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USF-1 inhibition protects against oxygen-and-glucose-deprivation-induced apoptosis via the downregulation of miR-132 in HepG2 cells

Wang Renjie [a,1], Liang Haiqian [b,1], Li Hui [c], Dou Herong [a], Zhang Minghua [a], Baobuhe [a], Du Zhenhua [a], Gao Mojie [a], Wang Ruimin [a,*]

[a] Department of Clinical Laboratory, Pingjin Hospital, Logistics College of Armed Police Forces, Tianjin, China
[b] Department of Neurosurgery, Pingjin Hospital, Logistics College of Armed Police Forces, Tianjin, China
[c] Department of Thoracic Surgery, Shandong Cancer Hospital and Institute, Jinan, Shandong, China

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ABSTRACT

Upstream stimulatory factor 1 (USF-1) is an important transcription factor that participates in glucose metabolism and tumorigenesis. The aim of the current study was to explore the regulatory mechanism of USF-1 in HepG2 cells exposed to oxygen and glucose deprivation (OGD). After the establishment of the OGD model in HepG2 cells, we determined that the cells treated with OGD exhibited a high apoptotic rate and that the introduction of siRNA against USF-1 protected the cells from OGD-induced apoptosis. The miRNA microarray results demonstrated that a set of miRNAs were deregulated in the cells transfected with USF-1 siRNA, and the set of downregulated miRNAs included a novel miRNA, miR-132. Further analyses indicated that miR-132 overexpression inhibits the protective roles of USF-1 siRNA in OGD-induced apoptosis. We also identified several binding sites for USF-1 in the miR-132 promoter. The silencing of USF-1 resulted in a reduction in miR-132 expression, and USF-1 overexpression increased the expression of this miRNA. Our study indicated that the silencing of USF-1 plays protective roles in OGD-induced apoptosis through the downregulation of miR-132, which indicates that the silencing of USF-1 may be a therapeutic strategy for the promotion of cancer cell survival under OGD conditions.

1. Introduction

Upstream stimulatory factors (USFs) are members of the basic helix-loop-helix leucine zipper family and function as important cellular transcription factors by binding to the E-box motif within genes. Therefore, the members of this family serve as key regulators of the expression of genes involved in cell proliferation, stress, and other cellular processes [1]. For instance, USFs can bind to the E-box in the cdc2 gene and regulate its expression during the processes of cell cycle and differentiation [2]. During the regulation of the p53 tumor suppressor gene, a USF can also bind to the promoter of p53 in a site-specific manner, resulting in the activation of p53 expression [3], which indicates that USFs also play roles in tumorigenesis. In addition, previous studies have demonstrated that USFs are involved in metabolism, including the response to glucose. USF-2 is reported to be involved in the regulation of thrombospondin 1 gene expression in response to exposure to high glucose concentrations [4]. The variant of USF-1 in hyperlipidemia and type 2 diabetes indicates that USF-1 is closely related to lipid and glucose metabolism [5]. Moreover, USF-1 mediates the upregulation of hepatic lipid expression induced by glucose in HepG2 cells [6]. However, the response of USF-1 to low glucose or glucose deprivation is poorly understood.

Oxygen and glucose play important roles in metabolism and tumorigenesis [7]. Previous studies have demonstrated that oxygen–glucose deprivation (OGD) results in cell damage. For example, OGD can induce injury to oligodendrocyte precursor cells, contributing to cell apoptosis [8]. The model of OGD in the central neuron system results in the inhibition of cell viability and the induction of inflammation [9]. Glucose deprivation increases the production of reactive oxygen species and causes cancer cell death [10]. USF-1 is validated as a glucose response element that also functions as a hypoxia response element [11], indicating the potential roles of USF-1 during the induction of OGD. Thus, the aim of the current study was to elucidate the regulatory mechanism of USF-1 in HepG2 cells treated with OGD.

The data obtained in this study demonstrate that OGD induces the apoptosis of HepG2 cells and that the silencing of USF-1 inhibits OGD-induced cell apoptosis. We then performed microarray analyses to explore the target genes of USF-1 that may play protective

* Corresponding author. Fax: +86 022 60577196.
E-mail address: wrm20142233@sohu.com (R. Wang).
[1] These two author contribute equal to this work.

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roles against cell apoptosis induced by OGD. Our results indicate that the silencing of USF-1 may function as a therapeutic strategy that results in cancer cell survival under OGD conditions.

2. Materials and methods

2.1. Cell culture and transfection

Hepatocarcinoma cells, namely, HepG2, Hep3B, Bel-7402, SK-Hep1, LM-6, and LM-3 cells, were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS) and maintained in a humidified incubator at 37 °C with 5% CO_2. The cells were transfected with USF-1 siRNA and scramble controls, miR-132 mimics and the respective controls, or pcMV-USF-1 and the respective controls using Lipofectamine™ 2000 (Invitrogen) according to the manufacturers' protocol. The sequences for USF-1 siRNA were the following: sense, 5′ GACCCAACCAGUGUGGCUATT 3′; antisense, 5′ UAGCCACACUGGUUGGGUCTT 3′. The miR-132 mimics and the pcMV-USF-1 plasmid, as well as the controls, were purchased from Saier Company (Tianjin, China).

