

# Serum Response Factor Protects Retinal Ganglion Cells Against High-Glucose Damage

Yan Cao<sup>1</sup> · Liang Wang<sup>1</sup> · Junhong Zhao<sup>1</sup> · Hongbing Zhang<sup>1</sup> · Ying Tian<sup>1</sup> · Houcheng Liang<sup>1</sup> · Qiang Ma<sup>1</sup>

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**Abstract** Serum response factor (SRF), which encodes the MADS-box family of related proteins, is a common transcription factor related to the expression of genes associated with cell survival. However, SRF's role in retinal ganglion cells (RGCs) after high-glucose injury remains unclear. In this study, we investigate the protective role of SRF after high-glucose injury and its underlying mechanism. The *in vitro* RGC model subjected to high glucose was established by employing a 50 mmol/L glucose culture environment. As detected by real-time quantitative PCR and Western blot, SRF was significantly upregulated in RGCs treated with high glucose. Overexpression of SRF significantly promoted survival among RGCs exposed to high glucose and inhibited RGC apoptosis. Knockdown of SRF exerted an inverse effect. Moreover, SRF upregulation enhanced expression of an antioxidant protein, nuclear factor erythroid 2-related factor (Nrf2), via control of the Fos-related antigen 1 (Fra-1). SRF upregulation also affected RGC survival after high-glucose treatment. Our findings showed that overexpression of SRF promoted survival of RGCs after high-glucose injury by regulating Fra-1 and Nrf2.

**Keywords** SRF · Overexpression · High glucose · Fra-1 · Nrf2

Yan Cao and Liang Wang contributed equally to this work.

✉ Qiang Ma  
qiangmaq@tom.com

<sup>1</sup> Department of Ophthalmology, Xi'an NO.1 Hospital, No. 30 Fen Alley South Street, Xi'an 710002, China

## Abbreviations

SRF	Serum response factor
RGC	Retinal ganglion cell
Nrf2	Nuclear factor erythroid 2-related factor
Fra-1	Fos-related antigen 1
DR	Diabetic retinopathy
PBS	Phosphate-buffered saline
siRNA	Small-interfering RNA
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

## Introduction

Diabetic retinopathy (DR) is an important cause of blindness. Research regarding the pathogenesis has focused on the changes observed in the retinal capillary microcirculation (Yau et al. 2012; Khan et al. 2013). Studies of retinal tissue, especially with respect to retinal ganglion cell (RGCs), have attracted research attention (Garrett and Dunham 1990; Isenmann et al. 2003; Jung et al. 2013). A better understanding of the underlying mechanism of RGC injury caused by high glucose may provide fresh insights for the treatment of DR.

Serum response factor (SRF) is a widely expressed transcription factor in the MADS-box gene family (Wong et al. 2012; Baarlink et al. 2013; Nam et al. 2013). SRF regulates genes by combining serum response elements demonstrated in the promoter of the immediate-early genes, like *c-fos* and *Egr-1*, and tissue-specific genes (Maggiolini et al. 2004). It has been implicated in cell proliferation, differentiation, and apoptosis (Aline and Sotiropoulos 2012; Wiese et al. 2015). SRF is also related to neural signal transmission (Kumar et al. 2012), muscle

development and function (Cenik et al. 2015), and tumor occurrence, such as squamous cell skin cancer in mice (Yamashita et al. 2008) and colon cancer in humans (He et al. 2013). The underlying mechanism of SRF in defending RGCs from high-glucose injury is unknown, and few studies have investigated high-glucose injury in RGCs. High glucose can induce oxidative stress in cells (Batchuluun et al. 2014), and SRF has shown involvement in cell survival (Schwartz et al. 2014) and oxidation resistance (Meyer et al. 1993). We investigated the role of SRF in defending RGCs against high-glucose injury.

Fos-related antigen 1 (Fra-1), which is an oncogene, encodes a nucleoprotein Fos, which is a family-related protein (Das et al. 2012; Motrich et al. 2013; Yang et al. 2014). The Fos family includes the genes c-Fos, FosB, Fra-1, and Fra-2, and it has been demonstrated to combine with the Jun family containing c-Jun, JunB, and JunD. Fos family and Jun family form the transcription factor AP-1 (De Bosscher et al. 2001; Dhillon and Tulchinsky 2015). Studies show that Fra-1 is particularly connected with cell differentiation (Grotsch et al. 2014), proliferation (Lu et al. 2012), apoptosis (Zhong et al. 2015), and the neoplastic transformation process (Belguise et al. 2012). Fra-1 has been reported to be functionally important in regulating oxidative stress processes and controlling expression of the antioxidant protein Nrf2 (Vaz et al. 2012). In the vascular smooth muscle cells, SRF regulation suppresses Fra-1 (Horita et al. 2011). We sought to test the hypothesis that SRF upregulates Nrf2 to protect against injury caused by high-glucose in the RGCs by controlling Fra-1 expression.

