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# Interferon Regulatory Factor-1 as a Positive Regulator for High Glucose-Induced Proliferation of Vascular Smooth Muscle Cells

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## ABSTRACT

High glucose-induced proliferation of vascular smooth muscle cells (VSMCs) plays an important role in the development of diabetic vascular diseases. However, molecular mediators responding for the proliferation of VSMCs remain to be determined. In this study, VSMCs were isolated from the rat thoracic aorta, and two cell models with Irf-1 knockdown and overexpression were established by transfecting cells with pGCsi-FU-Irf-1 and pGC-FU-Irf-1, respectively. Subsequently, high glucose was added to cells to induce proliferation. Proliferation assays were performed to see whether Irf-1 was involved in high glucose-induced proliferation of VSMCs. In addition, the expression of Irf-1 was detected in VSMCs stimulated with high glucose and the thoracic aorta of diabetic rats to confirm the relationship between Irf-1 expression and the proliferation of hyperglycemia-dependent VSMCs. The results showed that Irf-1 expression was significantly higher in the thoracic aorta of diabetic rats and VSMCs stimulated with high glucose than that in nondiabetic rats and untreated cells. Overexpression of Irf-1 accelerated the proliferation of VSMCs, and down-regulation of Irf-1 expression significantly depressed the proliferative ability of VSMCs under high-glucose conditions, indicating that Irf-1 was a positive regulator for high glucose-induced proliferation of VSMCs. It could be presumed that Irf-1 is associated with the accelerated proliferation of VSMCs in diabetic vascular diseases and may prove to be a potential target gene for disease treatment. J. Cell. Biochem. 113: 2671–2678, 2012. 

**KEY WORDS:** VASCULAR SMOOTH MUSCLE CELLS; CELL PROLIFERATION; INTERFERON REGULATORY FACTOR 1; DIABETIC VASCULAR DISEASES

Diabetes mellitus (DM) is a major risk factor for atherosclerosis and is associated with increased incidences of vascular diseases. Hyperglycemia may play an important role for high prevalence of vascular diseases in diabetic patients [Haller et al., 1996]. The proliferation and migration of vascular smooth muscle cells (VSMCs) within the vascular wall are reported to be increased in the presence of hyperglycemia [Vranes et al., 1999; Faries et al., 2001; Madi et al., 2009; Orasanu and Plutzky, 2009], which contributes to the formation of atherosclerosis and has been considered as an initiating factor of diabetic vascular diseases [Mikhail et al., 1993; Hsueh et al., 2001; Srivastava, 2002; Reusch and Wang, 2011]. High glucose-induced proliferation of VSMCs plays an important role in the development of diabetic vascular diseases. However, molecular mediators responding for the proliferation of VSMCs remain to be determined.

Currently, most research efforts concerning hyperglycemia-dependent mechanisms responsible for accelerated atherosclerosis and VSMCs proliferation in diabetes have been directed at upstream mediators, such as protein kinase C and advanced glycosylation end products [Aronson, 2008], while little is known about the transcription regulators mediating the process.

Sun Yu and Zhang Xi contributed equally to this work and should be considered co-first authors.

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Interferon regulatory factor 1 (Irf-1), originally recognized as a transcription factor involved in interferon beta regulation, has been defined by increasing evidence as having the effect of regulating proliferation of various cell types including tumor cells and somatic cells [Romeo et al., 2002; Eckert et al., 2006; Dror et al., 2007]. However, little attention has been paid to exploring the effect of Irf-1 in regulating the growth of VSMCs. Particularly, it remains unclear whether Irf-1 is involved in regulating the growth of VSMCs in pathological alterations observed in diabetic vascular diseases.

In this study, VSMCs were isolated from the rat thoracic aorta, and two cell models with Irf-1 knockdown and overexpression were established by transfecting cells with pGCsi-FU-Irf-1 and pGC-FU-Irf-1, respectively. Subsequently high glucose was added to cells to induce proliferation. Proliferation assays were performed to see whether Irf-1 was involved in high glucose-induced proliferation of VSMCs. The objective is to provide evidence for transcription regulators mediating VSMCs growth regulation in pathological alterations observed in diabetic vascular diseases.

## MATERIALS AND METHODS

### ANIMALS AND STZ-INDUCED DIABETES MODELS

A diabetic model was established in SD male rats aged 10 weeks and weighing $200 \pm 25$ g (Laboratory Animal Center of the Second Military Medical University, Shanghai, China) by intraperitoneal injection of streptozotocin (STZ; 65 mg/kg) dissolved in citrate buffer as described previously [Faries et al., 2001]. Control animals were injected intraperitoneally with citrate buffer alone. Blood glucose levels were determined by a blood glucose test meter (Super Glucocard II, Arkray, Inc., Japan). Rats with blood glucose levels $\geq$16 mM for two consecutive weeks were considered diabetic. All procedures were in accordance with the ethics guidelines for the care and use of laboratory animals of the said university.

The rats were killed by asphyxia at days 7, 14, and 21 after the initial administration of streptozotocin. The thoracic aorta was isolated and detected for Irf-1 expression by RT-PCR and immunofluorescence staining.

### CELL CULTURE AND GENE TRANSFER

VSMCs were grown from explants of the thoracic aorta from SD rats. The normal growth medium was DMEM supplemented with 10% fetal calf serum (Gibco), 0.375% NaHCO3, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mmol/L L-glutamine (Amresco). VSMCs between passages 3 and 6 were used for experiments.

