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PI3K/Akt Pathway Mediates High Glucose-Induced Lipid Accumulation in Human Renal Proximal Tubular Cells Via Spliced XBP-1

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ABSTRACT

In the present study, we investigated the effect of X-box-binding protein-1 (XBP-1) splicing on lipogenesis in high glucose-stimulated human renal proximal tubular cell line (HKC). The results revealed that high glucose promoted the splicing of XBP-1, concomitant with up-regulation of lipogenic genes including fatty acid synthase, acetyl-CoA carboxylase, adipocyte differentiation-related protein, and cellular triglyceride. Again, silence of XBP-1 with shRNA vector inhibited high glucose-caused increased lipogenesis. Furthermore, we confirmed that the inhibition of phosphotidyl inositol 3-kinase (PI3K)/Akt pathway with LY294002 or Akt shRNA vector blocked the effect of high glucose on XBP-1 splicing and cellular triglyceride. These above data suggest that spliced XBP-1 mediates high glucose-induced lipid accumulation in HKC cells and PI3K/Akt pathway may be involved in high glucose-caused XBP-1 splicing. J. Cell. Biochem. 113: 3288–3298, 2012.

KEY WORDS: XBP-1; HIGH GLUCOSE; LIPOGENESIS; RENAL PROXIMAL TUBULAR CELLS; PI3K/AKT

Endoplasmic reticulum stress also known as unfolded protein response (UPR) has been identified to be involved in pathogenesis of diabetic nephropathy, which is a complicated network including various regulators [Hummasti and Hotamisligil, 2010]. IRE1/XBP-1 is a classic UPR pathway that is activated to avoid cellular apoptosis when cells produce endoplasmic reticulum stress [Walter and Ron, 2011]. X-box-binding protein-1 (XBP-1) is a transcription factor involved in endoplasmic reticulum stress and it has two statuses: unspliced XBP-1 (XBP-1u) and spliced XBP-1 (XBP-1s). The XBP-1s has much stronger transcriptional activity than XBP-1u and regulates subsequent physiological activities through affecting protein transcription [Yoshida et al., 2001].

However, Lee et al. [2008] revealed unexpectedly another function of XBP-1 that XBP-1s regulates hepatitic lipid metabolism. XBP-1 deleted mice showed hypo-triglyceride and hypo-cholesterol through affecting hepatitic lipid metabolism. Subsequently, Nishina et al. [2010] also found that the transgenic mice, expressing the HCV polyprotein, fed the excess-iron diet showed a higher expression of sterol regulatory element-binding protein-1 (SREBP-1) and fatty acid synthase (FASN), and an activated UPR indicated by a higher expression of XBP-1u and XBP-1s, phosphorylated eukaryotic initiation factor-2alpha (p-eIF2α), CCAAT/enhancer-binding protein homology protein (CHOP), and abundant autophagosomes. However, the anti-oxidant *N*-acetyl cysteine dramatically reduced hepatic steatosis in transgenic mice fed the excess-iron diet through decreased expression of XBP-1u and XBP-1s, p-eIF2α, and CHOP. Therefore, it can be concluded that XBP-1 can mediate lipid metabolism via its transcriptional activity in the different animal models. In our previous study, it has been identified that high glucose can cause lipogenesis in renal tubular cells of diabetic rats via up-regulation of SREBP-1 [Hao et al., 2011b]. However, whether XBP-1 also involves in the lipid metabolism abnormality of diabetic nephropathy and the exact mechanism are still not known.

Therefore human renal proximal tubular cell line (HKC) was chosen to investigate the expression of XBP-1s under stimulation of high glucose. Furthermore, HKC cells were transfected with XBP-1s expression plasmid or shRNA plasmid aimed at XBP-1 genes to determine the effect of XBP-1 on high glucose-induced lipid

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deposition. Again, in order to explore the potential mechanism involved high glucose-caused XBP-1 splicing, phosphotidyl inositol 3-kinase (PI3K)/Akt signal transduction was detected in HKC cells treated with chemical inhibitor or Akt shRNA plasmid.

MATERIALS AND METHODS

REAGENTS AND CHEMICALS

Rabbit anti-Akt and phospho-Akt antibodies were purchased from Cell Signaling Technology Co. Rabbit anti-XBP-1 antibody was bought from Abcam, Inc. Rabbit anti-ADRP antibody was purchased from Epitomics, Inc. Triglyceride detecting kit was obtained from Dongou Biotechnology Co. The following products were from Santa Cruz Biotechnology Inc.: rabbit anti-β-actin antibody, goat anti-rabbit secondary antibody, XBP-1 shRNA plasmid (h): sc-38627-SH and control shRNA plasmid-A: sc-108060. Trizol reagent and reverse transcription kit were from invitrogen. GoTaq Green Master Mix was purchased from Promega Biosciences.

