repair factors. GLP1 has become the very important diabetes treatment drug that not only can well control blood glucose and repair islet cells, but also can improve diabetic complications, which include reduced lipid content in the liver, delayed progression of renal events, and significantly reduced incidence of serious adverse cardiovascular events in patients with T2DM. GLP1 can exert hypoglycemic effect by increasing the synthesis and secretion of insulin, inhibiting the emptying of gastric contents and inhibiting the excitation of feeding centers [10]. At the same time, GLP1 can correct the expression of GLUTs in the liver and muscles of patients with T1DM and T2DM [40]. Currently, the major analogues of GLP1 are Liraglutide and Trulicity. Trulicity is a protein fused with human antibody Fc, which leads to longer half-life and only needs to be injected once a week [9]. Therefore, we over-expressed the same sequence in MSCs in our study. FGF family is an important type of tissue growth factor, including 22 members. FGF21 is a member of the FGF19 subfamily of the FGF family and co-activates the downstream signaling pathway by binding the co-receptor β-Klotho with FGFRs [41]. It is mainly synthesized by liver, and plays important roles in regulating glucose and lipid metabolism in adipose tissue through endocrine pathways. In addition, FGF21 could also improve insulin sensitivity and insulin resistance by GSIS [7], and stimulate glucose uptake in skeletal muscles [8]. FGF21 has been developed as a drug for the treatment of metabolic diseases [6]. FGF21 reduces serum and hepatic triglyceride levels and ameliorates fatty liver in obese mice, through the suppression of the lipogenic gene, Srebp-1. FGF21 reduces hepatic cholesterol production by inhibiting Srebp-2 [18]. In vivo, GLP1 therapy can also activate the iNKTFGF21 axis, which contributes to weight loss [20]. Therefore, we think that GLP1 can further regulate the expression of srebp through FGF21 signaling. They may have synergies for regulating glucose and lipid metabolism. In our study, the combined application (FGF21+GLP1) could significantly reduce the expressions of srebp1 and srebp2, and significantly increase the expression of insulin, which was better than the single application. The results revealed the synergy between GLP1 and FGF21.

Although GLP1 and FGF21 can effectively alleviate glucose and lipid metabolism in DM patients, there are multiple application barriers when they are used. The most important barrier is the drug half-lives, even the best drug analogues developed at present, the half-lives of the two drugs are still extremely short, which makes patients need to inject two drugs in a large of quantities every day or every week, resulting in extremely high costs and drug resistance. Therefore, the use of FGF21 and GLP1 genetically modified MSC (MSC-FGF21+GLP1) cells is a better way to solve these problems. In a view of the perspective of factor secretion, MSCs secreted a lower concentration of factor (ng level), and the occurrence of cell infusions was lower, but the therapeutic effect
was much better than that of the drug therapy alone. That way, patients can get rid of long-term drug injections and reduce side effects.

Studies have shown that MSCs have the clinical potential to treat T2DM due to its multidirectional differentiation potential, tissue repair ability, immunomodulatory ability and the ability to secrete bioactive cytokines and growth factors. In vitro experiments have shown that MSCs can differentiate into islet beta cells under specific conditions [42, 43]. Animal experiments and clinical evidences have shown that MSCs infusion can effectively alleviate hyperglycemia in diabetic patients, improve insulin sensitivity and resistance in peripheral tissues such as muscle, fat and liver [44]. In addition, MSCs treatment basically has no serious adverse reactions, exhibiting good safety. Therefore, MSCs is accepted as an ideal seed cell for genetic engineering in management of DM. The molecular mechanism of MSCs in the treatment of diabetes is still unclear. The possible mechanisms may include: promoting islet cell regeneration, reducing peripheral tissue resistance to insulin, improving insulin sensitivity, regulating the immune system, protecting β cells of the islet, and improving the diabetes complications [26, 45]. MSCs normally secretes a series of bioactive cytokines and growth factors, such as HGF, VEGF and IGF1 to improve the local microenvironment, regulate the immune response and promote the repair and regeneration of damaged tissue. Particularly, a large number of reports have confirmed that MSCs can significantly improve diabetic complications caused by hyperglycemia, such as diabetic nephropathy, diabetic foot, lower limb vascular diseases, cardiovascular diseases and retinopathy [46]. However, high concentration of glucose may promote the expression level of PPAR-γ and C/EBP-α in the cells of MSCs to differentiate into adipocytes and osteoblasts [47, 48]. When using autologous MSC for cell therapy, MSCs function deteriorates as patients age[49, 50], and complications related to diabetic MSCs dysfunction contribute to the major pathological changes seen in the growing diabetic population [51].