Complementary oligonucleotide sequences were designed as small RNA interfering sequences (siRNA) according to IRF-1 cDNA sequences. The sequences were analyzed by BLAST to ensure that they did not have significant sequences homologous with other genes. Small RNA interfering sequences (siIRF-1) were: sense, 5′-GCC CAA CUC UCU ACU GUC Utt-3′; antisense, 5′-AGA CAG UAG AGA GUU GGG Ctt-3′. The siRNA sequences were inserted into the pGC-FU-GFP (GeneChem, Shanghai, China) to obtain the lentiviral vector pGCsi-FU-Irf-1. The full-length Irf-1 cDNA (GenBank accession No. NM_012591.1) was purchased commercially from GeneChem and subcloned into pGC-FU-GFP to obtain the lentiviral vector pGC-FU-Irf-1.

The pGCsi-FU-Irf-1 or pGC-FU-Irf-1 plasmid together with pHelper 1.0 and pHelper 2.0 plasmids (GeneChem) were co-transfected into 293T cells using lipofectamine 2000 (Invitrogen, Carlsbad, CA) to produce lentiviral stock. A blank vector pGC-FU was utilized as a negative control. After the titers were determined, the lentiviral particles were used to infect VSMCs. Colonies with GFP expression were selected to expand culture for further investigation.

### CELL PROLIFERATION AND APOPTOSIS ASSAY

Transfected VSMCs were seeded on 6-well plates at $1 \times 10^5$ cells/well in 2 ml DMEM containing 10% FBS with normal glucose (5.5 mM). After 24 h, $G_0$/early G1 synchronization was achieved by serum deprivation. Subsequently, the medium was switched to DMEM containing 10% FBS with normal glucose or high glucose (25 mM). For the measurement of cell proliferation under normal glucose and high glucose conditions after 5-day incubation, cell growth and viability were analyzed by cell counting and MTT assay. Additionally, flow cytometry was employed to detect apoptosis of transfected VSMCs in normal glucose and high glucose conditions. Data are expressed as mean $\pm$ SD. Differences were assessed by Dunnett-test. A value of $P < 0.05$ was considered significant.

### IMMUNOCYTOCHEMISTRY AND IMMUNOFLUORESCENCE STAINING

The thoracic aorta of diabetic rats was fixed in 4% paraformaldehyde for 5 h; paraffin embedded, and sliced to 4–5 μm sections. The sections were then dewaxed and rehydrated for immunostaining. Rabbit anti rat Irf-1 was applied to incubate the sections at 4°C overnight. The control staining was performed by omitting the primary antibody. After incubation of the sections with secondary antibodies of anti-rabbit IgG-TRITC (Sigma–Aldrich, Inc.) used at a 1:100 dilution for 60 min at room temperature, fluorescence imaging was visualized with an Olympus IX70 microscope.

VSMCs were plated at 50% confluence in 12-well plates, rinsed twice with PBS, and fixed in 95% ethanol for 30 min at room temperature. Rabbit anti rat Irf-1, rabbit anti rat factorVII cytokeratin and mouse anti rat α-SMA (Sigma–Aldrich, Inc.) were applied to incubate the cells at 4°C overnight. The control staining was performed by omitting the primary antibody. After incubation of the cells with secondary antibodies of anti-mouse (-rabbit) IgG-FITC (Sigma–Aldrich, Inc.) used at a 1:100 dilution for 60 min at room temperature, fluorescence imaging was visualized with an Olympus IX70 microscope.

The percentage of positive cells was determined by counting positive staining cells and total cells in one visual field under a microscope. At least three fields (around 300 cells) were counted in each sample. For the thoracic aorta of diabetic rats, Irf-1-positive cells located in media of the vessels were counted. Average data are presented as means $\pm$ SD and compared with Dunnett-test.

### RT-PCR ANALYSES FOR Irf-1

Irf-1 expression was detected in high glucose-stimulated VSMCs and the thoracic aorta of diabetic rats, from which total ribonucleic acid (RNA) was extracted by guanidinium thiocyanate method. After cDNA synthesis from 1 μg total RNA using AMV reverse transcriptase (Promege), cDNA samples were subjected to PCR

TABLE I. PCR Primers Used in This Study

| Gene | Primer | Reaction condition | Product size (bp) |
|---|---|---|---|
| Irf-1 | Sense: 5′-TTGGCGTTCTGAGGTT-3′<br>Antisense:5′-TAGTAGTTAGGTGGCGTTTC-3′ | 30 cycles at 55°C in 1 mM $MgCl_2$ | 559 |
| GAPDH | Sense: 5′-CCATGGAGAAGGCTGGGG-3′<br>Antisense: 5′-CAAAGTTGTCATGGATGACC-3′ | 30 cycles at 50°C in 1 mM $MgCl_2$ | 194 |

amplification with primers for Irf-1. The PCR primers and the reaction conditions used are described in Table I. PCR products were size fractionated by 1% agarose gel electrophoresis. The band intensities were digitally captured by AlphaImager and analyzed using Multi-Analyst software (BioRad). The level of GAPDH was used to normalize signal intensity. Intensity data were then subjected to statistical analyses using Microsoft Excel software. Average data are presented as means ± SD and compared with Dunnett-test.