CELL LINE AND GROUPS

Human renal proximal tubular cell line was a gift from Dr. Chen Xiangmei (301 Hospital, Beijing, China). HKC cells were cultured as described previously [Hao et al., 2011b]. To investigate the effect of high glucose on XBP-1 expression HKC cells were respectively stimulated with 30 mmol/L glucose for 0, 12, 24, and 48 h. To exclude the effect of high osmotic pressure caused by high glucose on XBP-1, HKC cells were respectively treated for 24 h with normal glucose medium (5.5 mmol/L glucose), normal glucose medium plus mannitol (5.5 mmol/L glucose + 24.5 mmol/L mannitol), and high glucose medium (30 mmol/L glucose). In addition, to determine the direct influence of XBP-1 on lipid metabolism HKC cells were respectively transfected with specific XBP-1s and XBP-1u vectors that were gifts from Dr. Kazutoshi Mori (Department of Biophysics, Graduate School of Science, Kyoto University, Japan) and Dr. Yao Chang (Division of Clinical Research, Division of Infectious Diseases, National Health Research Institutes, Tainan). Then the metabolic genes involved in lipid genesis including FASN and ACC were detected. Furthermore, XBP-1 shRNA plasmid was used to transfect HKC cells to explore whether XBP-1s up-regulation mediated high glucose-induced lipid metabolism abnormality. Then, LY294002, chemical inhibitor of PI3K/Akt pathway, was used to explore whether this pathway was involved in the regulation of XBP-1 splicing in high glucose-cultured HKC cells. Finally, human shRNA plasmid aimed at Akt gene (a gift from Dr. Myung-Haing CHO, Seoul National University, Korea) was transfected into HKC cell to further determine the effect of PI3K/Akt pathway on high glucose-stimulated increased XBP-1 splicing.

TRANSIENT TRANSFECTION

The transient transfection was performed with Lipofectamine 2000 as described previously [Hao et al., 2011b]. Briefly, HKC cells were cultured in six-well plates and the medium was changed the following day until 80% confluence was achieved. The cells were transfected with 4.0 μg vector DNA by 10 μl Lipofectamine 2000 in 2 ml serum-free DMEM medium. At 6 h after transfection the medium was replaced by normal DMEM medium with 10% fetal bovine serum (FBS) for 24 h. Then cells were cultured for 48 h under different medium to detect relevant changes.

WESTERN BLOT

Protein extracted from HKC cells was separated on 10% SDS–PAGE gel. Then protein was transferred onto PVDF membrane (Millipore Corporation, Bedford, MA) and the membrane was blocked for 1 h at 37°C with 5% BSA in Tris-buffered saline containing 0.05% Tween 20 (TBST). Next the membrane was incubated with primary antibodies 4°C overnight for XBP-1 (1:500, Abcam), Akt (1:800; Cell Signaling Technology, Beverly, MA), phospho-Akt (1:800; Cell Signaling Technology), and β-actin (1:1,000; Santa Cruz Biotechnology, CA). Subsequently, membrane was rinsed with TBST three times and then second antibody was used to incubate membrane at certain concentration (1:5,000) at room temperature for 2 h. After rinsing with TBST three times membrane was treated with ECL solution (Pierce, Rockford, IL) and bands were detected by exposing the blots to X-ray films. For quantitative analysis, bands were evaluated with IPP 5.0 software, normalized for β-actin density.

SEMI-QUANTITATIVE RT-PCR

Total RNA was extracted with Trizol (Invitrogen Co., CA) according to the instructions of the manufacturer. Total RNA (2 μg) was reverse transcribed using random primers and M-MLV at 42°C for 1 h and then heated to 94°C for 5 min in a total reaction volume of 20 μl. The PCR amplification began with a 5 min denaturation at 95°C, followed by 30 cycles of denaturation at 95°C for 40 s, annealing at 55°C for 40 s, and extension at 72°C for 40 s. The final extension was set for 10 min at 72°C. The products were electrophoresed on 1.5% agarose gel and the mRNA levels of FASN and ACC were normalized with GAPDH mRNA level. All PCR primers were shown in Table I.