To date, the metabolic kinetics cognition of MSCs in the body comes from animal experiments. Since MSC has the characteristics of chemotaxis to damaged tissue and organ parts, the metabolic kinetics of MSCs is different between the healthy body and the disease body. After the peripheral intravenous injection, most of the MSCs remained in the lungs and then reached the liver, kidney and spleen along with the blood flow [52, 53]. In another report, the autologous bone marrow MSCs of the patients with decompensated cirrhosis were amplified in vitro, and were infused via peripheral vein, the results showed that the lung stranded MSCs gradually migrated from lung to liver and spleen with the extension of time, and there are more cells in the spleen than in the liver [54]. This might be caused by the substance P released from damaged tissue to form a concentration gradient in vivo, which can attract MSCs to migrate to the damaged site along the concentration gradient for repair [55]. Therefore, we found that MSC-FGF21+GLP1 can significantly alleviate liver damage in this study.


In conclusion, the transplantation of double-gene modified MSCs (MSC-FGF21+GLP1) may be an effective treatment for patients with T2DM.

Conclusions

In conclusion, the present study has shown a new approach that combines FGF21 and GLP1 gene therapy with MSC cell therapy to treat type 2 diabetic mice effectively. We found that infusion of FGF21 and GLP1-transduced MSCs to T2D mice improved significantly insulin sensitivity and glucose disposal, enhanced the recovery of liver structures, increased plasma content of insulin, and exhibited synergistic effects in regulation of glucolipid metabolism.
Abbreviations

MSCs: Mesenchymal stem cell; CM: Culture medium; DM: Diabetes mellitus; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; GLP1: glucagon-like polypeptide 1; FGF21: fibroblast growth factor 21; SREBP: sterol regulatory element binding protein; GSIS: Glucose stimulated insulin secretion; TG: Triglyceride; TC: Cholesterol; LDL: Low-density lipoprotein cholesterol; HDL: High-density lipoprotein cholesterol.

Declarations

Declare to Stem Cell Research & Therapy

The laboratory animals were handled in accordance with Guidelines for the Care and Use of Laboratory Animals and the Animal Welfare Act in China and approved by the Animal Use and Care Committee of the Academy of Military Sciences. The protocol of MSCs preparation was approved by the General Logistics Department of the PLA.

Ethics approval and consent to participate

The care and use of laboratory animals were approved by the Animal Use and Care Committee of the Academy of Military Sciences.

Consent for publication

Not applicable.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Funding

This project was funded by a grant from the Postdoctoral Research Foundation of China.

Authors' contributions

BHX performed the scientific design, analyzed all experiments, and drafted the manuscript. HFD and LJQ performed the scientific design and revised the manuscript. XXX, TTY, JX, and TN performed the experiments and revised the manuscript critically; XHX, QHJ, LW and SFM contributed to the data and statistical analyses. All authors read and approved the final manuscript.

Acknowledgements

We thank Peiliang Geng, Ph.D., of the D Department of Experimental Hematology, Beijing Institute of Radiation Medicine, Academy of Military Sciences (Beijing, China), for his valuable comments and suggestions in writing and revising the manuscript.

Competing Interests

The authors declare that they have no competing interests.