### WESTERN BLOT ANALYSES FOR Irf-1

Two weeks after VSMCs were transfected with pGCsi-FU-Irf-1, pGC-FU-Irf-1, and pGC-FU vector, the cells were collected and lysed in RIPA lysis buffer (Beyotime). Then, the extracted proteins were resolved by SDS–PAGE and electroblotted onto PVDF membranes (Beyotime). Proteins of interest were detected using anti-Irf-1 by the ECL (Beyotime) chemiluminescence detection system. Band intensities were analyzed using Multi-Analyst software. The level of β-actin was used to normalize signal intensity. Intensity data were

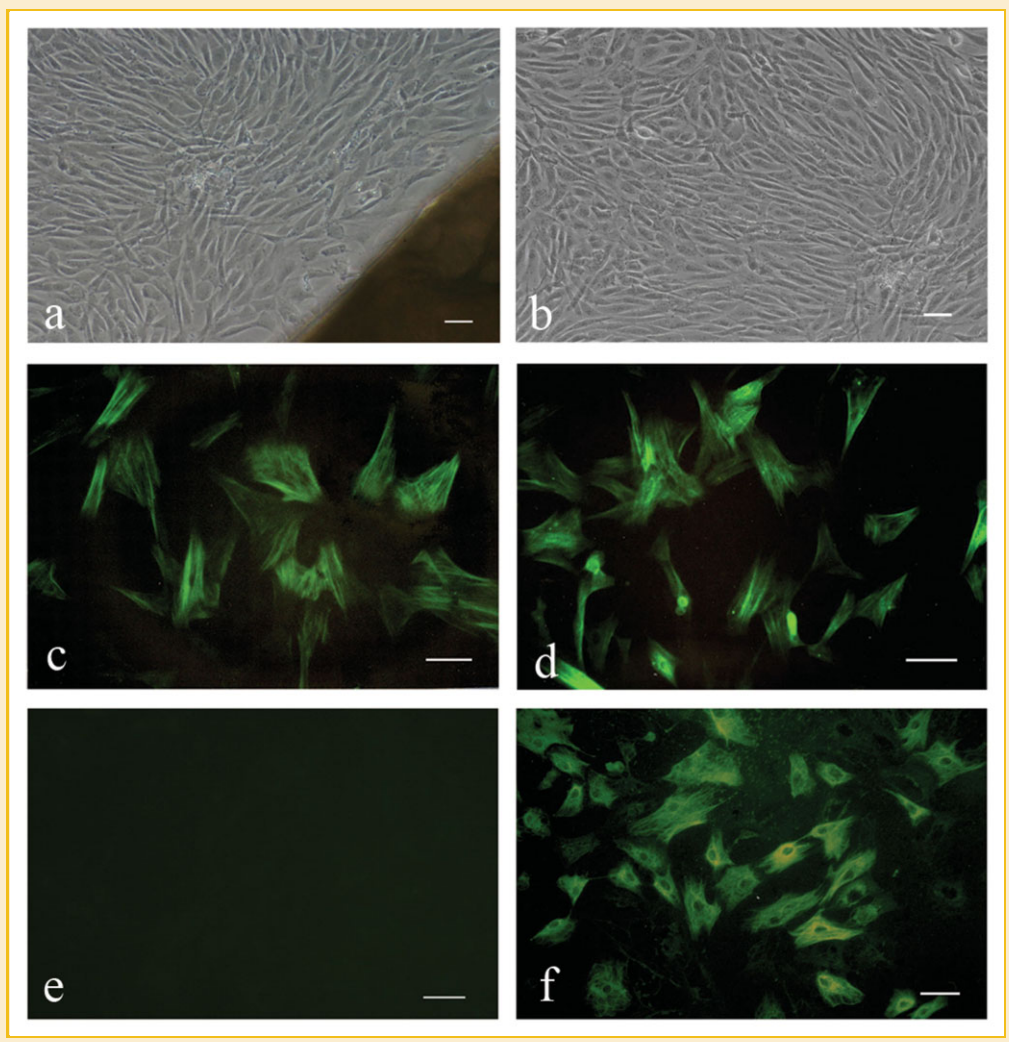


Fig. 1. Growth and identification of VSMCs. a: primary VSMCs; b: passaged VSMCs; c: representative positive staining of α-smooth muscle actin in VSMCs (3 passage); d: representative positive staining of α-smooth muscle actin in VSMCs (6 passage); e: representative negative staining of factorVIII in VSMCs (3 passage); f: representative positive staining of factorVIII in the positive control group (rat vascular endothelial cells). Scale bar 30 μm. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

then subjected to statistical analyses. Differences were assessed by Dunnett-test. A value of $P < 0.05$ was considered significant.

## RESULTS

### GROWTH AND IDENTIFICATION OF VSMCs

Cells were identified as VSMCs on the basis of their morphological and growth characteristics. Briefly, VSMCs exhibited a typical hill-and-valley growth pattern and also exhibited positive staining for a-smooth muscle actin but no staining for factor VIII antigen (Fig. 1a–f). All cells showed negative staining for factorVII in culture between passages 3 and 6.