Nrf2 is the most dynamic in the Cap'n'Collar transcription factor family. It is ubiquitously expressed in various tissue cells of the liver, kidney, digestive tract, lung, skin, and so on (Yu et al. 2012). Nrf2 and its cytoplasmic adapter protein, Kelch-like epichlorohydrin-associated protein 1 (Keap1), are the central regulators of the cellular antioxidant response (Kobayashi and Yamamoto 2005). Higher expression of Nrf2 can lower the reactive oxygen species (ROS) level, protecting cells from tumor formation, neuro-injury, inflammatory and oxidative stress, and so on (Chen and Kunsch 2004; Shibata et al. 2008; Rojo et al. 2010).

In this study, we focused on identifying the role and molecular mechanism of SRF in protecting the RGCs against high-glucose injury. We found that increased SRF expression in RGCs treated with high glucose and the overexpression of SRF promoted survival of RGCs with high-glucose injury. Further data indicated that this particular function of SRF occurred by regulating the Nrf2 through Fra-1. Therefore, a potential role of SRF in treating diabetes-related ocular complications was proposed.

## Materials and Methods

### Ethics Statement

The research was performed stringently and according to the guidelines established for the care and use of experimental animals by the National Health and Medical Research Council in China. The process of the study was authorized by Xi'an NO.1 Hospital.

### Culture of Retinal Ganglion Cells

Sprague-Dawley rats were purchased from the Animal Center of the Medical College, Shandong University, Shandong, China. The retinas were removed from postnatal rats within 24 h after birth. The retinas were washed thrice in phosphate-buffered saline (PBS) containing 100 kU/L penicillin-streptomycin and streptomycin (Invitrogen, Carlsbad, CA, USA) and placed in 0.25 % trypsin (Beyotime, Nantong, China) before incubation at 37 °C for 20 min. Next, the DMEM/F12 medium (Gibco, Rockville, MD, USA) containing 10 % fetal bovine serum (FBS; HyClone, Salt Lake City, UT, USA), 100 kU/L penicillin, and 50 kU/L streptomycin was added to halt the trypsin effect. The retinas were then blown using a pipette. The larger, undissociated pieces were allowed to settle. The cell suspension was then centrifuged at 1200 rpm/min for 5 min. Next, the cells were resuspended in the DMEM/F12 medium containing 10 % FBS and moved to a 6-well plate coated with OX-41. The cells were incubated at 37 °C for 30 min, and the plate was shaken for 10 min. Then, the cells were transferred without adhesion on the plate to a 24-well plate coated with OX-7 and incubated at room temperature for 30 min. The cell suspension in the 24-well plate was removed and the plate washed thrice using PBS. Eventually, 500 µL DMEM/F12 medium with 10 % FBS was added to the plate. RGCs were incubated under a humidified atmosphere of 5 % CO<sub>2</sub> at 37 °C.

### Construction of the Recombinant Plasmids

Human SRF full-length complementary DNA (cDNA) (Accession Number: J03161) and Fra-1 (Accession Number: NM005438) were amplified by reverse transcription PCR (RT-PCR) and inserted into the pcDNA.3.1/myc-His(-)Avector (Invitrogen). The restriction sites included *EcoR* and *BamH* in the two types of recombinant vectors. All recombinant plasmids were amplified in the DH5 *Escherichia coli*-competent cells (Tiangen, Beijing, China). Plasmids were extracted by TaKaRa MiniBEST Plasmid Purification Kit Ver.4.0 (Takara Biotechnology, Dalian, China). The correct plasmids, which had been sequenced, were labeled pcDNA.3.1-SRF and pcDNA.3.1-Fra-1.

