Low-dose ionizing radiation attenuates high glucose-induced hepatic apoptosis and immune factor release via modulation of a miR-155-SOCS1 axis

HONGQIONG FAN[1], SHANSHAN LIU[1], BENZHENG JIAO[2] and XINYUE LIANG[1]

Departments of [1]Hematology and [2]Nuclear Medicine, First Hospital of Jilin University, Changchun, Jilin 130021, P.R. China

Received March 8, 2023; Accepted July 12, 2023

DOI: 10.3892/mmr.2023.13058

Abstract. Diabetic liver injury (DLI) can result in several diseases of the liver, including steatohepatitis, liver fibrosis, cirrhosis, and liver cancer. Low-dose ionizing radiation (LDIR) has hormetic effects in normal/disease conditions. However, whether LDIR has a beneficial effect on DLI has not been assessed previously. MicroRNA (miR)-155 and its target gene suppressor of cytokine signaling 1 (SOCS1) play critical roles in modulating hepatic proliferation, apoptosis, and immunity. However, whether a miR-155-SOCS1 axis is involved in high glucose (HG) induced hepatic damage remains to be determined. In the present study, mouse hepatocyte AML12 cells were treated with 30 mM glucose (HG), 75 mGy X-ray (LDIR), or HG plus LDIR. The expression levels of miR-155 and SOCS1 were determined by reverse transcription-quantitative PCR and western blotting. Additionally, apoptosis was measured using flow cytometry. The release of inflammatory factors, including TNF-α, IL-1β, IL-6, IL-10, and IFN-γ, after HG and/or LDIR treatment was detected by ELISA. The results showed that HG may induce hepatic apoptosis by upregulating the levels of miR-155 and downregulating the levels of SOCS1. HG also stimulated the secretion of TNF-α, IL-1β, IL-6, and IL-10. However, LDIR blocked the HG-induced activation of a miR-155-SOCS1 axis and suppressed the release of inflammatory factors. These results indicated that a miR-155-SOCS1 axis plays a role in HG-induced liver injury, and LDIR may exert a hepatoprotective effect by regulating the miR-155-SOCS1 axis.

Introduction

In China, the morbidity and mortality rates of patients with diabetes are increasing on a yearly basis, and diabetes has become a major threat to the health of the Chinese populace (1). Diabetic liver injury (DLI) is a major complication of type 2 diabetes mellitus (T2DM) caused by a variety of factors, such as hyperglycemia, insulin resistance, dyslipidemia, oxidative stress, and inflammation (2). During the development of diabetes, DLI can progress from simple fatty liver to non-alcoholic steatohepatitis, liver fibrosis, cirrhosis, and even liver cancer (3).

Ionizing radiation (IR) is one of the most important environmental factors. High-dose ionizing radiation (HDIR) may cause severe tissue and organ damage and induce apoptosis. HDIR may also cause genomic instability and promote tumorigenesis (4). Low-dose ionizing radiation (LDIR) is typically defined as ≤0.2 Gy at low linear energy transfer (LET) or ≤0.05 Gy at high LET (5). LDIR has been shown to exhibit a hormetic and adoptive effect (6-8). Studies have shown that LDIR is associated with cell growth and development, immunoregulation, and disease prevention (9). Our previous study also indicated that LDIR stimulated the proliferation of normal cells, such as rat mouse bone marrow hematopoietic progenitor cells, mesenchymal stem cells, and several normal human cell lines (9,10), instead of promoting tumorigenesis (10).

MicroRNA-155 (miRNA/miR-155) resides within the B-cell integration cluster gene on human chromosome 21 (11). miR-155 is one of the most multifunctional miRNAs, and its sequence is highly conserved in evolution. It has been shown that miR-155 is abnormally expressed in several physiological and pathological processes, including hematopoietic lineage differentiation, immune response, inflammation, and tumorigenesis (12). miR-155 is also closely related to the proliferation, differentiation, invasion, and prognosis of tumor cells (13). A large number of studies have shown that abnormal expression of miR-155 is closely associated with viral hepatitis, alcoholic liver disease, non-alcoholic steatohepatitis, liver fibrosis, hepatocellular carcinoma, and other liver diseases (14-17).

Substantial evidence indicates that miR-155 plays a crucial role in the pathogenesis of diabetes and its complications (18). For example, El Samaloty *et al* (19) reported a higher expression level of miR-155 in patients with chronic hepatitis C virus (HCV) infection, but a significant decrease in diabetic HCV patients; Bai *et al* (20) reported that miR-155 expression was increased in patients with diabetic nephropathy compared to T2DM patients without diabetic nephropathy. However, there is limited research that focuses on the impact of LDIR on diabetes and its associated complications (21-23). In particular, whether LDIR may modulate the expression of miR-155

Correspondence to: Professor Xinyue Liang, Department of Hematology, First Hospital of Jilin University, 1 Xinmin Street, Changchun, Jilin 130021, P.R. China
E-mail: liangxinyue@jlu.edu.cn

Key words: low-dose ionizing radiation, high glucose, hepatic injury, microRNA-155, suppressor of cytokine signaling 1

during the treatment of diabetes and its complications should be assessed. Therefore, in the present study, the effect of HG on hepatocytes and how the miR-155-SOCS1 axis impacted the behavior of hepatocytes during this process was assessed. Additionally, the protective effect of LDIR on hepatocytes via the regulation of miR-155-SOCS1 axis was assessed. The aim of the present was to explore a novel potential strategy for protection against and treatment of DLI.

Material and methods

Cell lines and cell culture. Mouse AML12 hepatocytes were obtained from Procell Life Science and Technology Co., Ltd. Cells were grown in DMEM/F12 supplemented with 10% FBS (Procell Life Science and Technology Co., Ltd.) and penicillin (100 U/ml)-streptomycin (100 μg/ml) (Life-ilab). To construct a high glucose-induced hepatocyte injury model, AML12 cells were cultured in normal glucose (NG) (5.6 mM + 19.4 mM mannitol to adjust for osmotic pressure) or HG (30 mM) to 120 h (24,25). All cells were maintained in a humidified 37˚C incubator with 5% CO_2.

Low-dose ionizing radiation. AML12 cells received a total dose of 75 mGy X-ray at a dose rate of 25 mGy/min using an X-RAD 320 (Precision X-RAD; Precision X-Ray). Following LDIR, the tissue culture medium was replaced and the cells were further cultured under the same conditions until cells were required for subsequent experiments.

Apoptosis assay. Apoptosis was analyzed using an Annexin V-FITC Apoptosis Detection Kit (BD Biosciences). According to the manufacturer's instructions, $1x10^5$ AML12 cells were washed with binding buffer and stained with 50 μl staining solution containing 5 μl Annexin V-FITC and 5 μl PI at room temperature for 15 min in the dark. Apoptosis was measured using a FACScan flow cytometer (BD Biosciences) and analyzed using FlowJo software (version 10.0; FlowJo LLC).