### Irf-1 EXPRESSION IN THE THORACIC AORTA OF DIABETIC RATS

After streptozotocin administration, increased Irf-1 expression was observed in the rat thoracic aorta of rats. The mRNA level of Irf-1 was significantly increased in the rat thoracic aorta at days 7, 14, and 21 after the initial administration of streptozotocin, compared

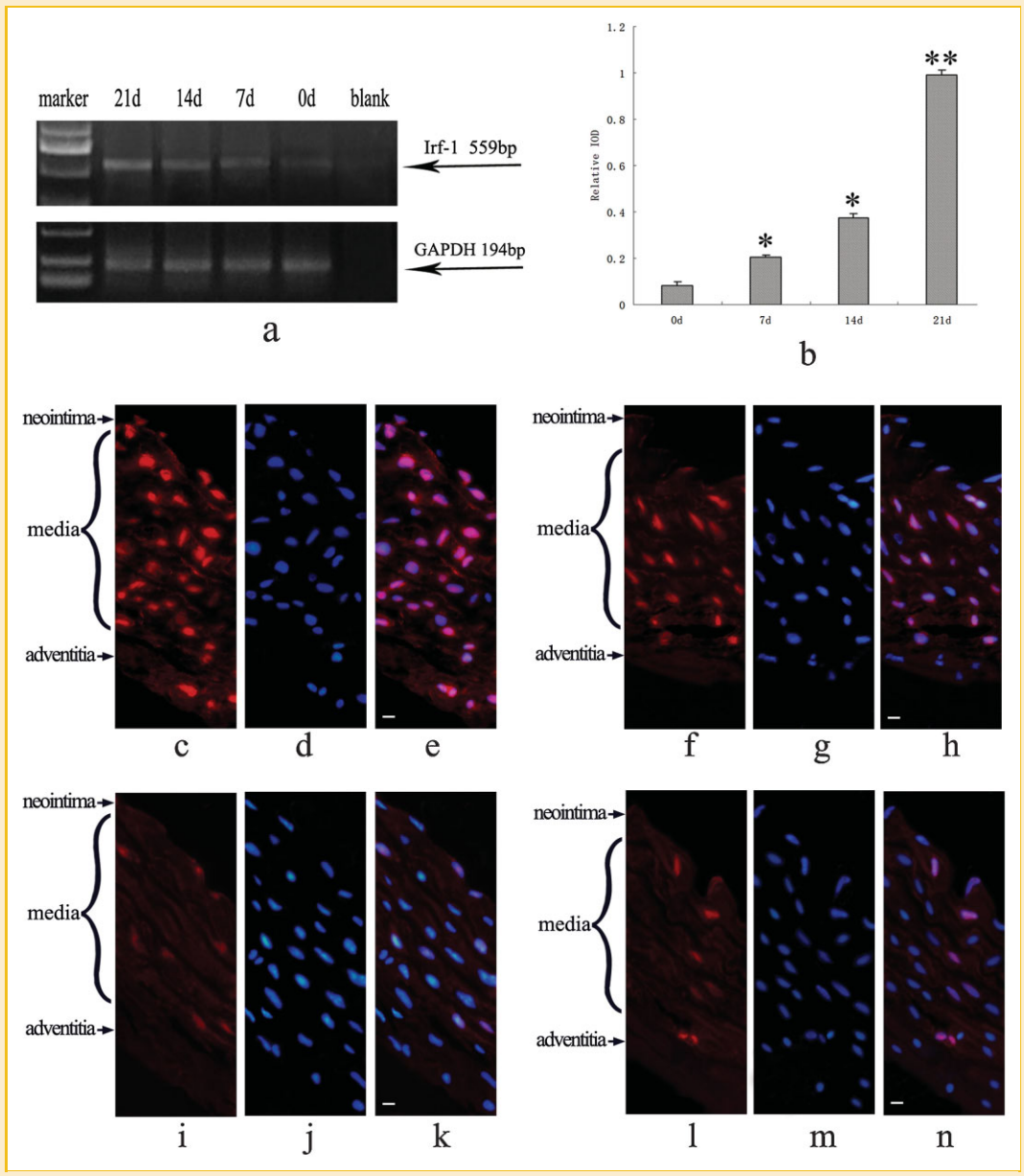


Fig. 2. Irf-1 expression in the thoracic aorta of diabetic rats. a: RT-PCR analysis showing expression of the mRNA coding Irf-1 in thoracic aorta of diabetic rats; 0d, rats without streptozotocin administration; 7d/14d/21d, rats administered with streptozotocin for 7/14/21 days. b: quantitative assessment for the level of mRNA coding Irf-1 in the thoracic aorta of diabetic rats by integrated optical density analyses; 0d, rats without streptozotocin administration, 7d/14d/21d, rats administered with streptozotocin for 7/14/21 days. c,f,i,l: representative positive staining of Irf-1 in the thoracic aorta wall of rats at days 21/14/7/0 after the initial administration of streptozotocin; d,g,j,m: cells in the thoracic aorta of diabetic rats were identified by their DAPI-labeled nuclei; e,h,k,n: co-staining of the Irf-1 with DAPI was shown by the merged images from the left column to the middle column. Scale bar 5 μm. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

with that in rats without streptozotocin administration (Fig. 2a,b). The significantly increased positive staining for Irf-1 protein was observed in the thoracic aorta at days 14 and 21 after the initial administration of streptozotocin (Fig. 2c–h, $P < 0.05$ versus the nondiabetic rats). The percentage of Irf-1 positive cells at days 14 and 21 after the initial administration of streptozotocin was $62.1 \pm 2.6\%$ and $74.5 \pm 2.2\%$ ($n = 12$), respectively. In contrast, less positive staining for the protein was seen in rats at day 7 after the initial administration of streptozotocin and without streptozotocin administration (Fig. 2i–n). The percentage of Irf-1 positive cells at day 7 after the initial administration of streptozotocin and without streptozotocin administration was $9.1 \pm 1.9\%$ and $10.3 \pm 1.7\%$ ($n = 12$), respectively.