**Table 1** The primers of RT-qPCR

Gene names	Primer sequences
SRF	Sense primer: 5'-TGGCACCAGTGTCTGCTA-3' Anti-sense primer: 5'-CAGATCCTCTTCTGAGATGAG-3'
Nrf2	Sense primer: 5'-TCAGCTACTCCCAGGTTGCCCA-3' Anti-sense primer: 5'-GGCAAGCGACTCATGGTCATCTAC-3'
Fra-1	Sense primer: 5'-GAGTAAGGCGCGAGCGGAACAA-3' Anti-sense primer: 5'-TGGAACATAGAGGGAAAGGGTCC-3'
GAPDH	Sense primer: 5'-CGTCTTACCACCATGGAGA-3' Anti-sense primer: 5'-CGGCCATCACGCCACAGTTT-3'

### Transfection of the Recombinant Plasmids and Small-Interfering RNA

The transfection process was performed following the standard instructions. The RGCs, which were in a good state, were plated in the 12-well plate. Next, the plate was transferred to the incubator with 5 % CO<sub>2</sub> at 37 °C for 24 h. The recombinant plasmid (pcDNA.3.1-SRF, pcDNA.3.1-Fra-1 + pcDNA.3.1-SRF, or pcDNA.3.1/myc-His(-)A), SRF small-interfering RNA (siRNA; 5'-GACCTGCCTCAACTCGCCAGAC-3'), or non-specific siRNA was separately diluted in 200 μL FBS-free DMEM/F12 medium with 6 μL TurboFect (Thermo Fisher Scientific, Waltham, MA, USA) per well when the RGCs reached 80 % fusion. The transfected and non-transfected RGCs were then cultured in the DMEM/F12 medium containing 10 % FBS and 50 mmol/L glucose under the conditions of 5 % CO<sub>2</sub> at 37 °C for 24 h.

### Cell Growth and Viability

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to measure growth and viability of the RGCs according to the instructions. The non-glucose RGCs (normal group) and glucose injury groups were cultured in the 96-well plate. Subsequently, the culture medium was replaced per well by 20 μL MTT (5 g/L) diluted in PBS and incubated at 37 °C for 5 h. Next, 150 μL of dimethyl sulfoxide was added per well, dissolving the crystal. The result was read using the microplate reader at 490 nm (Thermo Fisher Scientific).

### Annexin V-Fluorescein Isothiocyanate Conjugate and Propidium Iodide

RGC apoptosis was detected by using the Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit (Sigma, St. Louis, MO, USA). The operational approach was strictly followed using the instruction manual. In brief, the RGCs were pre-cooled by adding cold PBS to the buffer. Thereafter, 10 μL Annexin V-FITC (1 μL/

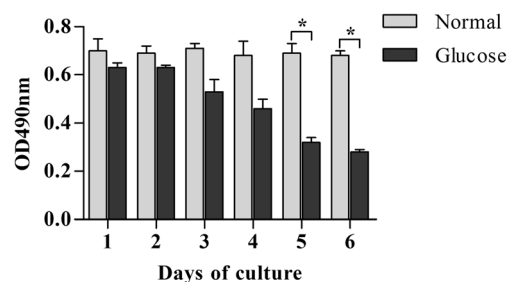
mL) was added and incubated with the RGCs at 4 °C for 28 min. Then, 10 μL of PI was added followed by incubation for 7 min. The RGC apoptosis was measured by the FACS analyzer (Beckman Coulter, Kraemer Boulevard Brea, CA, USA).

### Caspase-3 Activity Detection

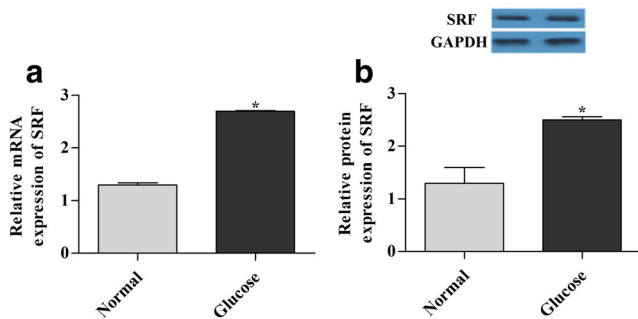
Caspase-3 activity assay of the RGCs was performed according to the instructions on the caspase-3 activity assay kit (Beyotime, Nantong, China) instructions.