2.2. Oxygen glucose deprivation (OGD) treatment

Prior to the induction of OGD, the medium was removed from the cells, and the cells were then rinsed twice with 1× PBS without Ca^{2+}/Mg^{2+}. The cells were then subjected to OGD for 2 h by being suspended in a glucose-free solution (121.7 mM NaCl, 0.8 mM $MgSO_4$, 20.7 mM $NaHCO_3$, 5.5 mM $KHCO_3$, 1 mM NaH_2PO_4, 1.8 mM $CaCl_2$, 0.01 mM glycine, and 10 mM HEPES at pH 7.4) in an anoxia incubator (95% N_2 and 5% CO_2). After 2 h of OGD treatment, the cells were removed from the anoxia incubator and cultured under normal conditions. Cells that were not exposed to OGD were used as controls and incubated under normal conditions. For the treatment of USF-1 siRNA, the cells were transfected with USF-1 siRNA, subjected to the OGD treatment, and then maintained under normal conditions for 24 h.

2.3. RNA isolation and real-time PCR

The total RNA from the cells was extracted using Trizol (Invitrogen) according to the manufacturer's protocol. The RNA concentration was measured, and 500 μg of RNA was used for cDNA synthesis. Real-time PCR was performed using the SYBR Premix Ex Taq™ Kit (TaKaRa) with the following conditions: 95 °C for 3 min and 40 cycles of 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s. U6 snRNA was used as an internal control for the normalization of miR-132 expression. GAPDH was used as an internal control for the normalization of USF-1 expression.

2.4. Western blotting

The transfected cells were subjected to a Western blotting assay for the analysis of the expression levels of USF-1. Briefly, the cells were washed with PBS and then lysed with RIPA buffer (50 mM Tris–HCl pH 7.2, 150 mM NaCl, 1% Triton X-100, and 0.1% SDS) for approximately 30 min at 4 °C. The concentration was determined, and 30 μg of the proteins were loaded into a 12% SDS–PAGE gel for the analysis of USF-1. GAPDH was used as the loading control. A rabbit polyclonal antibody to USF1 (Abcam, ab180717) and a rabbit polyclonal antibody to GAPDH (Abcam, ab9485) were used as the primary antibodies. An HRP-conjugated goat anti-rabbit antibody was used as the secondary antibody. The bound antibodies were detected using the ECL Plus Western Blotting Detection system (GE Healthcare), and the chemiluminescent signals were detected using a high-performance chemiluminescence film (GE Healthcare).

2.5. Annexin V-PE/7-AAD assay for apoptosis

Annexin V-FITC and 7-AAD (7-aminoactinomycin D) were used for the detection of apoptotic cells. The transfected cells were washed with ice-cold PBS, incubated with 10 μl of Annexin V-FITC solution and 20 μl of 7-AAD viability dye in the absence of light, and then covered with 400 μl of ice-cold Annexin V binding buffer. The cells were then analyzed within 30 min using a flow cytometer.

2.6. MTT assay

The cell viability was measured using the MTT assay. The cells were seeded at a density of 5000 cells/well in 96-well plates. Forty-eight hours after transfection, the cells were exposed to MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) for approximately 4 h and then incubated with DMSO for 10 min with shaking in the absence of light. The absorbance at 570 nm (OD_{570nm}) was then measured using a spectrophotometer.

2.7. miRNA microarray

The total RNA from the transfected cells was extracted using a miRCURY RNA isolation kit (Exiqon), and the RNAs were then subjected to an miRNA microarray according to the manufacturer's instruction, as previously described [12].

2.8. Statistics

All of the data were acquired from three independent experiments and are shown as the means ± standard variation (SD). The differences between groups were measured using Students' *t*-test, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. USF-1 knockdown protects against OGD-induced apoptosis in HepG2 cells

Because USF-1 is a glucose response element, we aimed to detect the roles of USF-1 in OGD-induced cell apoptosis. We knocked down the expression of USF-1 in HepG2 cells through the transfection of USF-1 siRNA, and the silenced USF-1 expression was confirmed by RT-PCR and Western blotting assay (Fig. 1A and B). The degree of cell apoptosis was then analyzed with Annexin V-FE/7-AAD. As shown in Fig. 3C and D, we discovered that OGD treatment increased the percentage of apoptotic cells from 0.49 ± 0.06% to 24.28 ± 2.01%. In contrast, the analysis of the cells that were transfected with USF-1 siRNA prior to OGD treatment showed that the cell apoptosis exhibited by these cells was reduced compared to that observed in the untransfected cells after OGD treatment. In line with the roles of USF-1 in cell apoptosis, the silencing of USF-1 increased the cell viability that was reduced by OGD (Fig. 1E). Taken together, these results suggest that the silencing of USF-1 protects HepG2 cells from OGD-induced apoptosis.