### Irf-1 EXPRESSION IN VSMCs STIMULATED WITH HIGH GLUCOSE

Irf-1 expression was increased in VSMCs stimulated with high glucose. The mRNA level of Irf-1 was significantly increased in VSMCs 6 h after the initial treatment of high glucose, compared with that in untreated cells (Fig. 3e,f). The significantly increased positive staining for Irf-1 protein was observed in VSMCs at 36 and 72 h after the initial treatment of high glucose (Fig. 4c,d; $P < 0.05$ versus the untreated VSMCs). The percentage of Irf-1 positive cells at 36 and 72 h after incubation with high glucose was $59.3 \pm 2.5\%$ and $63.6 \pm 3.1\%$ ($n = 12$), respectively. In contrast, less positive staining for the protein was seen in VSMCs at 12 h after the initial treatment of high glucose and the untreated cells (Fig. 3a,b). The percentage of Irf-1 positive cells at 12 h after incubation with high glucose and in the untreated cells was $10.1 \pm 1.3\%$ and $7.2 \pm 2.2\%$ ($n = 12$), respectively.

### SPECIFIC siRNA TARGETING Irf-1 INHIBITS HIGH GLUCOSE-INDUCED PROLIFERATION OF VSMCs AND LEADS TO THE CELLS APOPTOSIS IN HIGH GLUCOSE CONDITIONS

To determine the effect of down-regulated Irf-1 expression on high glucose-induced VSMCs proliferation, VSMCs were transfected with pGCsi-FU-Irf-1, and cell proliferation was examined by cell counting and MTT assay. Western blot confirmed that Irf-1 expression in VSMCs transfected with pGCsi-FU-Irf-1 was significantly lower than that in untransfected cells in high glucose conditions ($P < 0.01$, $n = 3$; Fig. 4a). The number of cells in VSMCs transfected with pGCsi-FU-Irf-1 was significantly less than that in untransfected VSMCs and VSMCs transfected with the blank pGC vector (pGC-FU) 5 days after the initial treatment of high glucose ($P < 0.01$, $n = 12$; Fig. 5a). This finding was also consistent with the result of MTT assay, which showed significantly decreased proliferation activity in transfected VSMCs with pGCsi-FU-Irf-1 compared with untransfected VSMCs and VSMCs transfected with the blank pGC vector 5 days after the initial treatment of high glucose ($P < 0.01$, $n = 12$; Fig. 5b). In contrast, there was no significant difference in cell number and proliferation activity between VSMCs transfected with pGCsi-FU-Irf-1 and cells of the control groups in normal glucose conditions ($P > 0.05$, $n = 12$; Fig. 5a,b).

Manipulation of the IRF-1 levels (silencing of the Irf-1) exerted effects on apoptosis of cells in the presence of high glucose. Flow cytometry showed that VSMCs transfected with pGCsi-FU-Irf-1 had more cell apoptosis than untransfected VSMCs and VSMCs transfected with the blank pGC vector in high glucose conditions ($P < 0.05$, $n = 12$; Table II), indicating that silencing of Irf-1 led to VSMCs apoptosis under high glucose conditions. In contrast, there was no significant difference in cell apoptosis between VSMCs transfected with pGCsi-FU-Irf-1 and cells of the control groups in normal glucose conditions ($P > 0.05$, $n = 12$; Table II).

### Irf-1 OVEREXPRESSION ENHANCES HIGH GLUCOSE-INDUCED PROLIFERATION CAPACITY OF VSMCs

To determine the effect of Irf-1 overexpression on high glucose-induced VSMCs proliferation, VSMCs were transfected with pGC-FU-Irf-1 to examine cell proliferation by cell counting and MTT assay. Western blot confirmed that Irf-1 expression in VSMCs transfected with pGC-FU-Irf-1 was significantly higher than that in untransfected cells ($P < 0.01$, $n = 3$; Fig. 4a,b). The number of cells in VSMCs transfected with pGC-FU-Irf-1 was significantly greater than that in untransfected VSMCs and VSMCs transfected with the blank pGC vector (pGC-FU) 5 days after the initial treatment of high glucose ($P < 0.01$, $n = 12$; Fig. 5a). This finding was consistent with the result of MTT assay, which showed significantly increased proliferation activity in VSMCs transfected with pGC-FU-Irf-1 compared with the untransfected VSMCs and VSMCs transfected with the blank pGC vector 5 days after the initial treatment of high glucose ($P < 0.01$, $n = 12$; Fig. 5b). In contrast, the number and proliferation activity in VSMCs transfected with pGC-FU-Irf-1 were significantly decreased in normal glucose conditions when compared with the untransfected VSMCs and VSMCs transfected with the blank pGC vector.