### Real-Time Quantitative Polymerase Chain Reaction Assay

First, the total RNA was extracted from the RGCs using TRIzol reagent (Takara Biotechnology, Dalian, China). Then, 3 μg RNA was used to synthesize the cDNA, per the manufacturer's protocol of the Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Next, the real-time quantitative polymerase chain reaction assay (RT-qPCR) was performed using the synthesized cDNA and SYBR Premix Ex Taq II (Takara Biotechnology, Dalian, China) as the template and the mix enzyme separately. The amplification was performed using the following cycling parameters: 94 °C for



**Fig. 1** Effect of high-glucose injury on cell viability. The RGCs were cultured following the high-glucose treatment for 1, 2, 3, 4, 5, and 6 days. RGC viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Normal RGCs subjected to neither high-glucose injury nor transfected treatment, Glucose RGCs treated with 50 mmol/L of high glucose for different days.  $N=6$ ,  $*P<.05$  for the glucose group versus the normal group indicates significant difference



**Fig. 2** Effect of high-glucose injury on serum response factor expression in retinal ganglion cells. The relative mRNA level (a) and protein level (b) of serum response factor were detected by qRT-PCR and Western blot analysis, respectively. The GAPDH was used as the control.  $N=5$ ,  $*P<.05$  for the high glucose group versus the normal group indicates significant difference

4 min; 35 cycles at 95 °C for 30 s, 55 °C (Enc1) or 58 °C (Nrf2, HO-1) for 30 s and 72 °C for 30 s; and 72 °C for 10 min. The relative levels of gene expression were estimated by the  $2^{-\Delta\Delta C_t}$  method. The primers were normalized to GAPDH. The primers of the genes are listed in Table 1.

**Western Blot**

The RGCs were treated with lysate and phenylmethanesulfonyl fluoride (Beyotime; 100:1), and the protein from the RGCs was quantified using the BCA kit (Pierce, Rockford, IL, USA). Next, 20 µg of protein was isolated in the 12 % sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to the nitrocellulose membrane (Bio-Rad, Hercules, CA, USA) by electrophoretic transfer. The nitrocellulose membrane with

the protein was blocked in 5 % skim milk diluted in Tris-buffered saline for 2 h followed by incubation with the primary antibodies (SRF, Nrf2, Fra-1, Bcl-2, and GAPDH; CST, Danvers, MA, USA; 1:500) overnight at 4 °C. Next, the nitrocellulose membrane was incubated with secondary antibody (1:800) diluted in the blocking buffer.

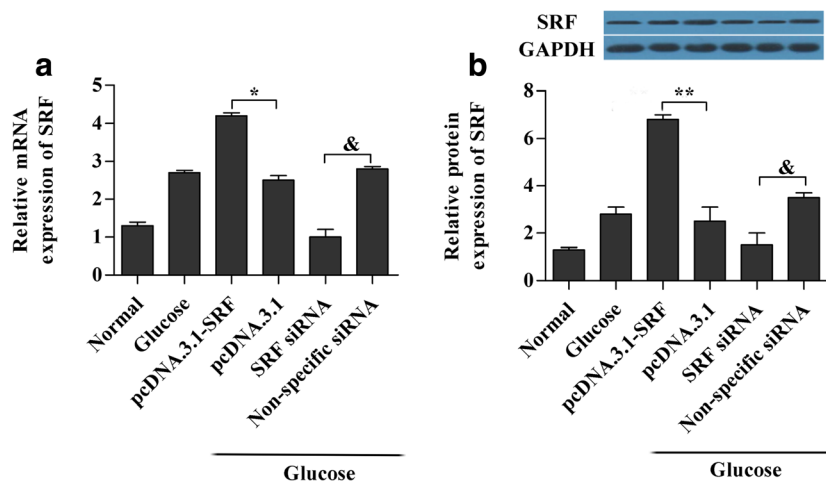
**Statistical Analysis**

Statistical significance was determined by the student *t* test between the two groups and by the one-way ANOVA for multiple groups. A *P* value of  $<.05$  was considered statistically significant. Data were expressed as the mean  $\pm$  standard deviation (SD).

**Results**

**High Glucose Increases Serum Response Factor Expression in Retinal Ganglion Cells**

First, we identified the maximum length of time that the high-glucose culturing could affect growth and viability of the RGCs, which was 5 days (Fig. 1). Therefore, we selected cells that were cultured for 5 days in the follow-up experiments. To investigate the change in SRF expression caused by the high glucose level in the RGCs, we measured the mRNA and protein from the RGCs treated only with high glucose by using RT-qPCR and Western blot methods, respectively. The results revealed that both