3.2. A set of miRNAs were regulated by USF-1 in HepG2 cells

Because miRNAs play important roles in cell apoptosis [13,14], we used a miRNA microarray to determine the deregulated miRNAs that may be involved in the protective roles of USF-1 siRNA

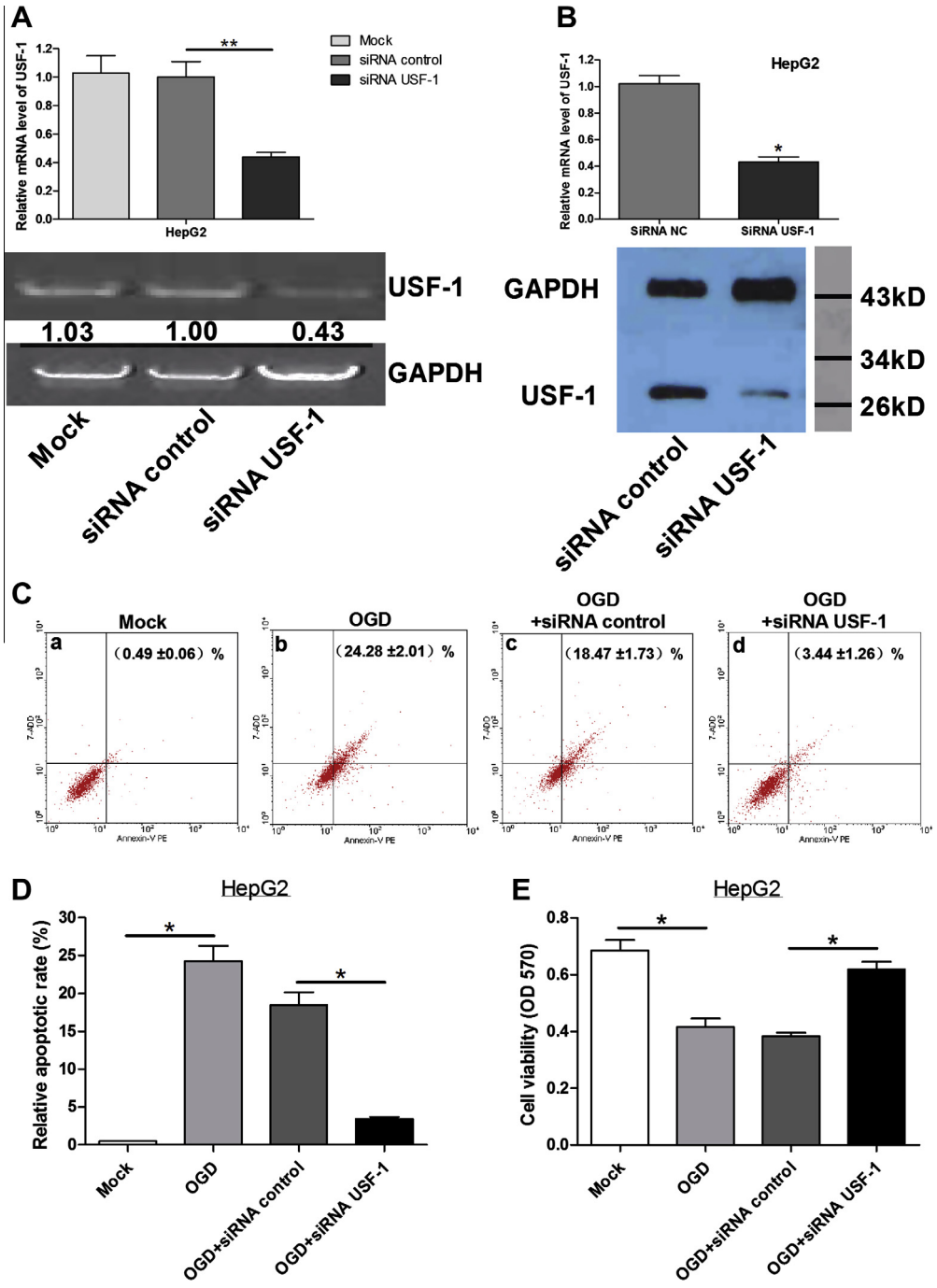

Fig. 1. Silencing of USF-1 protects HepG2 cells from OGD-induced apoptosis. (A and B) Cells transfected with USF-1 siRNA were subjected to RT-PCR and western blotting assays for the analysis of the expression levels of USF-1 mRNA and protein. GAPDH was used as the internal control. (C and D) Cells treated with OGD (or OGD and USF-1 siRNA) were subjected to Annexin V-PE/7-AAD staining for the analysis of cell apoptosis using flow cytometry. The images in (C) represent cells under different conditions, including live cells, early apoptotic cells, and dead cells. The graph in (D) indicates the percentages of apoptotic cells. (E) Cells treated with OGD (or OGD and USF-1 siRNA) were subjected to the MTT assay for the analysis of cell viability. Untransfected cells that were not exposed to OGD treatment were used as control cells in all of these experiments. The data are shown as the means ± SD. $^{*}P < 0.05$.

in OGD-induced apoptosis. As shown in Fig. 2A, we found that several miRNAs are regulated by USF-1 siRNA, including both downregulated and upregulated miRNAs, compared to the cells transfected with the USF-1 control siRNA. Based on the results of the miRNA microarray, we performed a real-time PCR analysis to confirm the effect of USF-1 on the expression of the miRNA identified from the microarray. As shown in Fig. 2B, USF-1 knockdown increased or reduced the expression of the miRNAs that were upregulated or downregulated in the miRNA microarray. These data indicate that the miRNA microarray is reliable and that the deregulated miRNAs may participate in the roles of USF-1 in cell apoptosis.