Transfection of miR-155 mimics, miR-155 inhibitor, and siRNA targeting SOCS1. AML12 cells were transfected with a 21 (siSOCS1#1) or 19 (siSOCS1#2) base-pair siRNA targeting SOCS1 [5'-CTACCTGAGTTCCTTCCCCTT-3' (26) and 5'-ACACTCACTTCCGCACCTT-3' (27)] using X-tremeGENE siRNA transfection reagent (MilliporeSigma), according to the manufacturer's protocol. At 48 h-post siRNA transfection, total protein was collected from the transfected cells and the expression levels of SOCS1 were western blotting. The siRNA sequence with the better interference result was chosen for subsequent experiments. miR-155 mimics, NC-mimics, miR-155 inhibitor, and NC-inhibitor were synthesized by Guangzhou RiboBio Co., Ltd. and transfected into cells at a concentration of 10 nmol/ml with X-tremeGENE siRNA transfection reagent. The subsequent experiments were performed at 48 h post-transfection.

RNA extraction and reverse transcription quantitative-PCR (RT-qPCR). According to the manufacturer's instructions, total RNA was extracted from cultured AML12 cells using an Eastep Super Total RNA Extraction kit (Promega Corporation), and 1 μg total RNA was used for cDNA synthesis using a TransScript miRNA RT Enzyme Mix (TransGen Biotech Co., Ltd.).

qPCR was used to detect the enrichment of miRNAs using an Eastep qPCR MasterMix (Promega Corporation) according to the manufacturer's instructions. Data were analyzed using the $2^{-\Delta\Delta Cq}$ method (28), and U6 snRNA expression was used as the endogenous control. The forward sequences of the primers were: miR-155, 5'-ttaatgctaattgtgatagg-3' and for U6, 5'-CTC GCTTCGGCAGCACATA-3'. The reverse primers were included in the cDNA synthesis kit.

Western blot analysis. AML12 cells were washed twice with ice-cold PBS and total protein was collected using RIPA lysis buffer (Beyotime Institute of Biotechnology). Protein concentration was determined with an enhanced bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology). A total of 25-50 μg protein was loaded on a 5-12% SDS gel and resolved SDS-PAGE (Epizyme) and transferred to a PVDF membrane (MilliporeSigma). Subsequently, the membranes were blocked in TBST containing 5% BSA at room temperature for 1 h after which the blots were probed with primary antibodies against SOCS1 (cat. no. 3950; 1:1,000; all from Cell Signaling Technology, Inc.) and β-actin (cat. no. 81115; 1:1,000; ProteinTech Group, Inc.) at 4˚C overnight. Subsequently, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (cat. no. PR30012; 1:3,000; ProteinTech Group, Inc.), and the peroxidase activity was visualized using an enhanced chemiluminescence reagent (Life-ilab). The mean band intensity was determined using densitometry analysis in Quantity One software (version 4.6; Bio-Rad Laboratories, Inc.).

Dual-luciferase reporter assay. Luciferase assays were implemented by adopting the Dual-Luciferase Reporter Assay System (Promega Corporation) in accordance with the manufacturer's instructions. The wild type (WT) or mutant (MT) SOCS1 3'-UTR sequences including the miR-155 targeting site were inserted into the pGL3 vector (Invitrogen; Thermo Fisher Scientific, Inc.) to construct pGL3-luc-SOCS1-WT and pGL3-luc-SOCS1-MT. AML12 cells were transfected with 50 ng pGL3-luc-SOCS1-WT/MT, 5 pmol miR-155 mimic or NC mimics (control,) and 5 ng Renilla luciferase using adopting Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) and seeded in a 96-well plate. The luciferase activities were detected 48 h post-transfection on a GloMax system (Promega Corporation).

ELISA. Mouse TNF-α (cat. no. MTA00B), IL-1β (cat. no. MLB00C), IL-6 (cat. no. M6000B), IL-10 (cat. no. M1000B), and IFN-γ (cat. no. MIF00) were detected according to the manufacturer's protocol of the ELISA kits (R&D Systems China Co., Ltd.).

Statistical analysis. Data are presented as the mean ± SD of at least three repeats. SPSS 19.0 (IBM Corp.) was used to analyze the experimental data. Differences between groups were compared using a Student's t-test or a one-way ANOVA followed by a post hoc LSD or Tukey's test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

LDIR attenuates HG-induced miR-155 upregulation. Previous studies indicated that the levels of miR-155 were upregulated

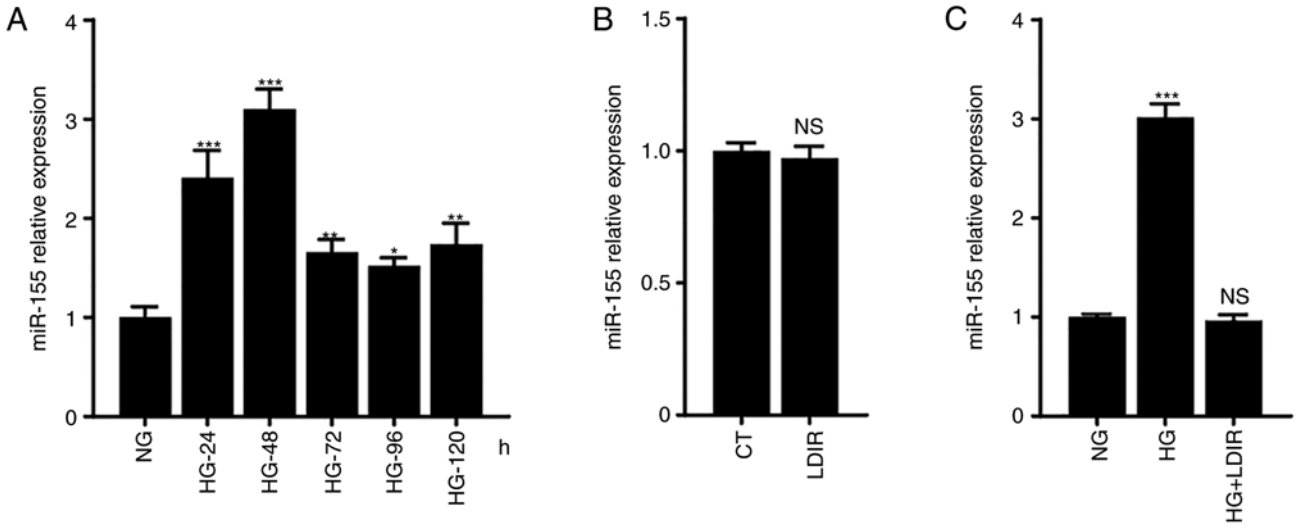

Figure 1. miR-155 expression levels following the treatment with HG and/or LDIR. AML12 cells were treated with HG for 24, 48, 72, 96, or 120 h, with 75 mGy LDIR, or with HG for 48 h followed by LDIR, after which the miR-155 expression levels were determined. (A) HG treatment increased the expression levels of miR-155, which peak at 48 h post-HG treatment. (B) LDIR alone did not affect the expression levels of miR-155. (C) LDIR suppressed the HG-induced miR-155 upregulation. *P<0.05, **P<0.01, ***P<0.001. NS, not significant; HG, high glucose; NG, normal glucose; LDIR, low-dose ionizing radiation; miR, microRNA.

in T1DM and downregulated in T2DM patients (19,29-31). However, whether miR-155 levels are altered in DLI remains to be determined. To determine whether miR-155 could be induced by HG in hepatocytes, AML12 cells were treated with 30 mM glucose for 24, 48, 72, 96, and 120 h, and the expression levels of miR-155 were measured. As shown in Fig. 1A, the levels of miR-155 were upregulated following HG treatment, with expression peaking at 48 h post-HG treatment, where it was 2x higher than that in the NG group, P<0.001.