References
1. Rossini, G., [Diagnosis and classification of diabetes mellitus]. Recenti Prog Med, 2010. 101(7-8): p. 274-6.
2. Cerf, M.E., Beta cell dysfunction and insulin resistance. Front Endocrinol (Lausanne), 2013. 4: p. 37.
3. Karalliedde, J. and L. Gnudi, Diabetes mellitus, a complex and heterogeneous disease, and the role of insulin resistance as a determinant of diabetic kidney disease. Nephrol Dial Transplant, 2016. 31(2): p. 206-13.
4. Kahn, S.E., M.E. Cooper, and S. Del Prato, Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. Lancet, 2014. 383(9922): p. 1068-83.
5. Basu, S., et al., Estimation of global insulin use for type 2 diabetes, 2018-30: a microsimulation analysis. Lancet Diabetes Endocrinol, 2019. 7(1): p. 25-33.
6. Kharitonenkov, A., et al., Rational design of a fibroblast growth factor 21-based clinical candidate, LY2405319. PloS One, 2013. 8(3): p. e58575.
7. Xu, J., et al., Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. Diabetes, 2009. 58(1): p. 250-9.
8. Gimeno, R.E. and D.E. Moller, FGF21-based pharmacotherapy--potential utility for metabolic disorders. Trends Endocrinol Metab, 2014. 25(6): p. 303-11.
9. Gläser, W., et al., Engineering and characterization of the long-acting glucagon-like peptide-1 analogue LY2189265, an Fc fusion protein. Diabetes Metab Res Rev, 2010. 26(4): p. 287-96.
10. Baggio, L.L. and D.J. Drucker, Biology of incretins: GLP-1 and GIP. Gastroenterology, 2007. 132(6): p. 2131-57.
11. Gallwitz, B., Glucagon-like peptide-1 analogues for Type 2 diabetes mellitus: current and emerging agents. Drugs, 2011. 71(13): p. 1675-88.
12. Inzucchi, S.E., et al., Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care, 2012. 35(S1): p. 1364-79.
13. O’Harte, F.P., et al., Improved stability, insulin-releasing activity and antidiabetic potential of two novel N-terminal analogues of gastric inhibitory polypeptide: N-acetyl-GIP and pGlu-GIP. Diabetologia, 2002. 45(9): p. 1281-91.
14. DeFronzo, R.A., Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes, 2009. 58(4): p. 773-95.
15. Gaich, G., et al., The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metab, 2013. 18(3): p. 333-40.
16. Hanssen, M.J., et al., Serum FGF21 levels are associated with brown adipose tissue activity in humans. Sci Rep, 2015. 5: p. 10275.
17. Kharitonenkov, A. and A.C. Adams, Inventing new medicines: The FGF21 story. Mol Metab, 2014. 3(3): p. 221-9.
18. Huang, Z., A. Xu, and B.M.Y. Cheung, The Potential Role of Fibroblast Growth Factor 21 in Lipid Metabolism and Hypertension. Curr Hypertens Rep, 2017. 19(4): p. 28.
19. Lin, Z., et al., Fibroblast growth factor 21 prevents atherosclerosis by suppression of hepatic sterol regulatory element-binding protein-2 and induction of adiponectin in mice. Circulation, 2015. 131(21): p. 1861-71.
20. Lynch, L., et al., iNKT Cells Induce FGF21 for Thermogenesis and Are Required for Maximal Weight Loss in GLP1 Therapy. Cell Metab, 2016. 24(3): p. 510-519.
21. Bunpetch, V., et al., From "Bench to Bedside": Current Advancement on Large-Scale Production of Mesenchymal Stem Cells. Stem Cells Dev, 2017. 26(22): p. 1662-1673.
22. Carlsson, P.O., et al., Preserved beta-cell function in type 1 diabetes by mesenchymal stromal cells. Diabetes, 2015. 64(2): p. 587-92.
23. Uccelli, A., L. Moretta, and V. Pistoia, Mesenchymal stem cells in health and disease. Nat Rev Immunol, 2008. 8(9): p. 726-36.
24. Song, L., et al., Adipose stem cells from chronic pancreatitis patients improve mouse and human islet survival and function. Stem Cell Res Ther, 2017. 8(1): p. 192.
25. Si, Y., et al., Infusion of mesenchymal stem cells ameliorates hyperglycemia in type 2 diabetic rats: identification of a novel role in improving insulin sensitivity. Diabetes, 2012. 61(6): p. 1616-25.
26. Mabed, M. and M. Shahin, Mesenchymal stem cell-based therapy for the treatment of type 1 diabetes mellitus. Curr Stem Cell Res Ther, 2012. 7(3): p. 179-90.
27. Li, X.Y., et al., Treatment of foot disease in patients with type 2 diabetes mellitus using human umbilical cord blood mesenchymal stem cells: response and correction of immunological anomalies. Curr Pharm Des, 2013. 19(27): p. 4893-9.