There was no significant difference in cell apoptosis between VSMCs transfected with pGC-FU-Irf-1 and cells of the control group ($P > 0.05$, $n = 12$; Table II), indicating that Irf-1 overexpression was not pro-apoptotic in high glucose conditions. In contrast, VSMCs transfected with pGCsi-FU-Irf-1 had more cell apoptosis than untransfected VSMCs and VSMCs transfected with the blank pGCsi vector in normal glucose conditions ($P < 0.05$, $n = 12$; Table II), indicating that Irf-1 overexpression was pro-apoptotic under normal glucose conditions.

## DISCUSSION

Irf-1 is a transcription factor that recognizes regulatory elements in the promoters of interferon (IFN)-beta and some IFN-inducible genes. Expression of the factor in different mammalian cell lines leads to down-regulation or arrest of proliferation depending on the extent of expression. Irf-1 mediates inhibition in cell growth and is regarded as a negative regulator of cell growth by activating downstream effector genes [Kirchhoff et al., 1993; Eckert et al., 2006; Li et al., 2009]. Interestingly, it remains unclear whether Irf-1 is involved in growth regulation of VSMCs in pathological alterations observed in diabetic vascular diseases.

To determine whether Irf-1 is related to pathological alterations of blood vessels in diabetes, Irf-1 expression was investigated in the thoracic aorta of diabetic rats in this study. The results showed that Irf-1 expression was significantly increased in the thoracic aorta of