**Fig. 3** Expression of serum response factor after overexpression and siRNA. The relative mRNA level (a) and protein level (b) of serum response factor (SRF) were detected by qRT-PCR and Western blot analysis, respectively. GAPDH was used as the control. *Normal* the retinal ganglion cells (RGCs) subjected to neither high glucose nor transfected treatment, *Glucose* the RGCs treated with 50 mmol/L of high glucose for different days, *pcDNA.3.1-SRF* the RGCs transfected with pcDNA.3.1-SRF, *pcDNA.3.1* the RGCs transfected with

pcDNA.3.1/myc-His(-)A, *SRF siRNA* the RGCs transfected with SRF siRNAs, *Non-specific siRNA* the RGCs transfected with non-specific siRNAs. The RGCs were cultured with the high-glucose treatment for 5 days after these transfections.  $N=5$ ,  $*P<.05$  and  $**P<.01$  for the pcDNA.3.1-SRF group versus the pcDNA.3.1 group indicate significant difference;  $\&P<.05$  for the SRF siRNA group versus the non-specific siRNA group

the mRNA (Fig. 2a) and protein (Fig. 2b) expression levels of the SRF significantly increased in the high-glucose-treated group, compared to the normal group.

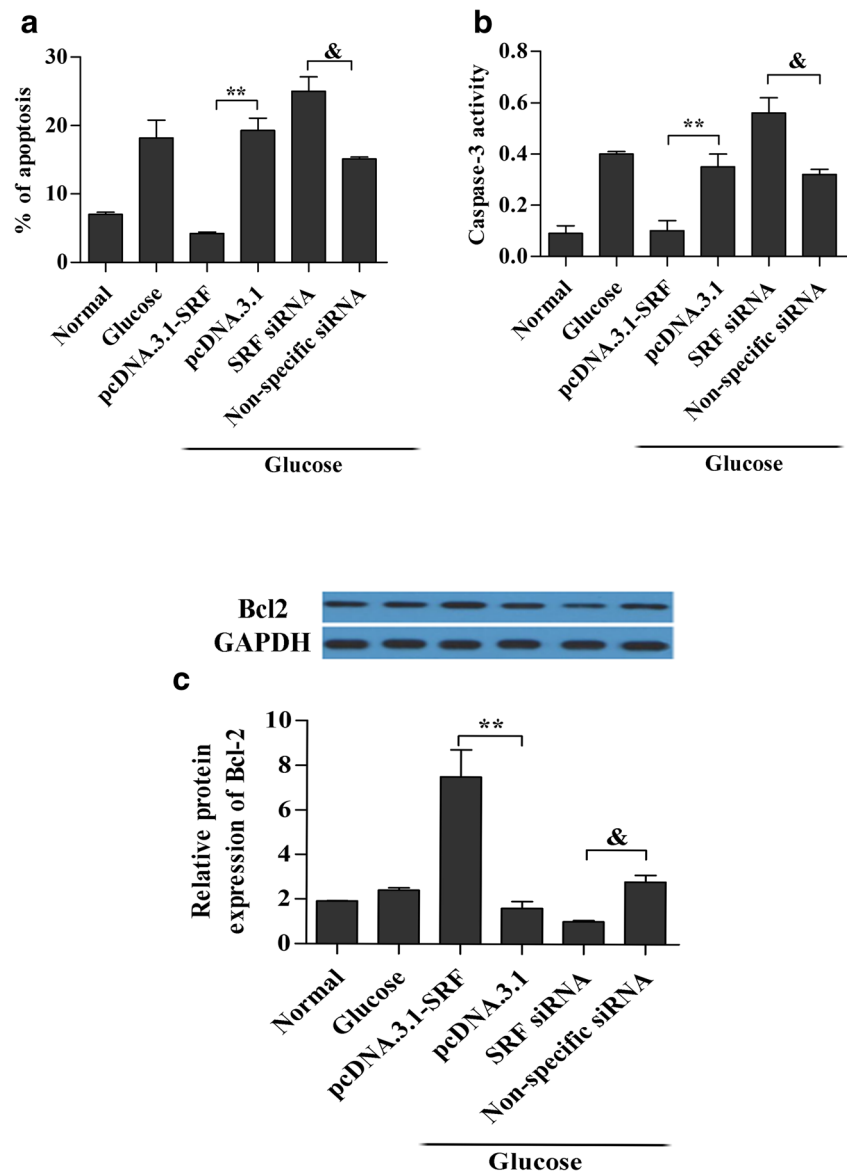
### SRF Overexpression Attenuates Apoptosis of Retinal Ganglion Cells Induced by High-Glucose Injury

To gain insight into the potential role of SRF in high glucose-induced RGC apoptosis, we utilized the technology involving SRF overexpression and siRNA. First, we detected the mRNA and protein expression of SRF to ensure that both technologies performed successfully. The detection results showed that SRF mRNA (Fig. 3a) and protein (Fig. 3b) expression of the pcDNA.3.1-SRF group increased, compared with the pcDNA.3.1/myc-His(-)A group. Meanwhile, SRF mRNA (Fig. 3a) and protein (Fig. 3b) expression of SRF

siRNA group were distinctly downregulated in contrast to the non-specific siRNA group.