28. Abdi, R., et al., Immunomodulation by mesenchymal stem cells: a potential therapeutic strategy for type 1 diabetes. Diabetes, 2008. 57(7): p. 1759-67.

29. Jiang, R., et al., Transplantation of placenta-derived mesenchymal stem cells in type 2 diabetes: a pilot study. Front Med, 2011. 5(1): p. 94-100.

30. Mead, B., et al., Mesenchymal stromal cell-mediated neuroprotection and functional preservation of retinal ganglion cells in a rodent model of glaucoma. Cytotherapy, 2016. 18(4): p. 487-96.

31. Liu, X., Inflammatory cytokines augments TGF-beta1-induced epithelial-mesenchymal transition in A549 cells by up-regulating TbetaR-I. Cell Motil Cytoskeleton, 2008. 65(12): p. 935-44.

32. Li, D., et al., Biological characteristics of human placental mesenchymal stem cells and their proliferative response to various cytokines. Cells Tissues Organs, 2007. 186(3): p. 169-79.

33. Horwitz, E.M. and W.R. Prather, Cytokines as the major mechanism of mesenchymal stem cell clinical activity: expanding the spectrum of cell therapy. Isr Med Assoc J, 2009. 11(4): p. 209-11.

34. Xu, Y.X., et al., Mesenchymal stem cells treated with rat pancreatic extract secrete cytokines that improve the glycometabolism of diabetic rats. Transplant Proc, 2009. 41(5): p. 1878-84.

35. Hu, J., M. Ye, and Z. Zhou, Aptamers: novel diagnostic and therapeutic tools for diabetes mellitus and metabolic diseases. J Mol Med (Berl), 2017. 95(3): p. 249-256.

36. Halcox, J. and A. Misra, Type 2 diabetes mellitus, metabolic syndrome, and mixed dyslipidemia: how similar, how different, and how to treat? Metab Syndr Relat Disord, 2015. 13(1): p. 1-21.

37. Davidson, M., A review of the current status of the management of mixed dyslipidemia associated with diabetes mellitus and metabolic syndrome. Am J Cardiol, 2008. 102(12A): p. 19L-27L.

38. Sumanasinghe, R.D., et al., Expression of proinflammatory cytokities by human mesenchymal stem cells in response to cyclic tensile strain. J Cell Physiol, 2009. 219(1): p. 77-83.

39. Kisiday, J.D., et al., Dynamic compression stimulates proteoglycan synthesis by mesenchymal stem cells in the absence of chondrogenic cytokines. Tissue Eng Part A, 2009. 15(10): p. 2817-24.

40. Villanueva-Penacarrillo, M.L., et al., Effect of GLP-1 treatment on GLUT2 and GLUT4 expression in type 1 and type 2 rat diabetic models. Endocrine, 2001. 15(2): p. 241-8.

41. Kilkenny, D.M. and J.V. Rocheleau, The FGF21 Receptor Signaling Complex: Klothobeta, FGFR1c, and Other Regulatory Interactions. Vitam Horm, 2016. 101: p. 17-58.

42. Tsai, P.J., et al., Transplantation of insulin-producing cells from umbilical cord mesenchymal stem cells for the treatment of streptozotocin-induced diabetic rats. J Biomed Sci, 2012. 19: p. 47.

43. Yuan, H., et al., Regulation of mesenchymal stem cell differentiation and insulin secretion by differential expression of Pdx-1. Mol Biol Rep, 2012. 39(7): p. 7777-83.

44. Liu, G.Y., et al., Adipose-Derived Mesenchymal Stem Cells Ameliorate Lipid Metabolic Disturbance in Mice. Stem Cells Transl Med, 2016. 5(9): p. 1162-70.