To determine whether LDIR affected the HG-induced miR-155 upregulation, AML12 cells were irradiated with 75 mGy X-ray, and it was found that single LDIR treatment did not affect the levels of miR-155 in AML12 cells (Fig. 1B). Next, the cells were treated with HG for 48 h, after which they were irradiated with 75 mGy X-ray, and the expression of miR-155 was determined. Interestingly, it was found that LDIR significantly suppressed the HG-induced miR-155 upregulation in AML-12 cells (Fig. 1C).

LDIR alleviates HG-induced AML12 cell apoptosis. Diabetes may induce apoptosis in multiple types of cells, including podocytes, cardiomyocytes, Schwann cells, and hepatocytes (32-35). In the present study, AML12 cell apoptosis following HG treatment was assessed. After AML12 cells were cultured in medium containing 30 mM glucose for 48 h, cell apoptosis was determined by flow cytometry. As shown in Fig. 2A, compared with the NG group, HG treatment significantly induced cell apoptosis (42.6 vs. 0.88%, P<0.001). Next, whether LDIR could alleviate the HG-induced cell apoptosis was assessed. AML12 cells treated with HG for 48 h were irradiated with 75 mGy X-ray. The flow cytometry results showed that LDIR did not induce cell apoptosis, but significantly decreased the HG-induced cell apoptosis (Fig. 2A, 42.6 vs. 13.84%, P<0.001). Transfection of miR-155 mimics induced apoptosis in AML12 cells and LDIR decreased the miR-155-induced cell apoptosis (Fig. 2B, 25.09 vs. 14.24%, P<0.001). Moreover, it was found that miR-155 inhibitor decreased the HG-induced cell apoptosis (Fig. 2C, 38.68 vs. 13.11%, P<0.001).

LDIR attenuates HG- or miR-155-induced SOCS1 suppression. SOCS1 has been reported to be a regulatory target protein of miR-155 (36); Fig. 3A shows the predicted miR-155 binding site on the 3' UTR of SOCS1. Next, luciferase reporter assays were performed to determine the relation between miR-155 and SOCS1. Luciferase reporter plasmids including WT or MT SOCS1 3'-UTR were constructed (Fig. 3A). As shown in Fig. 3B, the normalized luciferase activity of the WT SOCS1 3'-UTR was significantly reduced when co-transfected with miR-155 mimics. However, the luciferase activity of the MT 3'-UTR was not affected. These outcomes suggest SOCS1 is a direct target of miR-155 in AML12 cells.

Since it was demonstrated that miR-155 inhibitor may disturb HG-induced cell apoptosis, it was speculated that LDIR may alleviate HG-induced cell apoptosis through a miR-155-SOCS1 axis. First, the expression levels of SOCS1 in AML12 cells treated with HG and LDIR were determined. The western blotting results showed that HG significantly suppressed the expression of SOCS1, whereas LDIR attenuated this process (Fig. 3C, P<0.001). As a target of miR-155, the expression levels of SOCS1 were suppressed by the transfection of miR-155 mimics. However, LDIR disturbed the communication between miR-155 and SOCS1, and this resulted in the recovery of SOCS1 expression levels (Fig. 3D). Similarly, miR-155 inhibitor may have also disturbed the suppression of SOCS1 that was induced by HG (Fig. 3E).

LDIR suppresses the HG-induced release of inflammatory factors. It has been reported that HG treatment may significantly elevate the expression levels of inflammatory factors in endothelial cells (37,38). In the present study, the effects of LDIR on HG-induced inflammation in hepatocytes were detected using ELISA kits. The levels of TNF-α, IL-1β, IL-6, and IL-10 were significantly elevated by HG treatment