Next, we examined the effect of the SRF overexpression and SRF siRNA on the high-glucose-induced cell apoptosis. The results of the Annexin V-FITC/PI apoptosis assay showed that RGC apoptosis caused by high glucose was significantly decreased by SRF overexpression, whereas SRF siRNA further promoted cell apoptosis induced by high glucose (Fig. 4a). Caspase-3 activity, which was increased by the high glucose in the RGCs, was converted by SRF overexpression but increased by SRF siRNA (Fig. 4b). Meanwhile, the anti-apoptotic protein Bcl-2 was also measured by Western blot test. The findings showed that protein expression of Bcl-2 was significantly induced by overexpressing SRF, whereas it decreased by SRF siRNA (Fig. 4c). Thus, the results

**Fig. 4** Effect of serum response factor overexpression or silence on high-glucose-induced retinal ganglion cell apoptosis. **a** Annexin V-FITC/PI staining method was used to measure retinal ganglion cell (RGC) apoptosis induced by high glucose. **b** Caspase-3 activity assay kit was used to detect caspase-3 activity. **c** Bcl-2 protein expression was analyzed by Western blot analysis and normalized by GAPDH using Image-Pro Plus 6.0 software.  $N=3$ ,  $**P<.01$  for the pcDNA.3.1-SRF group versus the pcDNA.3.1 group;  $\&P<.05$  for the SRF siRNA group versus the non-specific siRNA group



predicated that apoptosis induced by high glucose can be reversed by SRF overexpression in RGCs.

### Overexpression of Serum Response Factor Preserves Cell Viability in High-Glucose Injured Retinal Ganglion Cells

To further estimate the influence of SRF on RGC viability under a high glucose state, we performed the MTT assay. The results revealed that cell viability of RGCs under the high-glucose condition was significantly conserved in the pcDNA.3.1-SRF group, compared with the pcDNA.3.1/myc-His(-)A group (Fig. 5). In contrast, knockdown of SRF further decreased cell viability of the high glucose-treated RGCs (Fig. 5).

### Upregulated Serum Response Factor Augments the Nrf2 Through the Decreasing Fra-1 Expression

It has been reported that high glucose could induce oxidative stress (Quagliaro et al. 2003). Apoptosis is related to oxidative stress (Chandra et al. 2000). Therefore, oxidative stress plays a crucial role in high-glucose-induced injury. Nrf2 has an important function in combating oxidative stress. Here, we investigated whether SRF played any role in Nrf2. We found that mRNA (Fig. 6a) and protein (Fig. 6b, c) expression of Nrf2 was enhanced by SRF overexpression or significantly decreased by SRF knockdown. Meanwhile, SRF overexpression significantly suppressed Fra-1 expression at the mRNA level (Fig. 6a), whereas knockdown of SRF markedly increased levels of mRNA and protein expression of the Fra-1 (Fig. 6a–c). Additionally, according to the studies reported, we assume that SRF regulates Nrf2 expression through decreasing Fra-1. Therefore, we performed the co-overexpression of SRF and Fra-1 in the RGCs in a high-glucose state to investigate if SRF regulates Nrf2 by controlling expression of Fra-1. Finally, we demonstrated that overexpression of Fra-1 apparently abolished the promoted effect of SRF overexpression on Nrf2 expression (Fig. 6a–c). Furthermore, overexpression of Fra-1 significantly abrogated the protective effect of SRF overexpression on cell survival of the RGCs treated with high glucose (Fig. 6d). To summarize, these results suggest that SRF regulated Nrf2 expression through inhibition of Fra-1, which could be involved in regulating RGC survival after high-glucose injury.

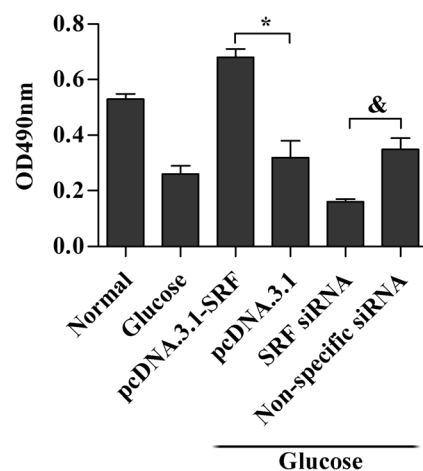
## Discussion

Diabetes has emerged as the third largest serious threat to human health as a chronic non-communicable disease after cancer and cardiovascular disease, and it is a rapidly growing public health problem (Callow 2006). DR is the most common chronic complication of diabetes (Klein 2007). DR can cause

blindness (Khan et al. 2013). Diabetic macular edema and proliferative DR are the main causes of visual impairment in DR (Kempner et al. 2004; Porta et al. 2011). Studies about DR have focused on retinal capillary microcirculation rather than on the retina. Thus far, the molecular mechanism of DR in the retina, especially regarding RGCs, remains unclear. In this study, we have detected a molecular mechanism that protects RGCs against high glucose states.