45. Zang, L., et al., Mesenchymal stem cell therapy in type 2 diabetes mellitus. Diabetol Metab Syndr, 2017. 9: p. 36.

46. Cho, J., et al., A review of clinical trials: mesenchymal stem cell transplant therapy in type 1 and type 2 diabetes mellitus. Am J Stem Cells, 2018. 7(4): p. 82-93.

47. Li, Y.M., et al., Effects of high glucose on mesenchymal stem cell proliferation and differentiation. Biochem Biophys Res Commun, 2007. 363(1): p. 209-15.

48. Keats, E. and Z.A. Khan, Unique responses of stem cell-derived vascular endothelial and mesenchymal cells to high levels of glucose. PLoS One, 2012. 7(6): p. e38752.

49. Li, Y., et al., Senescence of mesenchymal stem cells (Review). Int J Mol Med, 2017. 39(4): p. 775-782.

50. Ganguly, P., et al., Age-related Changes in Bone Marrow Mesenchymal Stromal Cells: A Potential Impact on Osteoporosis and Osteoarthritis Development. Cell Transplant, 2017. 26(9): p. 1520-1529.

51. Pijany, A., et al., Mesenchymal stem cell dysfunction in diabetes. Mol Biol Rep, 2018.

52. Zangl, I., et al., Direct imaging of immune rejection and memory induction by allogeneic mesenchymal stromal cells. Stem Cells, 2009. 27(11): p. 2865-74.

53. Schmuck, E.G., et al., Biodistribution and Clearance of Human Mesenchymal Stem Cells by Quantitative Three-Dimensional Cryo-Imaging After Intravenous Infusion in a Rat Lung Injury Model. Stem Cells Transl Med, 2016. 5(12): p. 1668-1675.
54. Gholamrezanezhad, A., et al., *In vivo tracking of 111In-oxine labeled mesenchymal stem cells following infusion in patients with advanced cirrhosis*. Nucl Med Biol, 2011. 38(7): p. 961-7.

55. Dubon, M.J. and K.S. Park, *The mechanisms of substance P-mediated migration of bone marrow-derived mesenchymal stem cell-like ST2 cells*. Int J Mol Med, 2016. 37(4): p. 1105-11.
Morphology and multilineage differentiation capacity of MSCs. A: Adipose derived MSCs showed a homogeneous spindle-shaped morphology. B: Osteogenesis was examined by von Kossa staining for mineral nodule deposition. Adipogenesis as observed by the presence of lipid vesicles and confirmed by oil red O staining. C Immunophenotype of MSCs by flow cytometric analysis. Representative histograms are demonstrated. MSCs were positive for CD90, CD105, and CD73 and negative for CD34, CD45, and HLA-DR.
Transduction of MSCs with lentiviral vector particles. A: Expressing of EGFP in transduced MSCs with MOI of 40 under fluorescence microscopy. B: Analysis of EGFP fluorescence by flow cytometry at 48 h after transduction with different MOI. C: Lentiviral transduced MSCs could differentiate into osteoblasts and lipids. D: Immunophenotype of lentiviral transduced MSCs by flow cytometric analysis. Representative histograms are demonstrated. Lentiviral transduced MSCs were positive for CD90, CD105, and CD73 and negative for CD34, CD45, and HLA-DR. Lentivirus transduction did not affect the expression of MSCs surface markers.
Figure 3