3.3. miR-132 participates in the roles of USF-1 in OGD-induced apoptosis in HepG2 cells

In the miRNA microarray, miR-132 was found to be downregulated in the cells transfected with USF-1 siRNA. To determine

(A) Differentially expressed miRNAs in HepG2 cells transfected with siRNA USF-1 compared with HepG2 cells transfected with siRNA control.

Up-regulated miRNAs ID	Fold changes: (siRNA USF-1 /siRNA control)	*Down-regulated miRNAs ID*	Fold changes: (siRNA USF-1 /siRNA control)
miR-1974	202.29	miR-569	0.02
miR-509-5p	11.99	miR-1207-5p	0.03
let-7b	10.50	miR-650	0.04
miR-21	9.35	miR-566	0.04
miR-1975	6.71	**miR-132**	**0.08**
miR-629	5.71	miR-24	0.09
miR-663b	5.05	miR-570	0.11
miR-484	4.67	miR-1268	0.11
miR-1245	3.08	miR-1207-3p	0.13
miR-1206	3.08	miR-572	0.13

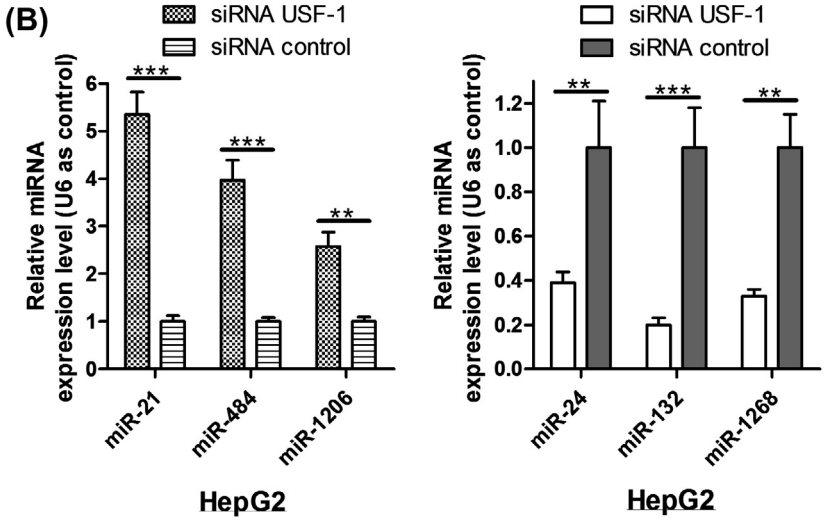

Fig. 2. Silencing of USF-1 affects the expression of a set of miRNAs. (A) Cells transfected with USF-1 siRNA were subjected to a miRNA microarray assay to identify miRNAs that are regulated by USF-1. The table shows several miRNAs that are either up- or downregulated due to the transfection of USF-1 siRNA. (B) The expression levels of six miRNAs that were deregulated in the cells transfected with USF-1 siRNA were analyzed by real-time PCR. U6 RNA was used as the internal control. Untransfected cells were used as the control cells. The data are shown as the means ± SD. $^{**}P < 0.01$ and $^{***}P < 0.001$.

whether miR-132 is involved in the roles of USF-1 in OGD-induced apoptosis, we analyzed the roles of miR-132 in cell apoptosis and cell viability. As shown in Fig. 3A and B, we discovered that the silencing of USF-1 protected against OGD-induced apoptosis and that this protective effect was disrupted by miR-132 overexpression. Similar miR-132-induced effects were also observed with cell viability. Overall, these results suggest that miR-132 is involved in the protective roles of USF-1 siRNA in OGD-induced cell apoptosis.

3.4. USF-1 may increase miR-132 by binding to its promoter

To investigate the mechanism through which USF-1 regulates the miR-132 expression level, we used a bioinformatics tool (TF SEARCH software) to screen for potential transcription factors that bind to the miR-132 promoter. As shown in Fig. 4A, we found that there are several binding sites for USF-1 on the miR-132 promoter. We then used real-time PCR assays to confirm the transcription factor roles of USF-1 in the activation of miR-132 expression. We discovered that the silencing of USF-1 decreased the expression level of miR-132 in several hepatocarcinoma cells, including HepG2, Hep3B, Bel-7402, SK-Hep1, LM-6, and LM-3 cells (Fig. 4B). To confirm the effect of USF-1 in miR-132 expression, we overexpressed USF-1 and found that ectopic USF-1 expression increases miR-132 expression (Fig. 4C). These data suggest that USF-1 may increase miR-132 expression by binding to its promoter, but the evidence of the interaction between the miR-132 promoter and USF-1 needs to be further investigated.