SRF belongs to the class of highly conserved transcription factors in the MADS evolutionary family and is widely expressed in a variety of regulatory processes (Zhao et al. 2013). SRF combines with various cofactors to form protein complexes that control DNA transcription (Long et al. 2013). SRF also plays a crucial role in vascular smooth muscle cell differentiation, cardiomyopathy, and tumor development (Joung et al. 2012; Cenik et al. 2015). SRF functions as the primary antioxidant-responsive transcription factor relevant to cell survival (Schwartz et al. 2014). However, the exact molecular mechanism of SRF in DR that induces oxidative stress remains unclear. In our study, we found that SRF expression was upregulated in RGCs with high-glucose injury. Next, we performed transfection techniques to upregulate SRF expression and found that cell survival was significantly enhanced, whereas SRF inhibition exerted the reverse effect. Our data further demonstrated that SRF overexpression effectively reduced injury induced by high glucose levels in RGCs.

Nrf2 is a widely expressed transcription factor and a member of the Cap'n'Collar family (Higgins and Hayes 2011). The antioxidant activity of Nrf2 was restricted by binding Keap1 (Villeneuve et al. 2010). However, Nrf2 combines with the antioxidant-responsive element (ARE) following dissociation from Keap1 and initiates downstream proteins containing anti-oxidation and anti-inflammatory proteins in the oxidation state (Tkachev et al. 2011). The Nrf2/ARE pathway protects



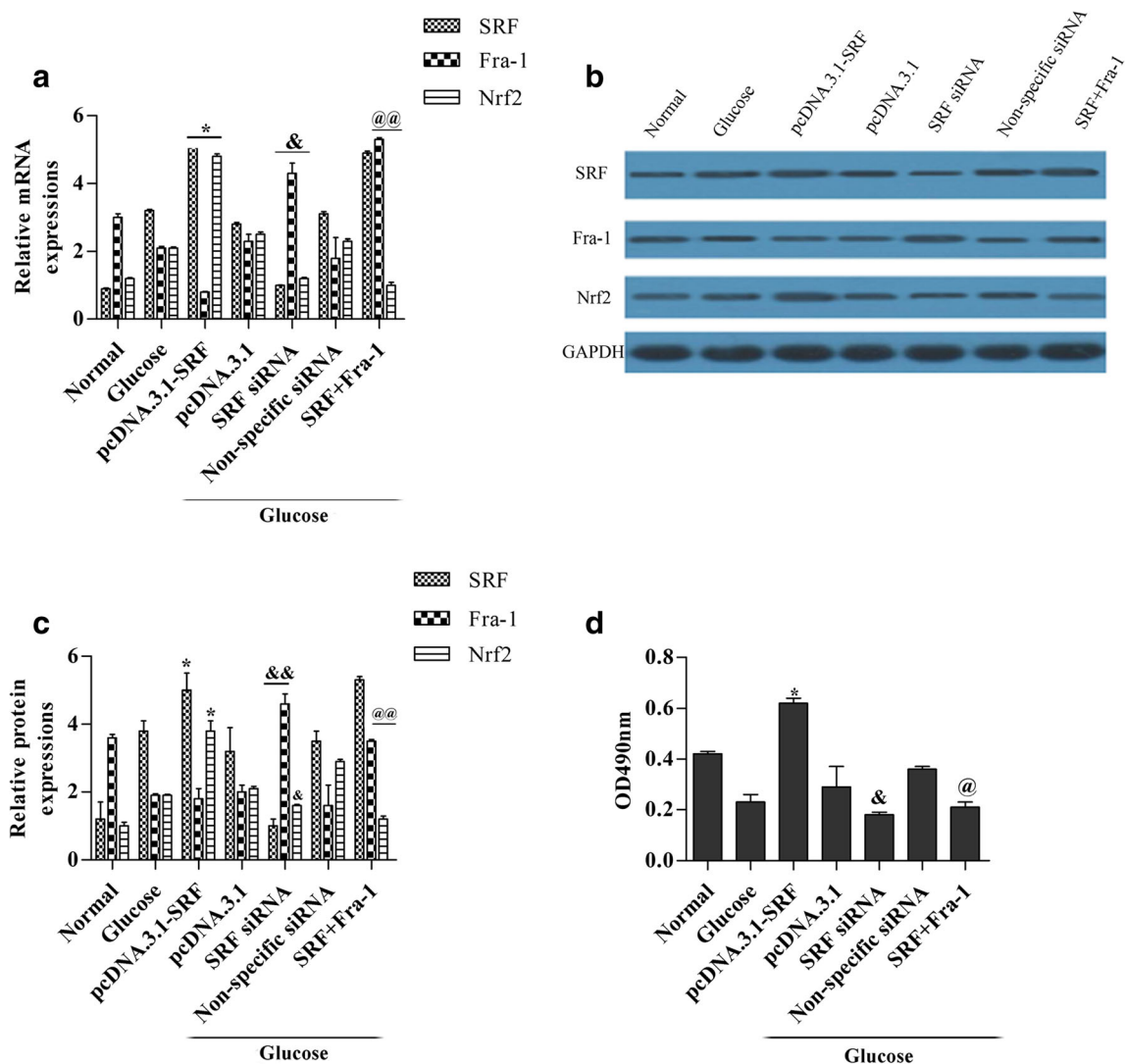
**Fig. 5** Effect of serum response factor overexpression or silence on retinal ganglion cell viability treated with high glucose. RGC viability with high-glucose treatment was determined using MTT. \* $P < .05$  for the pcDNA.3.1-SRF group versus the pcDNA.3.1 group; & $P < .05$  for the SRF siRNA group versus the non-specific siRNA group