IMMUNOCYTOCHEMISTRY

HKC cells were planted on cover slides in six-well plates. Cells were fixed with 10% formalin at room temperature for 15 min. After pretreatment of 0.3% Triton X-100 for 20 min at 37°C, cells were blocked with goat serum for 30 min at 37°C. Cells were incubated with anti-ADRP monoclonal antibody (1:100) overnight at 4°C. At the following day, the sections were washed with PBS for three times and incubated with reagent 1 (Santa Cruz Biotechnology) for 30 min

TABLE I. Primers and Product for FASN, ACC, and GAPDH

Gene	Sense	Antisense	Product (bp)
FASN	5′-CCCAAGGGAAGCACATT-3′	5′-GAGCGAGAAGTCAACACGA-3′	223
ACC	5′-GAACATCCCTACGCTAAAC-3′	5′-CAGCCTGTCATCCTCAATA-3′	337
GAPDH	5′-AACGGATTTGGTCGTATTG-3′	5′-GCTCCTGGAAGATGGTGAT-3′	214

and reagent 2 for 30 min at 37°C. Then slides were observed after rinsed with PBS for three times. Negative controls were obtained by replacing specific antibody with PBS.

OIL RED O STAINING

Cultured cells were fixed for 15 min in 4% paraformaldehyde (in PBS), and stained for 15 min in 1% Oil Red O. Then, the sections were washed with 70% alcohol for 5 s to remove background staining. Finally, the cells were rinsed in tap water, counterstained with Harris hematoxylin (10 s). The stained sections were imaged with an Olympus microscope and examined in a blinded manner by the renal pathologist.

TRIGLYCERIDE QUANTITATIVE DETECTION

Lipid was extracted according to the Folch method from HKC cells [Folch et al., 1957]. Triglyceride was detected with the kit from Zhejiang Dongou Company.

STATISTICAL ANALYSIS

All data were expressed as mean ± standard deviation (SD) and analyzed with SPSS 11.0 for Windows. Statistical analyses were performed using one-way analysis of variance (ANOVA) and Student–Newman–Keuls test was used to determine statistically significant differences within and between groups. $P < 0.05$ was considered statistically significant.

RESULTS

HIGH GLUCOSE INCREASED SPLICED XBP-1 EXPRESSION IN HKC CELLS IN TIME-DEPENDENT MANNER

Compared with HKC cells cultured in normal glucose medium (5.5 mmol/L glucose), HKC cells treated with high glucose medium (containing 30 mmol/L glucose) showed the increased splicing of XBP-1. As shown in Figure 1, XBP-1s protein was enhanced with the addition of high glucose and the most expression presented at 24 h after stimulation of high glucose. Again, XBP-1u was revealed to supposedly show the high expression in HKC cells grown in normal medium. Corresponding with the increase of XBP-1s, it can be seen that high glucose led to the significant down-regulation of XBP-1u in time-dependent manner. Again, we determined whether the changes of XBP-1s and XBP-1u were resulted from high glucose itself not the high osmotic pressure caused by high glucose. The results showed that no difference in XBP-1s and XBP-1u expression was found between normal glucose medium-cultured HKC cells and normal glucose medium plus mannitol-cultured cells. However, high glucose medium-cultured cells presented evident