4. Discussion

Illustrating the molecular mechanism underlying the survival of tumor cells under stress conditions is useful in the search for novel drugs for tumor therapy. The purpose of this study was to evaluate the protective effect of USF-1 siRNA in OGD-induced cell apoptosis.

The results of the current study show that OGD induces cell apoptosis and inhibits cell viability. These results are in agreement with the findings of previous studies [8,10]. The cells that were transfected with the siRNA of USF-1 prior to OGD treatment exhibited decreased cell apoptosis and increased cell viability in response to OGD treatment compared to the untransfected cells, which indicates that the silencing of USF-1 exerts a protective effect in HepG2 cells against OGD-induced cell apoptosis. USF-1

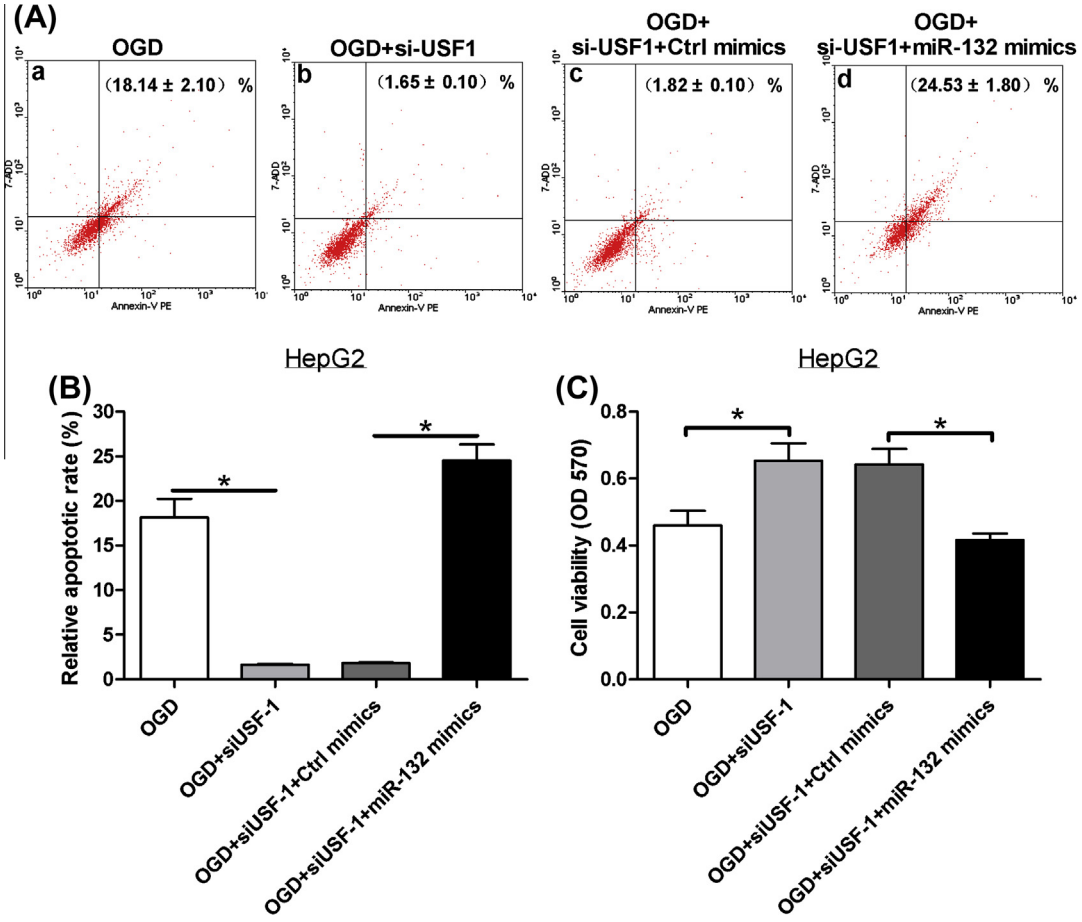

Fig. 3. Overexpression of miR-132 inhibits the protective roles of UFS-1 siRNA in OGD-induced apoptosis. (A and B) Cells co-transfected USF-1 siRNA and miR-132 mimics and exposed to OGD were subjected to Annexin V-PE/7-AAD staining for the analysis of cell apoptosis using flow cytometry. The images in (A) represent cells under different conditions, including live cells, early apoptotic cells, and dead cells. The graph in (B) shows the percentages of apoptotic cells. (C) Cells co-transfected USF-1 siRNA and miR-132 mimics and exposed to OGD were subjected to an MTT assay for the analysis of cell viability. Untransfected cells were used as control cells in all of these experiments. The data are shown as the means ± SD. $^{*}P < 0.05$.

is a novel transcription factor that can bind to the promoter of target genes and activate their transcription. Thus, the identification of downstream genes of USF-1 will aid the investigation of the regulatory mechanism of USF-1 in OGD-induced apoptosis.