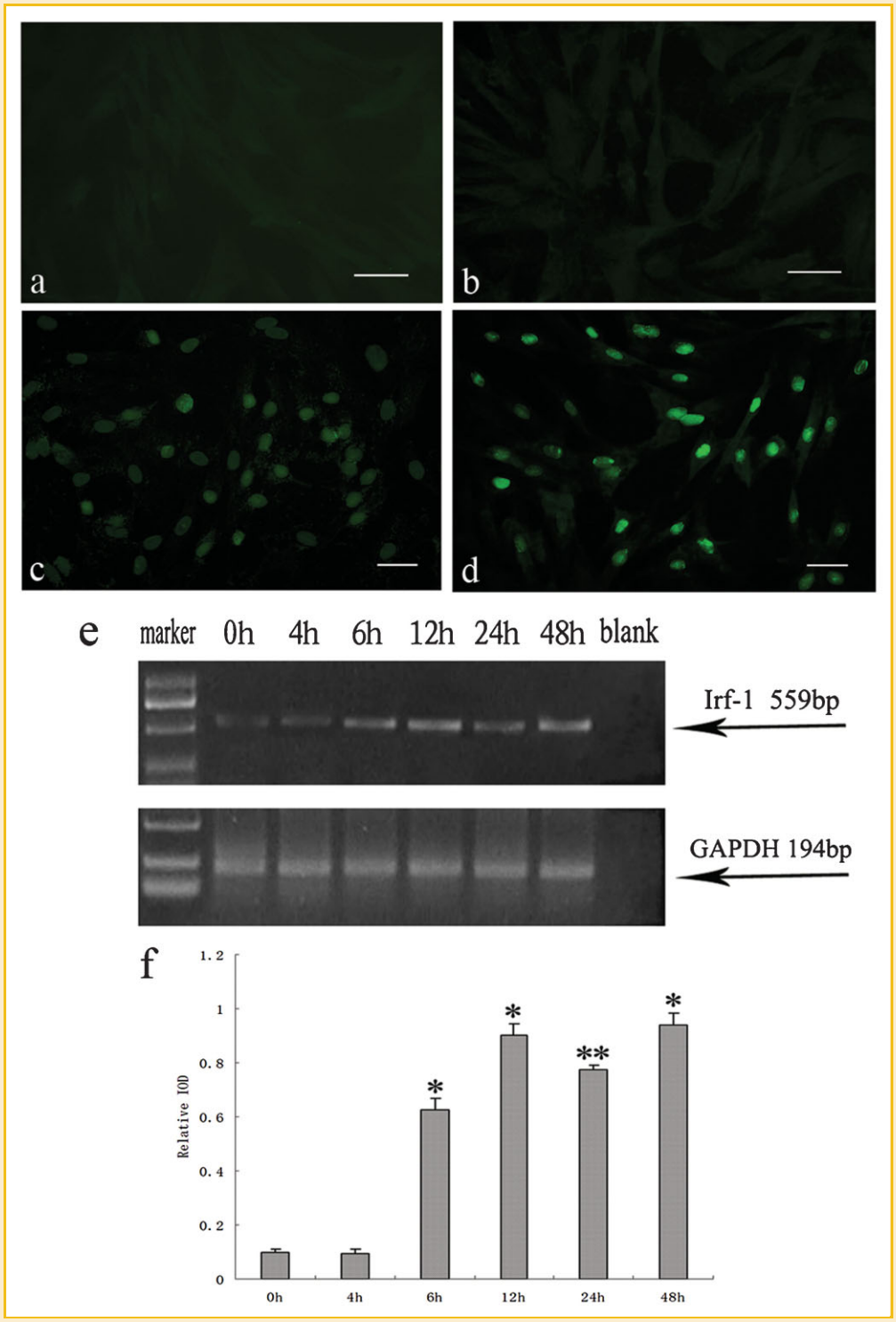


Fig. 3. Irf-1 expression in VSMCs stimulated with high glucose (25 mM). a: Representative negative staining of Irf-1 in untreated VSMCs; b: representative negative staining of Irf-1 in VSMCs 12 h after the initial treatment of high glucose; c: representative positive staining of Irf-1 in VSMCs 36 h after the initial treatment of high glucose; d: representative positive staining of Irf-1 in VSMCs 72 h after the initial treatment of high glucose. Scale bar 30 μm. e: RT-PCR analysis showing expression of the mRNA coding Irf-1 in VSMCs stimulated with high glucose; 0d, untreated VSMCs; 4h/6h/12h/24h/48h, VSMCs treated with high glucose for 4/6/12/24/48 h. d: Quantitative assessment for the level of mRNA coding Irf-1 in VSMCs stimulated with high glucose; 0d, untreated VSMCs; 4h/6h/12h/24h/48h, VSMCs treated with high glucose for 4/6/12/24/48 h. $^{*}P < 0.05$, $^{**}P < 0.01$. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

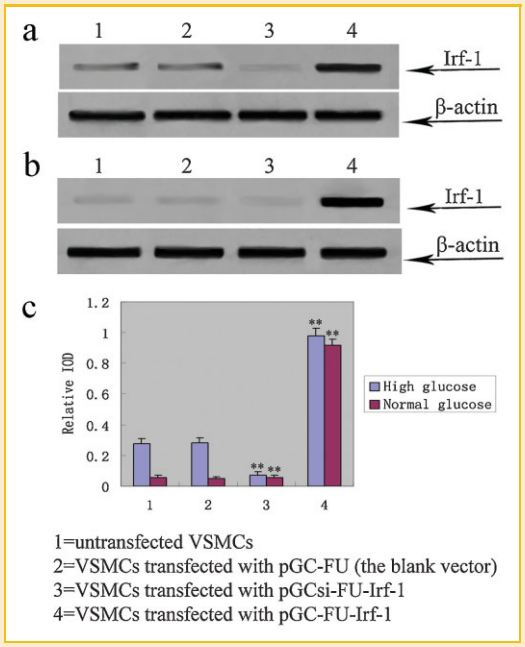


Fig. 4. Examination of Irf-1 expression in VSMCs transfected with pGCsi-FU-Irf-1 and pGC-FU-Irf-1 by Western blot. a: Western blot analysis showing expression of Irf-1 protein in the transfected VSMCs in high glucose conditions; b: Western blot analysis showing expression of Irf-1 protein in the transfected VSMCs in normal glucose conditions (c) quantitative assessment for the level of Irf-1 protein by integrated optical density analyses. $^{*}P < 0.05$; $^{**}P < 0.01$ versus corresponding values in untransfected VSMCs. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

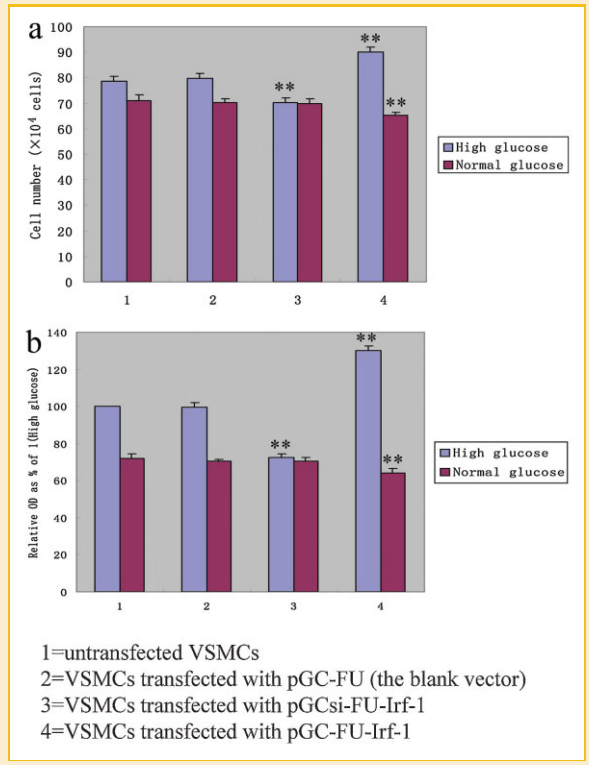


Fig. 5. Comparison of high glucose-induced cell proliferation in VSMCs with Irf-1 overexpression and downregulation. a: Cell number was counted with a hemocytometer; b: cell proliferation was also measured by MTT assay; Data are mean ± SD from triplicate determinations repeated in four separate experiments. $^{*}P < 0.05$; $^{**}P < 0.01$ versus corresponding values in untransfected VSMCs. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

diabetic rats, compared with that in nondiabetic ones, probably due to the role that Irf-1 plays in the pathological alterations of diabetic vascular diseases.

To confirm the relationship between Irf-1 expression and hyperglycemia-dependent VSMCs proliferation, Irf-1 expression was investigated in VSMCs stimulated with high glucose in vitro. The results indicated that the level of Irf-1 expression was significantly increased in VSMCs after the treatment of high glucose, compared with that in the untreated cells, confirming that there is a relationship between Irf-1 expression and high glucose-induced proliferation of VSMCs, and suggesting that Irf-1 may be involved in regulating the proliferation of hyperglycemia-dependent VSMCs in diabetic vascular diseases.