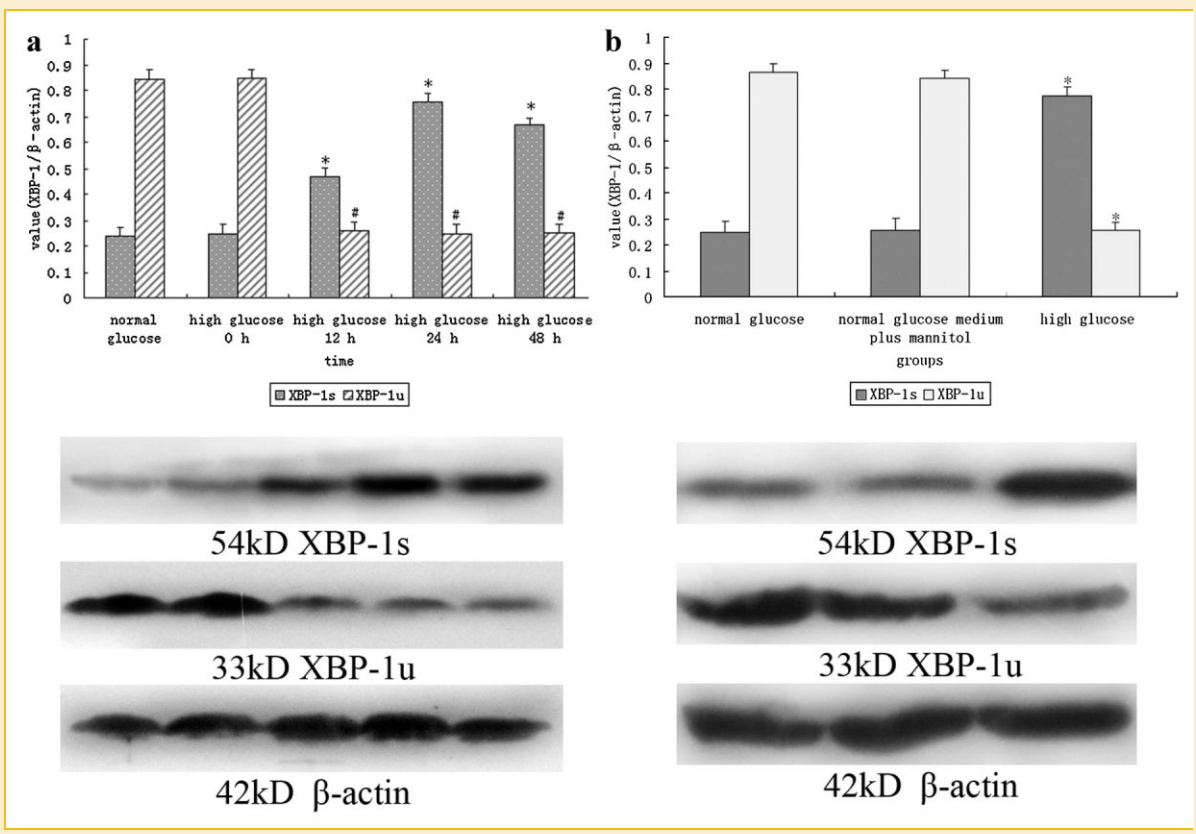

Fig. 1. High glucose increased spliced XBP-1 expression in HKC cells in time-dependent manner. a: Western blot is used to analyze the time-dependent effect of high glucose on XBP-1s and XBP-1u protein in HKC cells. The levels of XBP-1s and XBP-1u were quantified by densitometry (mean ± SD, n = 6). *$P < 0.05$ versus normal glucose group. b: Effect of high osmotic pressure caused by high glucose on XBP-1s and XBP-1u was determined by Western blot. *$P < 0.05$ versus normal glucose plus mannitol group.

down-regulation of XBP-1u and up-regulation of XBP-1s compared with normal glucose medium plus mannitol-treated HKC cells (Fig. 1).

INTRODUCTION OF SPLICED XBP-1 CAUSED DIRECTLY CELLULAR TRIGLYCERIDE ACCUMULATION THROUGH REGULATING LIPID GENESIS IN HKC CELLS

In order to determine the effect of splicing of XBP-1 on lipid metabolism of HKC cells, XBP-1s plasmid and XBP-1u plasmid were respectively transfected into HKC cells. At 48 h after transfection XBP-1 protein was detected and the results confirmed that XBP-1s plasmid enhanced effectively XBP-1s expression in HKC cells without variation of XBP-1u protein. On the contrary XBP-1u plasmid only caused the significant up-regulation of XBP-1u protein and had no influence on XBP-1s protein. Furthermore, FASN and ACC, the key enzymes involved in triglyceride genesis, were investigated by the method of RT-PCR and it can be illustrated in Figure 2 that XBP-1s plasmid led to the increased FASN and ACC mRNA. As seen in Figure 3, Oil Red O staining revealed that the visible red-stained granule was found in HKC cells transfected with XBP-1s plasmid. However, no visible stained lipid droplet could be seen in HKC cells of other three groups. Furthermore, the