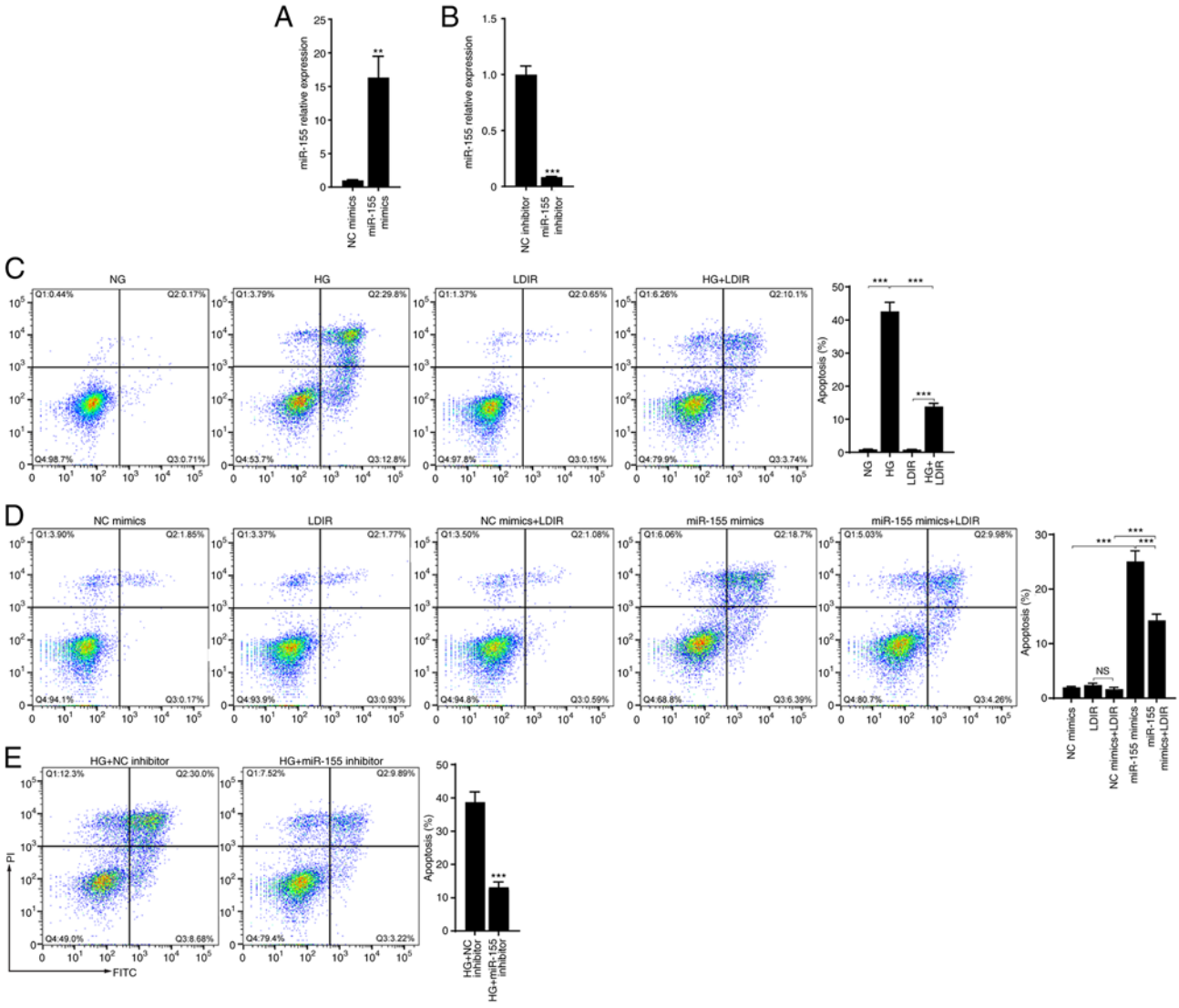

Figure 2. LDIR attenuated HG or miR-155-induced cell apoptosis. AML12 cells were treated with HG for 48 h, with 75 mGy LDIR, or transfected with miR-155 mimics or miR-155 inhibitor, and cell apoptosis was determined by flow cytometry. (A and B) Relative miR-155 expression levels following transfection with miR-155 mimics and miR-155 inhibitor. (C) LDIR significantly attenuated the HG-induced cell apoptosis. (D) Transfection of miR-155 mimics induced cell apoptosis, which was attenuated by LDIR. (E) Transfection of miR-155 attenuated the HG-induced cell apoptosis. **P<0.01, ***P<0.001. NS, not significant; HG, high glucose; LDIR, low-dose ionizing radiation; miR, microRNA; NC, negative control.

(Fig. 4A; P<0.001, P<0.001, P<0.001, and P<0.05, respectively). However, the levels of IFN-γ were not affected by HG treatment (P>0.05). LDIR reduced the levels of TNF-α, IL-1β, IL-6, and IL-10, compared with cells treated with HG alone (Fig. 4A, P<0.001, P<0.01, P<0.01, and P<0.05, respectively).

SOCS1 has been reported to negatively regulate the expression of several inflammatory factors (39,40). In the present study, two siRNA sequences were used to knockdown SOCS1 expression, independently, and siSOCS1#1, which exhibited the better knockdown efficiency, was used for subsequent experiments (Fig. 4B). The release of inflammatory factors in SOCS1 knockdown cells was measured, and it was found that the levels of TNF-α, IL-1β, IL-6, and IL-10 were significantly increased (Fig. 4C, P<0.01). Since miR-155 is a negative regulator of SOCS1, here, it was demonstrated that in HG-treated AML12 cells, the overexpression of miR-155 mimics significantly reduced the levels of TNF-α, IL-1β, IL-6, and IL-10 (Fig. 4D, P<0.001, P<0.001, P<0.05, and P<0.01, respectively).

Discussion

The liver is the largest gland in the body and is pivotal for substance metabolism. The liver is one of the organs that is involved in diabetes injury. In patients with T2DM, the incidence of non-alcoholic fatty liver disease ranges from 50-70% (41), and approximately 20-30% of these patients show abnormal liver function (42). In addition, the rate of cirrhosis in diabetics is 1-3x higher than that in healthy individuals (42). Several drugs, such as liraglutide, exenatide, and lixisenatide, have been synthesized with the aim of reducing triacylglycerol, total cholesterol, and low-density lipoprotein cholesterol levels in the blood of T2DM patients. These drugs have also been shown to exert a protective effect against T2DM-induced hepatic steatosis and liver damage by inhibiting oxidative stress and various inflammatory responses (43-45). Compared with the development of drugs, there are no studies that have focused on the treatment of DLI patients using physical

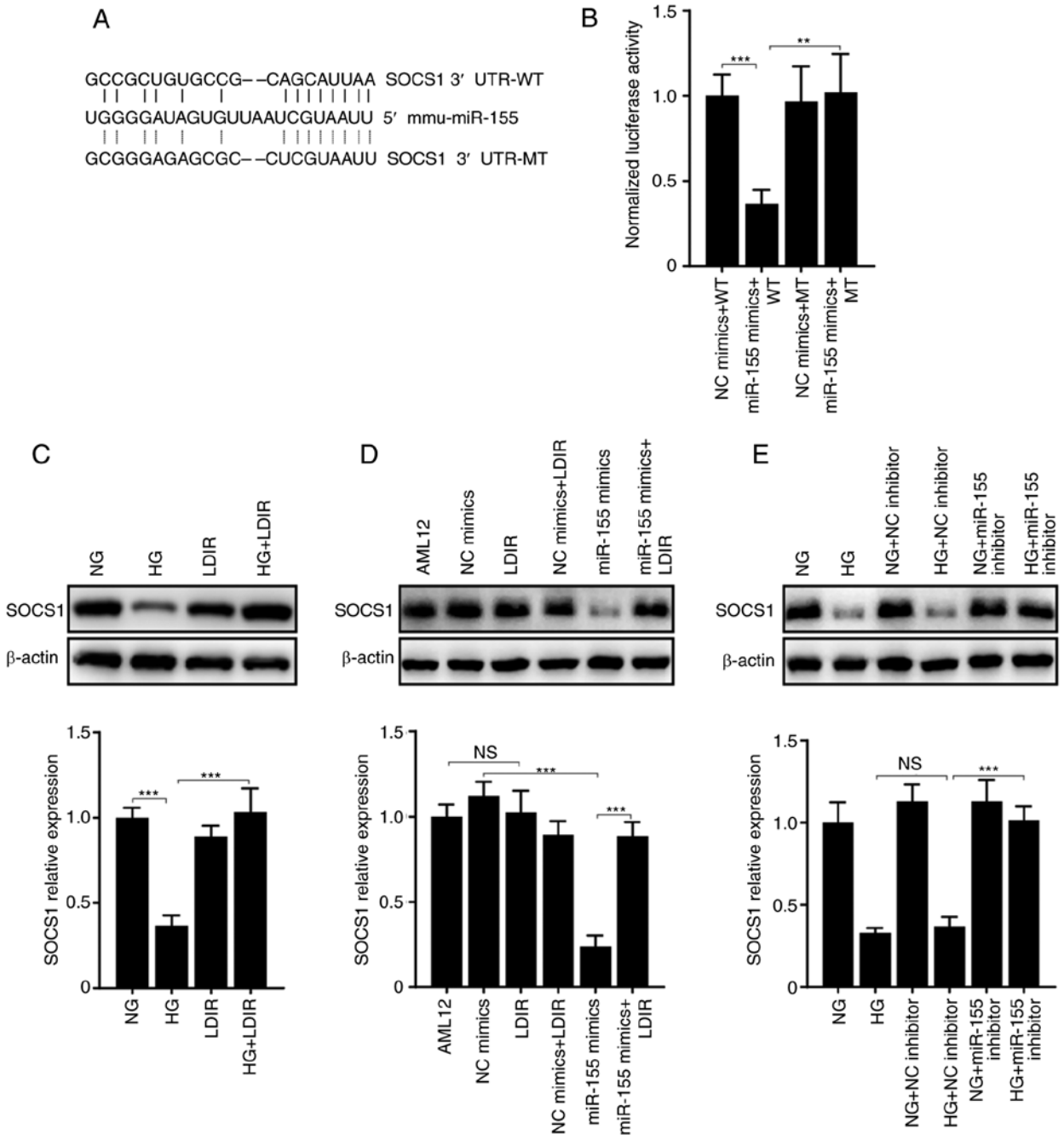

Figure 3. LDIR attenuates the HG- or miR-155-induced reduction in SOSC1 expression. (A) Bioinformatics analysis showed that the 3' UTR of SOCS1 contained a likely miR-155 binding site that was conserved in mice. (B) Dual-luciferase assays confirmed that the SOCS1 3'-UTR was a direct target of miR-155 in AML12 cells. (C) Western blotting results indicated that HG suppressed the expression of SOCS1, which was reversed by LDIR. (D) Transfection of miR-155 mimics reduced the expression of SOCS1 and LDIR reversed this. (E) Transfection of miR-155 inhibitor reversed the HG-induced SOCS1 suppression. **P<0.01, ***P<0.001. HG, high glucose; LDIR, low-dose ionizing radiation; miR, microRNA; SOCS1, suppressor of cytokine signaling 1.