Although Irf-1 expression is known to be related to high glucose-induced proliferation of VSMCs, it remained unclear whether Irf-1 mediated VSMCs growth regulation by inhibition or proliferation. Our study showed that overexpression of Irf-1 led to the accelerated proliferation of VSMCs and down-regulation of Irf-1 expression depressed significantly the proliferation ability of VSMCs and induced more cell apoptosis in conditions of high glucose, suggesting that Irf-1 is pro-proliferate and anti-apoptotic. The result of our study is contrary to previous studies, which demonstrated that Irf-1 mediated cell growth inhibition and is a negative regulator of cell growth [Kirchhoff et al., 1993; Eckert et al., 2006; Li et al., 2009]. Interestingly, our data demonstrated that in normal glucose conditions Irf-1 overexpression is anti-proliferate and pro–apoptotic, suggesting that the contradiction is due to high glucose. The probable reason for this discrepancy is that in high

TABLE II. Analysis of VSMCs Apoptosis by Flow Cytometry

| | Untransfected VSMCs (%) | VSMCs transfected with pGC-FU (%) | VSMCs transfected with pGCsi-FU-Irf-1 (%) | VSMCs transfected with pGC-FU-Irf-1 (%) |
|---|---|---|---|---|
| High glucose | 2.06 ± 0.15 | 1.99 ± 0.11 | 9.33 ± 0.36* | 2.15 ± 0.12 |
| Normal glucose | 3.74 ± 0.24 | 3.85 ± 0.14 | 3.67 ± 0.22 | 8.24 ± 0.35* |

Values are mean ± SE from triplicate determinations repeated in four separate experiments.
*$P < 0.05$ versus corresponding values in untransfected VSMCs.

glucose conditions Irf-1, as a transcriptional activator, activates down-stream effector genes different from those reported in previous studies. Of course, this supposition needed to be testified in future study.

In conclusion, we have demonstrated that Irf-1 is a positive regulator for high glucose-induced proliferation of VSMCs and associated with accelerated proliferation of VSMCs in diabetic vascular diseases. Irf-1 may prove to be a potential target gene for disease treatment.

## REFERENCES


Aronson D. 2008. Hyperglycemia and the pathobiology of diabetic complications. Adv Cardiol 45:1–16.

Dror N, Alter-Koltunoff M, Azriel A, Amariglio N, Jacob-Hirsch J, Zeligson S, Morgenstern A, Tamura T, Hauser H, Rechavi G, Ozato K, Levi BZ. 2007. Identification of IRF-8 and IRF-1 target genes in activated macrophages. Mol Immunol 44:338–346.

Eckert M, Meek SE, Ball KL. 2006. A novel repressor domain is required for maximal growth inhibition by the IRF-1 tumor suppressor. J Biol Chem 281:23092–23102.

Faries PL, Rohan DI, Takahara H, Wyers MC, Contreras MA, Quist WC, King GL, Logerfo FW. 2001. Human vascular smooth muscle cells of diabetic origin exhibit increased proliferation, adhesion, and migration. J Vasc Surg 33:601–607.

Haller H, Drab M, Luft FC. 1996. The role of hyperglycemia and hyperinsulinemia in the pathogenesis of diabetic angiopathy. Clin Nephrol 46:246–255.

Hsueh WA, Jackson S, Law RE. 2001. Control of vascular cell proliferation and migration by PPAR-gamma: A new approach to the macrovascular complications of diabetes. Diabetes Care 24:392–397.

Kirchhoff S, Schaper F, Hauser H. 1993. Interferon regulatory factor 1 (IRF-1) mediates cell growth inhibition by transactivation of downstream target genes. Nucleic Acids Res 21:2881–2889.

Li Z, Wang ZG, Bian C, Chen XD, Li JW, Chen X, Han B, Hou GF, Chu J, Cui Q. 2009. Interferon regulatory factor-1 exerts inhibitory effect on neointimal formation after vascular injury. Chin Med Sci J 24:91–96.

Madi HA, Riches K, Warburton P, O'Regan DJ, Turner NA, Porter KE. 2009. Inherent differences in morphology, proliferation, and migration in saphenous vein smooth muscle cells cultured from nondiabetic and Type 2 diabetic patients. Am J Physiol Cell Physiol 297:C1307–C1317.

Mikhail N, Fukuda N, Tremblay J, Hamet P. 1993. Platelets, growth factors, and vascular smooth-muscle cells in hypertension and diabetes. J Cardiovasc Pharmacol 22(Suppl. 6):S64–S74.

Orasanu G, Plutzky J. 2009. The pathologic continuum of diabetic vascular disease. J Am Coll Cardiol 53:S35–S42.

Reusch JE, Wang CC. 2011. Cardiovascular disease in diabetes: Where does glucose fit in? J Clin Endocrinol Metab 96:2367–2376.

Romeo G, Fiorucci G, Chiantore MV, Percario ZA, Vannucchi S, Affabris E. 2002. IRF-1 as a negative regulator of cell proliferation. J Interferon Cytokine Res 22:39–47.

Srivastava AK. 2002. High glucose-induced activation of protein kinase signaling pathways in vascular smooth muscle cells: A potential role in the pathogenesis of vascular dysfunction in diabetes (review). Int J Mol Med 9:85–89.

Vranes D, Cooper ME, Dilley RJ. 1999. Cellular mechanisms of diabetic vascular hypertrophy. Microvasc Res 57:8–18.