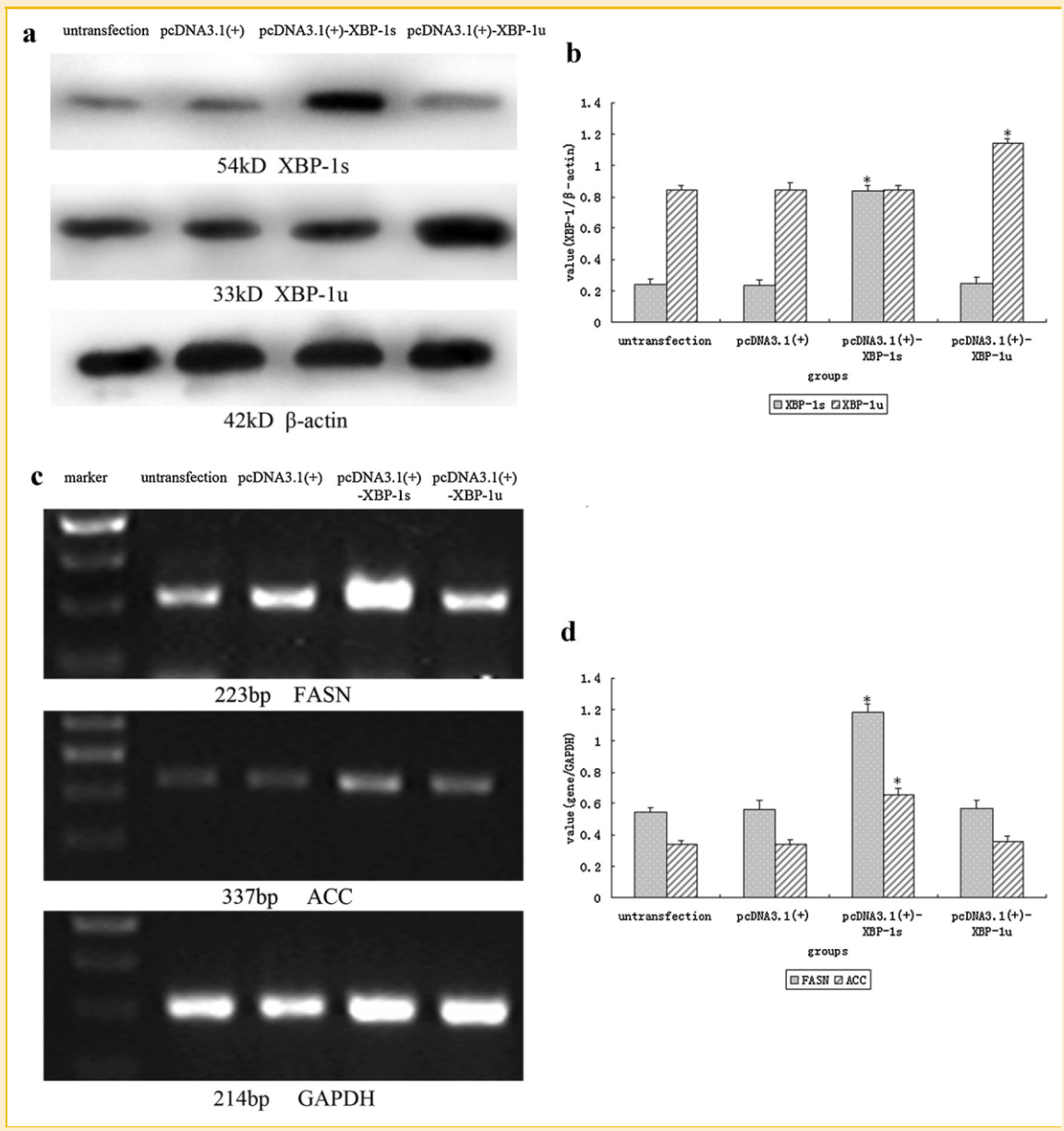

Fig. 2. XBP-1s expression vector increased FASN and ACC expression in HKC cells. a,b: Expression of XBP-1 protein was determined by Western blot analysis and quantified by densitometry (mean ± SD, n = 6) in HKC cells respectively transfected with specific XBP-1s vector and XBP-1u vector. $^{*}P < 0.05$ versus untransfection group. c,d: The up-regulation of FASN and ACC mRNA was determined by RT-PCR in HKC cells transfected with human XBP-1s vector. $^{*}P < 0.05$ versus untransfection group.