miRNAs are a family of conserved, small non-protein-coding molecules that can guide gene expression in a sequence-specific manner [15]. These molecules can bind to a complementary sequence in the 3′UTR of the target mRNA to induce target mRNA cleavage [16,17] or translational repression [18]. It has been reported that approximately 60% of protein-coding genes are regulated by miRNAs, indicating that miRNAs are widely involved in diverse biological processes, including cell growth, apoptosis, and metabolism [19,20]. In addition, aberrant miRNA expression is closely related to the pathogenesis of tumors. To date, approximately 30% of the identified miRNAs have been found to be located in genomic regions that are associated with tumors [21]. For instance, miR-125b suppresses the proliferation and metastasis of liver cancer cells by targeting LIN28B2 [22]. miR-301 modulates the proliferation and invasion of human breast cancer cells [23]. Because miRNAs are widely involved in cell apoptosis and growth [20], we performed an miRNA microarray to determine the downstream targets of USF-1.

The miRNA microarray results revealed a set of miRNAs that may be regulated by USF-1. A more detailed analysis of the downregulated miRNAs revealed that the expression of miR-132 if downregulated in the cells transfected with USF-1 siRNA. This miRNA has been reported to be downregulated in some cancers and functions as a tumor-suppressive miRNA. For instance, miR-132 has been demonstrated to be downregulated in benign breast cancer but not malignant breast cancer, indicating its tumor-suppressive roles [24]. In the ductal carcinoma *in situ* form of breast cancer, miR-132 is downregulated and can suppress cell proliferation [25]. Based on the inhibitory roles of miR-132 in cell proliferation, we selected to further investigate the involvement of miR-132 in the mechanism through which USF-1 protects against cell apoptosis. Our data show that the silencing of USF-1 decreases the expression of miR-132 and that USF-1 overexpression has the opposite effect. In addition, through functional studies of miR-132 in cell apoptosis and cell viability, we found that miR-132 overexpression increases the cell apoptosis that was inhibited by USF-1 siRNA and inhibits the cell viability induced by USF-1 knockdown. These data are in agreement with the findings in previous reports [24–26].

To explore the mechanism through which USF-1 regulates miR-132 expression, we analyzed the promoter of miR-132 and discovered several binding sites for USF-1. A previous study indicated that USF-1 can activate the expression of GATA through the E-box motif and that the hypermethylation of the E-box inhibits the activation of GATA5 by USF-1 [27]. It has been reported that the promoter of miR-132 is hypermethylated in tumors [28]. Therefore, we hypothesized that the hypermethylation of the E-box in the miR-132 promoter may disrupt the interaction between miR-132 and USF-1 and that USF-1 may inhibit miR-132 expression. Conversely, our data demonstrate that USF-1 increases miR-132 expression, suggesting that the E-box in the miR-132 promoter