methods, to the best of our knowledge. Several studies have tried to use LDIR to treat diabetes, where it has been shown to exert a favorable effect in animal experiments (21,22,46); however, using LDIR to treat DLI has not been previously reported on, to the best of our knowledge.

Epidemiological, preclinical, and clinical studies highlight the beneficial effects of LDIR in healthy and diseased conditions (47,48). Several studies have reported the hormetic effects of LDIR, which increases the longevity of organisms (49), reduces tumor metastasis and mortalities (50,51), improves neuronal function (52), improves the condition of patients with T2DM (53), and even improves the health of patients with COVID-19 (54). Takehara *et al* (21) reported that in a diabetic mouse model, pre-treatment with LDIR inhibited alloxan-induced diabetes and delayed the onset of hyperglycemia by elevating antioxidant levels and protecting pancreatic cells. Zhang *et al* (46) reported that in a diabetic nephropathy (DN) mouse model, treatment with LDIR cured the DN by upregulating the expression of the renal antioxidant enzyme superoxide dismutase-1 (SOD-1). In the present study, it was first demonstrated that LDIR may protect against DLI through suppression of a miR-155/SOCS1 axis. HG may induce hepatic

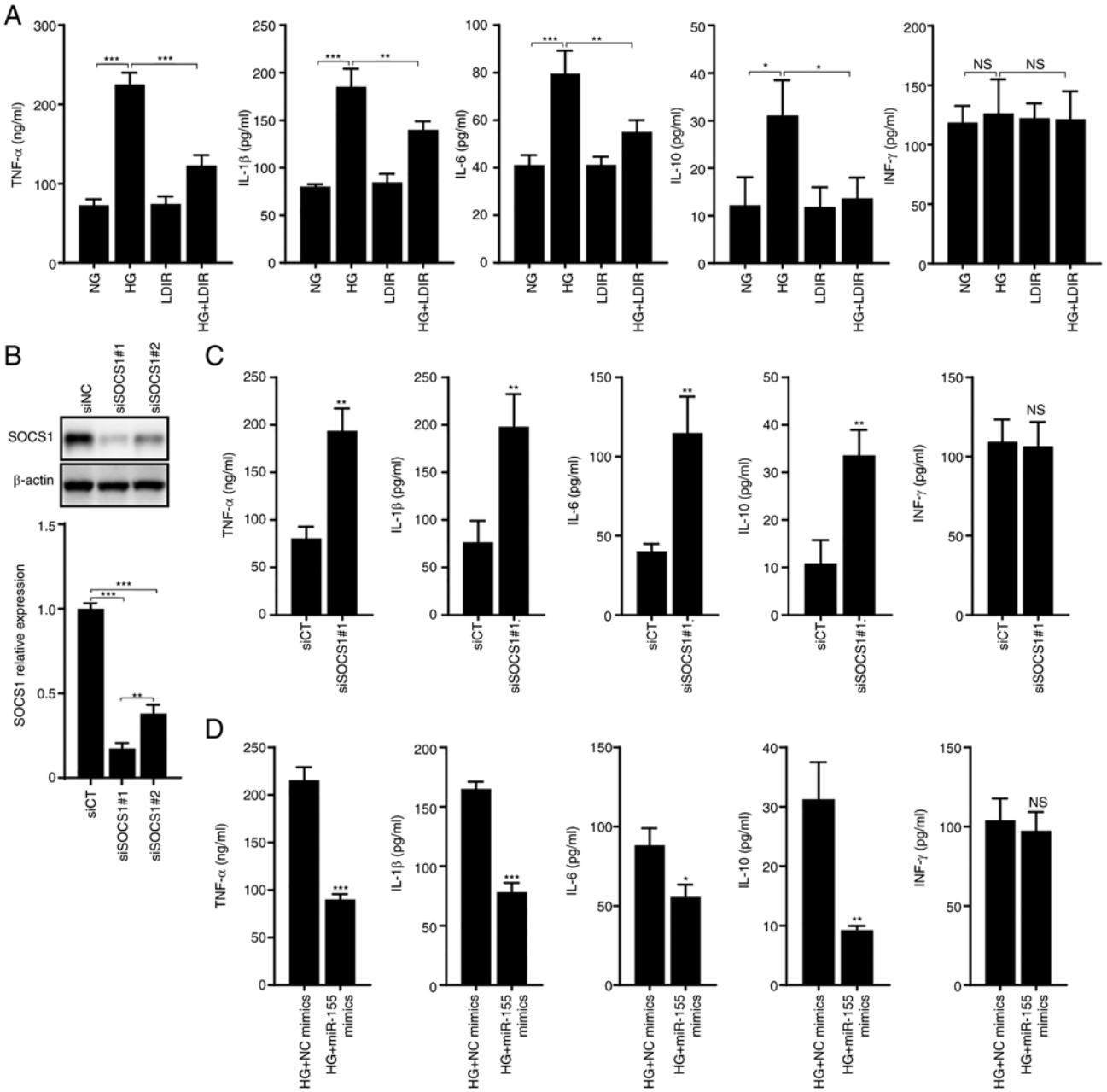

Figure 4. LDIR attenuated the HG-induced release of inflammatory factors through regulation of a miR-155-SOCS1 axis. AML12 cells were treated by HG for 48 h, 75 mGy LDIR, transfected with siSOCS1, or transfected with miR-155 mimics, after which, the release of five inflammatory factors, including TNF-α, IL-1β, IL-6, IL-10, and IFN-γ was detected by ELISA. (A) LDIR suppresses the HG-induced release of TNF-α, IL-1β, IL-6, and IL-10, but did not affect the release of IFN-γ. (B) SOCS1 expression was knocked down by transfection of siSOCS1 in AML12 cells. (C) Knockdown of SOCS1 expression upregulated the release of TNF-α, IL-1β, IL-6, and IL-10. (D) Transfection of miR-155 mimics suppressed the HG-induced release of TNF-α, IL-1β, IL-6, and IL-10. *P<0.05, **P<0.01, ***P<0.001. HG, high glucose; NG, normal glucose; zLDIR, low-dose ionizing radiation; miR, microRNA; SOCS1, suppressor of cytokine signaling 1; si, small interfering; CT, control.

apoptosis by upregulating the levels of miR-155 and downregulating the levels of SOCS1 protein. HG also stimulated the secretion of TNF-α, IL-1β, IL-6, and IL-10. However, LDIR blocked the HG-induced activation of miR-155/SOCS1 axis and suppressed the release of inflammatory factors (Fig. 5).

miR-155 exerts different biological functions in different cell types and disease models. In certain tumors, it acts as an oncogene to promote cell proliferation, invasion, and metastasis (55-58), whereas, in other tumors, it acts as a tumor suppressor to inhibit cell proliferation and promote apoptosis (59,60). Li *et al* (61) reported that triptolide could significantly induce the expression of miR-155 both in normal human hepatocytes and in rodent liver tissues, and inhibition of miR-155 mitigated the hepatic damage caused by triptolide. According to the results of the present study, HG-induced miR-155 could also damage AML12 hepatocytes, and inhibition of miR-155 mitigated this damage, consistent with the findings of Li *et al* (61).