against a variety of oxidative stress in the body. In this study, we observed that Nrf2 expression was significantly downregulated by SRF overexpression. Nevertheless, the drop in Nrf2 was reversed in the situation of Nrf2 inhibition. The results indicated that SRF regulated Nrf2 expression to perform the oxidant function in the RGCs under the high-glucose environment. Other studies have proven that SRF could repress Fra-1 expression in vascular smooth muscle cells (Horita et al. 2011) and that Fra-1 can suppress Nrf2 (Vaz et al. 2012). Therefore, we speculate that SRF increased Nrf2 expression by downregulating Fra-1.

Fra-1 has been posited as a transcription factor in the Fos family (Das et al. 2012). The transcription factor activity of Fra-1 is realized by combining AP-1 proteins with the Jun family and the element responsive to 12-*O* tetradecanoyl-

phorbol-13 acetate-responsive in target genes (Moquet-Torcy et al. 2014). Fra-1 regulates the genes that are related to cell differentiation, proliferation, and angiogenesis processes, as well as tumor invasion and antioxidative response (Vaz et al. 2012). In this research, we demonstrated that Fra-1 was negatively regulated by SRF. Fra-1 was distinctly downregulated, and SRF was overexpressed. Meanwhile, co-elevated SRF and Fra-1 expression significantly decreased Nrf2, whereas only overexpressed SRF exerted an effect convert to Nrf2. The results demonstrated that SRF overexpression enhances the expression of Nrf2 via controlling Fra-1 in high-glucose RGCs.

This study revealed that SRF overexpression positively regulates Nrf2 expression and facilitates RGC survival during high-glucose injury. Additionally, SRF regulation of Nrf2 is



**Fig. 6** Expression of Fra-1 and Nrf2 in transfected retinal ganglion cells. **a** The mRNA level and protein levels of **b** Fra-1 and **c** Nrf2 were measured by RT-qPCR and Western blot analysis, respectively. **d** The RGC apoptosis induced by high glucose was detected by MTT. The SRF + Fra-1, RGCs transfected with pcDNA.3.1-Fra-1, and

pcDNA.3.1-SRF.  $N=6$ ,  $*P<.05$  for the pcDNA.3.1-SRF group versus the pcDNA.3.1 group;  $\&P<.05$  and  $\&\&P<.01$  for the SRF siRNA group versus the non-specific siRNA group.  $\&\&P<.01$  and  $\&P<.05$  for the SRF + Fra-1 group versus the pcDNA.3.1-SRF group

relevant to Fra-1. Our study revealed the unique role of SRF in regulating RGC survival following a high-glucose injury and provided novel insight for developing a potential therapeutic strategy for the treatment of DR.

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