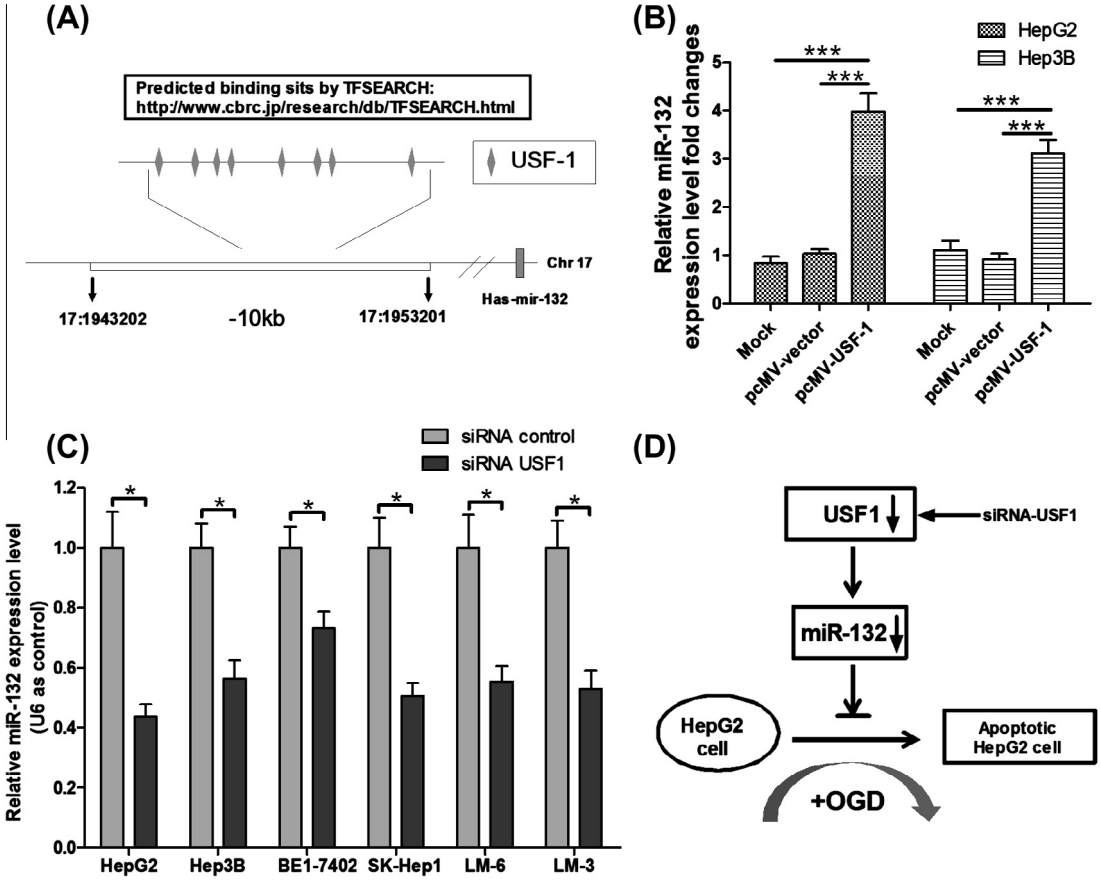

Fig. 4. USF-1 regulates the expression levels of miR-132. (A) Several binding sites for USF-1 were identified in the promoter of miR-132. (B) The effect of USF-1 on miR-132 expression in several hepatocarcinoma cell lines transfected with USF-1 siRNA was analyzed by real-time PCR. The cells transfected with USF-1 siRNA exhibited reduced miR-132 expression levels. (C) Cells transfected with an USF-1 overexpression plasmid (pcMV-USF-1) were subjected to real-time PCR for the analysis of miR-132 expression. The cells transfected with the pcMV-USF-1 plasmid exhibited increased miR-132 expression. Untransfected cells were used as the control cells in all of these experiments. All of the data are shown as the means ± SD. $^{*}P < 0.05$ and $^{***}P < 0.001$. (D) Model of the mechanism through which USF-1 regulates OGD-induced apoptosis. OGD causes HepG2 cell apoptosis, and the silencing of USF-1 decreases miR-132 expression. However, the inhibition of miR-132 by USF-1 knockdown suppresses the cell apoptosis induced by OGD.

may not be hypermethylated, but the detailed methylated regions or the methylated status of miR-132 in HepG2 cells needs to be further investigated.

In conclusion, our results indicate that USF-1 knockdown protects HepG2 cells from apoptosis induced by OGD via the downregulation of miR-132 (Fig. 4D). The study indicates that the silencing of USF-1 or the inhibition of miR-132 may allow HepG2 cells to survive under OGD conditions, which indicates the potential of USF-1 and miR-132 as therapeutic targets for hepatocarcinoma. However, the roles of miR-132 and its targets in HepG2 cells need to be investigated further.

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References

[1] S. Corre, M.D. Galibert, USF as a key regulatory element of gene expression, Med. Sci. (Paris) 22 (2006) 62–67.
[2] S. North, X. Espanel, F. Bantignies, B. Viollet, V. Vallet, P. Jalinot, G. Brun, G. Gillet, Regulation of cdc2 gene expression by the upstream stimulatory factors (USFs), Oncogene 18 (1999) 1945–1955.
[3] D. Reisman, V. Rotter, The helix-loop-helix containing transcription factor USF binds to and transactivates the promoter of the p53 tumor suppressor gene, Nucleic Acids Res. 21 (1993) 345–350.
[4] S. Wang, J. Skorczewski, X. Feng, L. Mei, J.E. Murphy-Ullrich, Glucose up-regulates thrombospondin 1 gene transcription and transforming growth factor-beta activity through antagonism of cGMP-dependent protein kinase repression via upstream stimulatory factor 2, J. Biol. Chem. 279 (2004) 34311–34322.
[5] W. Putt, J. Palmen, V. Nicaud, D.A. Tregouet, N. Tahri-Daizadeh, D.M. Flavell, S.E. Humphries, P.J. Talmud, Variation in USF1 shows haplotype effects, gene: gene and gene: environment associations with glucose and lipid parameters in the European Atherosclerosis Research Study II, Hum. Mol. Genet. 13 (2004) 1587–1597.
[6] D. van Deursen, H. Jansen, A.J. Verhoeven, Glucose increases hepatic lipase expression in HepG2 liver cells through upregulation of upstream stimulatory factors 1 and 2, Diabetologia 51 (2008) 2078–2087.
[7] T. Kietzmann, A. Krones-Herzig, K. Jungermann, Signaling cross-talk between hypoxia and glucose via hypoxia-inducible factor 1 and glucose response elements, Biochem. Pharmacol. 64 (2002) 903–911.
[8] X. Wu, X. Qu, Q. Zhang, F. Dong, H. Yu, C. Yan, D. Qi, M. Wang, X. Liu, R. Yao, Quercetin promotes proliferation and differentiation of oligodendrocyte precursor cells after oxygen/glucose deprivation-induced injury, Cell. Mol. Neurobiol. (2014).
[9] H. Zhao, Y. Liu, L. Cheng, B. Liu, W. Zhang, Y.J. Guo, L. Nie, Mesencephalic astrocyte-derived neurotrophic factor inhibits oxygen–glucose deprivation-induced cell damage and inflammation by suppressing endoplasmic reticulum stress in rat primary astrocytes, J. Mol. Neurosci. 51 (2013) 671–678.
[10] N.A. Graham, M. Tahmasian, B. Kohli, E. Komisopoulou, M. Zhu, I. Vivanco, M.A. Teitell, H. Wu, A. Ribas, R.S. Lo, I.K. Mellinghoff, P.S. Mischel, T.G. Graeber, Glucose deprivation activates a metabolic and signaling amplification loop leading to cell death, Mol. Syst. Biol. 8 (2012) 589.
[11] A. Krones, K. Jungermann, T. Kietzmann, Cross-talk between the signals hypoxia and glucose at the glucose response element of the L-type pyruvate kinase gene, Endocrinology 142 (2001) 2707–2718.
[12] A.J. Trindade, D.A. Medvetz, N.A. Neuman, F. Myachina, J. Yu, C. Priolo, E.P. Henske, MicroRNA-21 is induced by rapamycin in a model of tuberous sclerosis (TSC) and lymphangioleiomyomatosis (LAM), PLoS ONE 8 (2013) e60014.