Additionally, miR-155 is an important regulator of inflammation and immunity. Preclinical studies and clinical trials have indicated that miR-155 levels are altered in several types of liver disease, such as alcoholic liver injury, infectious liver injury, and

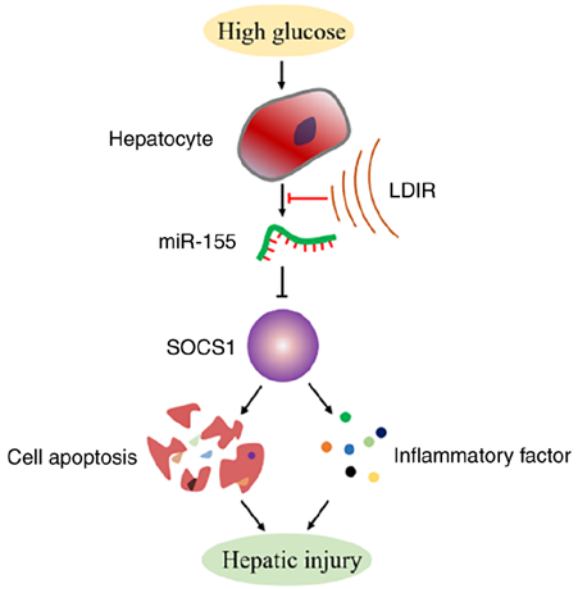

Figure 5. Proposed model on how LDIR protects against HG-induced liver injury. LDIR, low-dose ionizing radiation; HG, high glucose. HG, high glucose; LDIR, low-dose ionizing radiation; miR, microRNA; SOCS1, suppressor of cytokine signaling 1.

viral hepatitis (62). Sarkar *et al* (63) reported that miR-155 expression was downregulated in HBV-infected serum samples and liver biopsy; Bala *et al* (64) demonstrated that chronic alcohol treatment induced a time-dependent increase in the levels of miR-155 in macrophages *in vivo* and *in vitro*; and Dai *et al* (65) demonstrated that the expression of miR-155 was decreased in serum and liver tissue samples from patients with cirrhosis. SOCS is an important inhibitor of cytokine signaling pathways and also a key physiological regulator of natural and acquired immunity systems (66). SOCS1 is one of the most frequent modulatory targets of miR-155. The miR-155/SOCS1 axis, which is known to inhibit the JAK-STAT pathway, plays a critical role in the regulation of cell proliferation, inflammatory responses, and viral replication (15,36,67,68). The miR-155/SOCS1 axis is also reported to play a role in diabetes. Lin *et al* (69) reported that the levels of miR-155 are lower in T2DM patients. They indicated that miR-155 positively modulated glucose uptake through coordination with SOCS1, and overexpression of miR-155 in transgenic mice resulted in hypoglycemia, and improved glucose tolerance and insulin sensitivity. Several transcription factors, including NF-κB, AP-1, and STAT3, have been identified as positive regulators of miR-155; however, the exact signaling pathways remain unclear (18).

In conclusion, in the present study, it was shown that LDIR may exert a hepatoprotective effect by regulating a miR-155-SOCS1 axis. Pretreatment with LDIR may abrogate the HG-induced activation of the miR-155-SOCS1 axis and suppress the release of inflammatory factors. Considering that there are many diabetic patients in China and there is still a lack of effective preventative measures for diabetic liver disease, the present study provides a potential therapeutic strategy for the treatment of DLI. However, due to the lack of direct human studies, the therapeutic efficacy of LDIR remains contested. Moreover, the present study was a preliminary *in vitro* study, and to obtain a more accurate conclusion, further *in vivo* experiments are still required.

Acknowledgements

Not applicable.

Funding

The present study was supported in part by grants from the Science and Technology Department of Jilin Province (grant no. 20200201309JC) and the National Natural Science Foundation of China (grant no. 81903250).

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XL conceived and designed the study. HF, SL, and BJ acquired, analyzed, and interpreted the data. HF drafted the manuscript. All authors read and approved the final manuscript. HF and XL confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Wang J, Wu W, Dong G, Huang K and Fu J: Pediatric diabetes in China: Challenges and actions. Pediatr Diabetes 23: 545-550, 2022.
2. Bedi O, Aggarwal S, Trehanpati N, Ramakrishna G and Krishan P: Molecular and pathological events involved in the pathogenesis of diabetes-associated nonalcoholic fatty liver disease. J Clin Exp Hepatol 9: 607-618, 2019.
3. Assuncao SNF, Sorte NCB, Alves CD, Mendes PSA, Alves CRB and Silva LR: Nonalcoholic fatty liver disease (NAFLD) pathophysiology in obese children and adolescents: Update. Nutr Hosp 34: 727-730, 2017.
4. Farhood B, Goradel NH, Mortezaee K, Khanlarkhani N, Najafi M and Sahebkar A: Melatonin and cancer: From the promotion of genomic stability to use in cancer treatment. J Cell Physiol 234: 5613-5627, 2019.
5. Mettler FA, Sinclair WK, Anspaugh L, Edington C, Harley JH, Ricks RC, Selby PB, Webster EW and Wyckoff HO: The 1986 and 1988 UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation) reports: Findings and implications. Health Phys 58: 241-250, 1990.
6. Luckey TD: Physiological benefits from low levels of ionizing radiation. Health Phys 43: 771-789, 1982.
7. Feinendegen LE: Evidence for beneficial low level radiation effects and radiation hormesis. Br J Radiol 78: 3-7, 2005.
8. Olivieri G, Bodycote J and Wolff S: Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. Science 223: 594-597, 1984.
9. Averbeck D, Salomaa S, Bouffler S, Ottolenghi A, Smyth V and Sabatier L: Progress in low dose health risk research: Novel effects and new concepts in low dose radiobiology. Mutat Res Rev Mutat Res 776: 46-69, 2018.