The expressions of FGF21 and GLP1-fc in the transduced MSCs. A: Quantitative real-time PCR detected the expressions of FGF21 and GLP1-fc mRNA in the FGF21 or/and GLP1-fc transduced cells, the nontransduced MSCs and vector transduced cells were controls. The intracellular β-actin gene was used as a reference gene, ***P<0.05. B: ELISA analysis of the FGF21 and GLP1-Fc level in the cell culture media of FGF21 or/and GLP1-fc transduced MSCs compared to the media of the nontransduced MSCs and vector transduced MSCs, ***P<0.001, *P<0.05. C: Western blot analysis shows strong FGF21 and GLP1-IgG4-fc band in the MSCs transduced with FGF21or/and GLP1-fc expressing lentiviral particles compare to the vector lentiviral particles or nontransduced MSCs.
FGF21 and GLP1 transduced MSCs could reduce blood glucose and weight in T2DM mice. A: The time course of body weight during 4 weeks in BKS mice injected i.p. with saline (Con), MSCs, liraglutide, FGF21 or/and GLP1-fc
transduced MSCs. The cells were injected three times, as indicated, *P<0.05, **P<0.01. B: Gross appearance of BKS mice injected i.p. with saline (Con), MSCs, liraglutide, FGF21 or/and GLP1-fc transduced MSCs, *P<0.05, **P<0.01. C: Time course of the fasting blood glucose concentrations of BKS mice injected i.p. with saline (Con), MSCs, liraglutide, FGF21 or/and GLP1-fc transduced MSCs. D: Blood glucose concentration from oral glucose tolerance tests in BKS mice injected i.p. with saline (Con), MSCs, liraglutide, FGF21 or/and GLP1-fc transduced MSCs, different superscripts (*, # and @) represent significant differences between groups, P<0.05. E: The serum insulin levels in each group, *P<0.05.
Figure 5
FGF21 and GLP1 transduced MSCs could improve lipid metabolism in T2DM mice. A: Hematoxylin and eosin staining of representative liver and adipose sections obtained from mice of the indicated groups (scale bars = 100 mm). B: Statistics on the diameter of fat cells, different superscripts (*, # and @) represent significant differences between groups, P<0.05. C: The serum TG, TC, HDL-C, and LDL-C levels in the indicated groups, different superscripts (*, #, @ and &) represent significant differences between groups, P<0.05.

Figure 6

FGF21 and GLP1 transduced MSCs could improve glucolipid metabolism in vitro. A: Quantitative real-time PCR detected the effect of FGF21 or/and GLP1-fc transduced MSCs on the expressions of srebp1c mRNA in the HepG2 cells. Vector transduced MSCs was negative control, and liraglutide was employed as positive control. The intracellular β-actin gene was used as reference genes, different superscripts (*, #, @ and &) represent significant differences between groups, P<0.05. B: Quantitative real-time PCR detected the influence of FGF21 or/and GLP1-fc transduced MSCs on the expressions of insulin mRNA in the INS-1 cells. Vector transduced MSCs was negative control and liraglutide was positive control. The intracellular GAPDH gene was used as reference genes, different superscripts (*, #, @, & and $) represent significant differences between groups, P<0.05. C: Insulin secretion in INS-1 cells incubated at conditioned medium of different transduced MSCs. Blank group was KRBH medium without any added reagent. Vector transduced MSCs and liraglutide acted the negative and positive controls, respectively. Different superscripts (*, #, @ and &) represent significant differences between groups, P<0.05.
Figure 7

Signaling pathways of MSC-FGF21+GLP1 regulating lipid metabolism. Western blot verified the signaling pathway of MSC-FGF21+GLP1 cells regulating lipid metabolism. β-actin and GAPDH were reference proteins. p-AMPK, p-ACC and p-HSL respectively represented the phosphorylation levels of these proteins. Cytoplasmic proteins were extracted for detecting AMPK, ACC, FAS, and HSL. Nuclear proteins were extracted for detecting SREBP1 and SREBP2. AMPK: adenosine monophosphate activated protein kinase, SREBP: sterol regulatory element binding proteins, ACC: acetyl-coenzyme A carboxylase, FAS: fatty acid synthase, HSL: hormone sensitive lipase.
The mechanisms of FGF21 and GLP1 synergistically improving lipid metabolism. The diagram illustrates three existing approaches to regulate lipid metabolism. The red box represents the known metabolism regulation aimed at PCSK9 and HMC-CoA reductase. Currently, antibodies and statins are mainly used to block the expression of PCSK9 and HMC-CoA reductase so as to reduce cholesterol and lipid synthesis. In this study, we focused on the cytokines that could regulate lipid metabolism. FGF21 and GLP1 bind to their receptor, then they synergistically increased AMPK phosphorylation levels. Activated AMPK further inhibits the expression of SREBP1/2 genes and the entry of mature SREBP1/2 protein into the nucleus. At last the expression of enzymes directly involved in lipid metabolism is significantly inhibited.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementarymaterials.tif