[13] A. Zhao, Q. Zeng, X. Xie, J. Zhou, W. Yue, Y. Li, X. Pei, MicroRNA-125b induces cancer cell apoptosis through suppression of Bcl-2 expression, J. Genet. Genom. 39 (2012) 29–35.
[14] Q. Wei, Y.X. Li, M. Liu, X. Li, H. Tang, MiR-17-5p targets TP53INP1 and regulates cell proliferation and apoptosis of cervical cancer cells, IUBMB Life 64 (2012) 697–704.
[15] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[16] C. Llave, Z. Xie, K.D. Kasschau, J.C. Carrington, Cleavage of Scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA, Science 297 (2002) 2053–2056.
[17] S. Yekta, I.H. Shih, D.P. Bartel, MicroRNA-directed cleavage of HOXB8 mRNA, Science 304 (2004) 594–596.
[18] T. Dalmay, Mechanism of miRNA-mediated repression of mRNA translation, Essays Biochem. 54 (2013) 29–38.
[19] C.Z. Chen, L. Li, H.F. Lodish, D.P. Bartel, MicroRNAs modulate hematopoietic lineage differentiation, Science 303 (2004) 83–86.
[20] V. Ambros, The functions of animal microRNAs, Nature 431 (2004) 350–355.
[21] G.A. Calin, C. Sevignani, C. Dan Dumitru, T. Hyslop, E. Noch, S. Yendamuri, M. Shimizu, S. Rattan, F. Bullrich, M. Negrini, C.M. Croce, Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers, Proc. Natl. Acad. Sci. USA 101 (2004) 2999–3004.
[22] L. Liang, C.M. Wong, Q. Ying, D.N. Fan, S. Huang, J. Ding, J. Yao, M. Yan, J. Li, M. Yao, I.O. Ng, X. He, MicroRNA-125b suppressesed human liver cancer cell proliferation and metastasis by directly targeting oncogene LIN28B2, Hepatology 52 (2010) 1731–1740.
[23] W. Shi, K. Gerster, N.M. Alajez, J. Tsang, L. Waldron, M. Pintilie, A.B. Hui, J. Sykes, C. P'ng, N. Miller, D. McCready, A. Fyles, F.F. Liu, MicroRNA-301 mediates proliferation and invasion in human breast cancer, Cancer Res. 71 (2011) 2926–2937.
[24] A. Tahiri, S.K. Leivonen, T. Luders, I. Steinfeld, M. Ragle Aure, J. Geisler, R. Makela, S. Nord, M.L. Riis, Z. Yakhini, K. Kleivi Sahlberg, A.L. Borresen-Dale, M. Perala, I.R. Bukholm, V.N. Kristensen, Deregulation of cancer-related miRNAs is a common event in both benign and malignant human breast tumors, Carcinogenesis 35 (2014) 76–85.
[25] S. Li, H. Meng, F. Zhou, L. Zhai, L. Zhang, F. Gu, Y. Fan, R. Lang, L. Fu, L. Gu, L. Qi, MicroRNA-132 is frequently down-regulated in ductal carcinoma in situ (DCIS) of breast and acts as a tumor suppressor by inhibiting cell proliferation, Pathol. Res. Pract. 209 (2013) 179–183.
[26] X. Wei, C. Tan, C. Tang, G. Ren, T. Xiang, Z. Qiu, R. Liu, Z. Wu, Epigenetic repression of miR-132 expression by the hepatitis B virus x protein in hepatitis B virus-related hepatocellular carcinoma, Cell. Signal. 25 (2013) 1037–1043.
[27] A.E. Caccamo, R. Hsu, Z. Li, P.C. Kogut, Q. Du, K. Rouser, B. Camoretti-Mercado, J. Solway, Upstream stimulatory factor 1 activates GATA5 expression through an E-box motif, Biochem. J. 446 (2012) 89–98.
[28] S. Zhang, J. Hao, F. Xie, X. Hu, C. Liu, J. Tong, J. Zhou, J. Wu, C. Shao, Downregulation of miR-132 by promoter methylation contributes to pancreatic cancer development, Carcinogenesis 32 (2011) 1183–1189.