10. Jiang H, Xu Y, Li W, Ma K, Cai L and Wang G: Low-dose radiation does not induce proliferation in tumor cells in vitro and in vivo. Radiat Res 170: 477-487, 2008.
11. Bedewy AML, Elmaghraby SM, Shehata AA and Kandil NS: Prognostic value of miRNA-155 expression in B-cell Non-Hodgkin Lymphoma. Turk J Haematol 34: 207-212, 2017.
12. Bayraktar R and Van Roosbroeck K: miR-155 in cancer drug resistance and as target for miRNA-based therapeutics. Cancer Metastasis Rev 37: 33-44, 2018.
13. Mashima R: Physiological roles of miR-155. Immunology 145: 323-333, 2015.
14. Bala S, Csak T, Saha B, Zatsiorsky J, Kodys K, Catalano D, Satishchandran A and Szabo G: The pro-inflammatory effects of miR-155 promote liver fibrosis and alcohol-induced steatohepatitis. J Hepatol 64: 1378-1387, 2016.
15. Chen L, Ming X, Li W, Bi M, Yan B, Wang X, Yang P and Yang B: The microRNA-155 mediates hepatitis B virus replication by reinforcing SOCS1 signalling-induced autophagy. Cell Biochem Funct 38: 436-442, 2020.
16. Babuta M, Furi I, Bala S, Bukong TN, Lowe P, Catalano D, Calenda C, Kodys K and Szabo G: Dysregulated autophagy and lysosome function are linked to exosome production by Micro-RNA 155 in alcoholic liver disease. Hepatology 70: 2123-2141, 2019.
17. Wang J and Che J: CircTP63 promotes hepatocellular carcinoma progression by sponging miR-155-5p and upregulating ZBTB18. Cancer Cell Int 21: 156, 2021.
18. Jankauskas SS, Gambardella J, Sardu C, Lombardi A and Santulli G: Functional role of miR-155 in the pathogenesis of diabetes mellitus and its complications. Noncoding RNA 7: 39, 2021.
19. El Samaloty NM, Hassan ZA, Hefny ZM and Abdelaziz DHA: Circulating microRNA-155 is associated with insulin resistance in chronic hepatitis C patients. Arab J Gastroenterol 20: 1-7, 2019.
20. Bai X, Luo Q, Tan K and Guo L: Diagnostic value of VDBP and miR-155-5p in diabetic nephropathy and the correlation with urinary microalbumin. Exp Ther Med 20: 86, 2020.
21. Takehara Y, Yamaoka K, Hiraki Y, Yoshioka T and Utsumi K: Protection against alloxan diabetes by low-dose 60Co gamma irradiation before alloxan administration. Physiol Chem Phys Med NMR 27: 149-159, 1995.
22. Zhang C, Tan Y, Guo W, Li C, Ji S, Li X and Cai L: Attenuation of diabetes-induced renal dysfunction by multiple exposures to low-dose radiation is associated with the suppression of systemic and renal inflammation. Am J Physiol Endocrinol Metab 297: E1366-E1377, 2009.
23. Zhao Y, Kong C, Chen X, Wang Z, Wan Z, Jia L, Liu Q, Wang Y, Li W, Cui J, *et al*: Repetitive exposure to low-dose X-irradiation attenuates testicular apoptosis in type 2 diabetic rats, likely via Akt-mediated Nrf2 activation. Mol Cell Endocrinol 422: 203-210, 2016.
24. Zhang Y, Li Y, Zhao J, Wang C, Deng B, Zhang Q and Shi C: Protective effects and mechanisms of Polyethylene Glycol Loxenatide Against Hyperglycemia and liver injury in db/db diabetic mice. Front Pharmacol 12: 781856, 2021.
25. Lizotte F, Denhez B, Guay A, Gevry N, Cote AM and Geraldes P: Persistent insulin resistance in podocytes caused by epigenetic changes of SHP-1 in diabetes. Diabetes 65: 3705-3717, 2016.
26. Shen L, Evel-Kabler K, Strube R and Chen SY: Silencing of SOCS1 enhances antigen presentation by dendritic cells and antigen-specific anti-tumor immunity. Nat Biotechnol 22: 1546-1553, 2004.
27. Shi D, Li D, Yin Q, Qiu Y, Yan H, Shen Y, Lu G and Liu W: Silenced suppressor of cytokine signaling 1 (SOCS1) enhances the maturation and antifungal immunity of dendritic cells in response to Candida albicans in vitro. Immunol Res 61: 206-218, 2015.
28. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
29. Polina ER, Oliveira FM, Sbruzzi RC, Crispim D, Canani LH and Santos KG: Gene polymorphism and plasma levels of miR-155 in diabetic retinopathy. Endocr Connect 8: 1591-1599, 2019.
30. Garcia-Diaz DF, Pizarro C, Camacho-Guillen P, Codner E, Soto N and Perez-Bravo F: Expression of miR-155, miR-146a, and miR-326 in T1D patients from Chile: Relationship with autoimmunity and inflammatory markers. Arch Endocrinol Metab 62: 34-40, 2018.
31. Mostahfezian M, Azhir Z, Dehghanian F and Hojati Z: Expression pattern of microRNAs, miR-21, miR-155 and miR-338 in patients with type 1 diabetes. Arch Med Res 50: 79-85, 2019.
32. Chen X, Wang J, Lin Y, Liu Y and Zhou T: Signaling pathways of podocyte injury in diabetic kidney disease and the effect of sodium-glucose cotransporter 2 inhibitors. Cells 11: 3913, 2022.
33. Ouyang C, You J and Xie Z: The interplay between autophagy and apoptosis in the diabetic heart. J Mol Cell Cardiol 71: 71-80, 2014.
34. Liu YP, Shao SJ and Guo HD: Schwann cells apoptosis is induced by high glucose in diabetic peripheral neuropathy. Life Sci 248: 117459, 2020.
35. Schattenberg JM and Schuchmann M: Diabetes and apoptosis: Liver. Apoptosis 14: 1459-1471, 2009.
36. Lin X, Chen L, Li H, Liu Y, Guan Y, Li X, Jia Z, Lin X, Jia J, Sun Y and Xiao D: miR-155 accelerates proliferation of mouse hepatocytes during liver regeneration by directly targeting SOCS1. Am J Physiol Gastrointest Liver Physiol 315: G443-G453, 2018.
37. Zhou X, Wang L, Zhang Z, Liu J, Qu Q, Zu Y and Shi D: Fluorometholone inhibits high glucose-induced cellular senescence in human retinal endothelial cells. Hum Exp Toxicol 41: 9603271221076107, 2022.
38. Li B, Li H, Dai L, Liu C, Wang L, Li Q and Gu C: NIK-SIX1 signalling axis regulates high glucose-induced endothelial cell dysfunction and inflammation. Autoimmunity 55: 86-94, 2022.
39. He Y, Zhang W, Zhang R, Zhang H and Min W: SOCS1 inhibits tumor necrosis factor-induced activation of ASK1-JNK inflammatory signaling by mediating ASK1 degradation. J Biol Chem 281: 5559-5566, 2006.
40. Wu XY, Yu J and Tian HM: Effect of SOCS1 on diabetic renal injury through regulating TLR signaling pathway. Eur Rev Med Pharmacol Sci 23: 8068-8074, 2019.
41. Lee YH, Cho Y, Lee BW, Park CY, Lee DH, Cha BS and Rhee EJ: Nonalcoholic fatty liver disease in diabetes. Part I: Epidemiology and diagnosis. Diabetes Metab J 43: 31-45, 2019.
42. Cusi K, Sanyal AJ, Zhang S, Hartman ML, Bue-Valleskey JM, Hoogwerf BJ and Haupt A: Non-alcoholic fatty liver disease (NAFLD) prevalence and its metabolic associations in patients with type 1 diabetes and type 2 diabetes. Diabetes Obes Metab 19: 1630-1634, 2017.
43. Voukali M, Kastrinelli I, Stragalinou S, Tasiopoulou D, Paraskevopoulou P, Katsilambros N, Kokkinos A, Tentolouris N and Ioannidis I: Study of postprandial lipaemia in type 2 diabetes mellitus: Exenatide versus liraglutide. J Diabetes Res 2014: 304032, 2014.
44. Sun F, Wu S, Wang J, Guo S, Chai S, Yang Z, Li L, Zhang Y, Ji L and Zhan S: Effect of glucagon-like peptide-1 receptor agonists on lipid profiles among type 2 diabetes: A systematic review and network meta-analysis. Clin Ther 37: 225-241 e228, 2015.
45. Roca-Rodriguez MM, Muros de Fuentes MT, Piedrola-Maroto G, Quesada-Charneco M, Maraver-Selfa S, Tinahones FJ and Mancha-Doblas I: Lixisenatide in patients with type 2 diabetes and obesity: Beyond glycaemic control. Aten Primaria 49: 294-299, 2017 (In Spanish).
46. Zhang C, Xing X, Zhang F, Shao M, Jin S, Yang H, Wang G, Cui J, Cai L, Li W and Lu X: Low-dose radiation induces renal SOD1 expression and activity in type 1 diabetic mice. Int J Radiat Biol 90: 224-230, 2014.
47. Shibamoto Y and Nakamura H: Overview of biological, epidemiological, and clinical evidence of radiation hormesis. Int J Mol Sci 19: 2387, 2018.
48. Scott BR: Radiation-hormesis phenotypes, the related mechanisms and implications for disease prevention and therapy. J Cell Commun Signal 8: 341-352, 2014.
49. Lopez-Martinez G and Hahn DA: Early life hormetic treatments decrease irradiation-induced oxidative damage, increase longevity, and enhance sexual performance during old age in the Caribbean fruit fly. PLoS One 9: e88128, 2014.
50. Cheda A, Wrembel-Wargocka J, Lisiak E, Nowosielska EM, Marciniak M and Janiak MK: Single low doses of X rays inhibit the development of experimental tumor metastases and trigger the activities of NK cells in mice. Radiat Res 161: 335-340, 2004.
51. Doss M: Evidence supporting radiation hormesis in atomic bomb survivor cancer mortality data. Dose Response 10: 584-592, 2012.
52. Cuttler JM, Moore ER, Hosfeld VD and Nadolski DL: Update on a patient with Alzheimer disease treated with CT scans. Dose Response 15: 1559325817693167, 2017.
53. Kojima S, Cuttler JM, Shimura N, Koga H, Murata A and Kawashima A: Radon therapy for autoimmune diseases pemphigus and diabetes: 2 case reports. Dose Response 17: 1559325819850984, 2019.
54. Dhawan G, Kapoor R, Dhawan R, Singh R, Monga B, Giordano J and Calabrese EJ: Low dose radiation therapy as a potential life saving treatment for COVID-19-induced acute respiratory distress syndrome (ARDS). Radiother Oncol 147: 212-216, 2020.

55. Qu YL, Wang HF, Sun ZQ, Tang Y, Han XN, Yu XB and Liu K: Up-regulated miR-155-5p promotes cell proliferation, invasion and metastasis in colorectal carcinoma. Int J Clin Exp Pathol 8: 6988-6994, 2015.
56. Bhattacharya S, Chalk AM, Ng AJ, Martin TJ, Zannettino AC, Purton LE, Lu J, Baker EK and Walkley CR: Increased miR-155-5p and reduced miR-148a-3p contribute to the suppression of osteosarcoma cell death. Oncogene 35: 5282-5294, 2016.
57. McDonald SJ, Cranford TL, VanderVeen BN, Cardaci TD, Velázquez KT, Enos RT, Chatzistamou I, Fan D and Murphy EA: miR155 deficiency reduces breast tumor burden in the MMTV-PyMT mouse model. Physiol Genomics 54: 433-442, 2022.
58. Su N, Li L, Zhou E, Li H, Wu S and Cao Z: Resveratrol down-regulates miR-155-5p to block the malignant behavior of gastric cancer cells. Biomed Res Int 2022: 6968641, 2022.
59. Li S, Zhang T, Zhou X, Du Z, Chen F, Luo J and Liu Q: The tumor suppressor role of miR-155-5p in gastric cancer. Oncol Lett 16: 2709-2714, 2018.
60. Yao LY, Ma J, Zeng XM and Ou-Yang J: MicroRNA-155-5p inhibits the invasion and migration of prostate cancer cells by targeting SPOCK1. Oncol Lett 20: 353, 2020.
61. Li Y, Guo L, Hou Z, Gong H, Yan M and Zhang B: Role of MicroRNA-155 in Triptolide-induced hepatotoxicity via the Nrf2-dependent pathway. J Ethnopharmacol 281: 114489, 2021.
62. Xue X, Wang J, Fu K, Dai S, Wu R, Peng C and Li Y: The role of miR-155 on liver diseases by modulating immunity, inflammation and tumorigenesis. Int Immunopharmacol 116: 109775, 2023.
63. Sarkar N, Panigrahi R, Pal A, Biswas A, Singh SP, Kar SK, Bandopadhyay M, Das D, Saha D, Kanda T, *et al*: Expression of microRNA-155 correlates positively with the expression of Toll-like receptor 7 and modulates hepatitis B virus via C/EBP-β in hepatocytes. J Viral Hepat 22: 817-827, 2015.
64. Bala S and Szabo G: MicroRNA signature in alcoholic liver disease. Int J Hepatol 2012: 498232, 2012.
65. Dai W, Zhao J, Tang N, Zeng X, Wu K, Ye C, Shi J, Lu C, Ning B, Zhang J and Lin Y: MicroRNA-155 attenuates activation of hepatic stellate cell by simultaneously preventing EMT process and ERK1 signalling pathway. Liver Int 35: 1234-1243, 2015.
66. Liang YB, Tang H, Chen ZB, Zeng LJ, Wu JG, Yang W, Li ZY and Ma ZF: Downregulated SOCS1 expression activates the JAK1/STAT1 pathway and promotes polarization of macrophages into M1 type. Mol Med Rep 16: 6405-6411, 2017.
67. Tan L, Jiang W, Lu A, Cai H and Kong L: miR-155 aggravates liver Ischemia/reperfusion injury by suppressing SOCS1 in mice. Transplant Proc 50: 3831-3839, 2018.
68. Ren Y, Cui Y, Xiong X, Wang C and Zhang Y: Inhibition of microRNA-155 alleviates lipopolysaccharide-induced kidney injury in mice. Int J Clin Exp Pathol 10: 9362-9371, 2017.
69. Lin X, Qin Y, Jia J, Lin T, Lin X, Chen L, Zeng H, Han Y, Wu L, Huang S, *et al*: MiR-155 enhances insulin sensitivity by coordinated regulation of multiple genes in mice. PLoS Genet 12: e1006308, 2016.