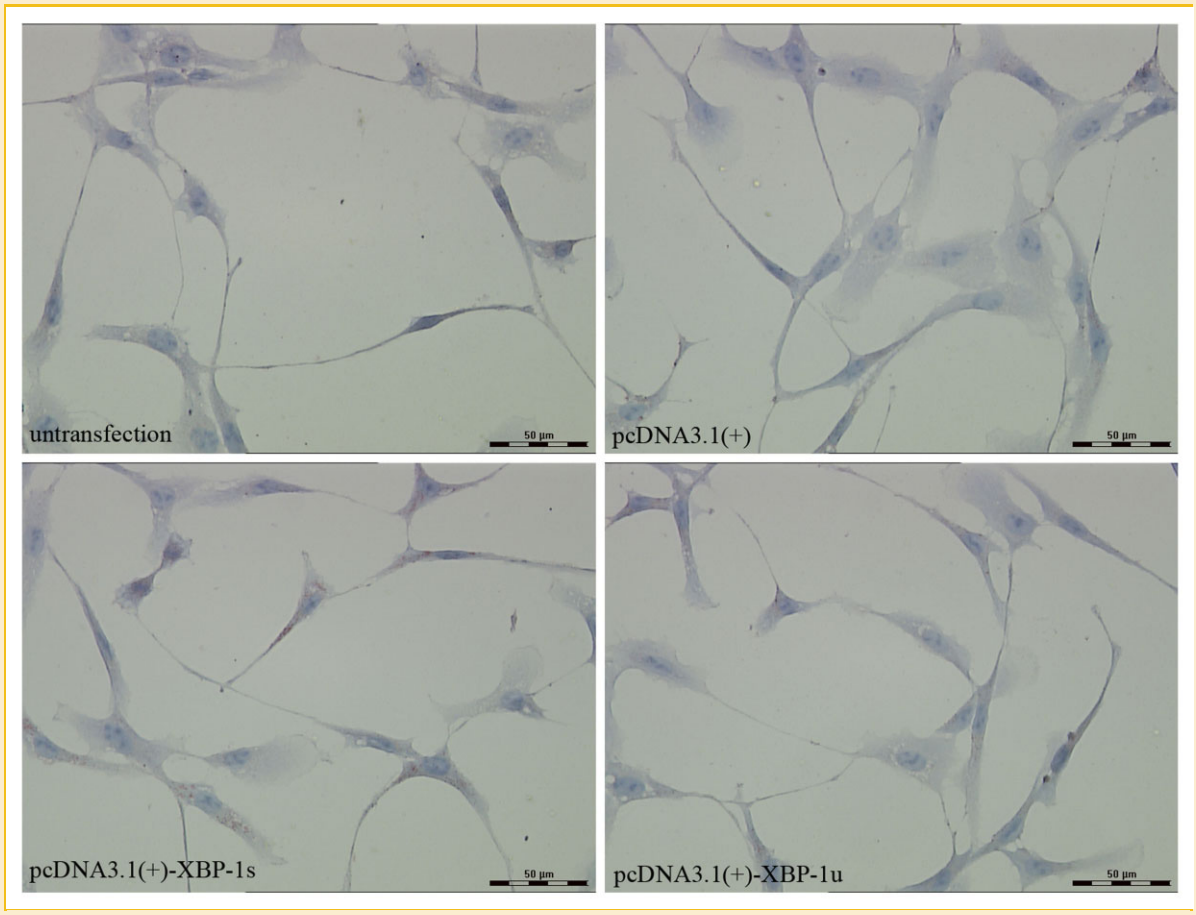

Fig. 3. Oil Red O staining of HKC cells transfected with specific human XBP-1s and XBP-1u vectors ×400.

quantitative triglyceride detection testified a similar result that splicing of XBP-1 was a positive factor of the triglyceride accumulation (Table II).

SILENCE OF SPLICED XBP-1 EXPRESSION EFFECTIVELY PREVENTED HIGH GLUCOSE-INDUCED LIPID DEPOSITION IN HKC CELLS VIA AFFECTING LIPID GENESIS

To explore whether the up-regulation of XBP-1s mediates high glucose-induced triglyceride accumulation, we transfected the HKC cells with specific XBP-1 shRNA plasmid. The results showed that XBP-1 shRNA plasmid evidently decreased XBP-1s protein and XBP-1u protein as seen in Figure 4. Furthermore, the down-regulation of FASN and ACC mRNA were also revealed in HKC cells transfected with XBP-1 shRNA plasmid, which was absent in control shRNA plasmid-treated HKC cells. Again, Figure 5 showed that ADRP, the marker of lipid droplet, was decreased illustrated as mild yellow staining in HKC cells transfected with XBP-1 shRNA plasmid compared with untransfection cells and control shRNA plasmid-transfected cells that presented the evident strong yellow or brown staining. Oil Red O staining revealed that the marked red-stained granule could be found in untransfected-HKC cells stimulated by high glucose. However control shRNA plasmid did not cause significant change of red-stained lipid droplet in high glucose-treated HKC cells. On the contrary the specific XBP-1 shRNA plasmid produced the apparent reduction of lipid droplet. The triglyceride quantitative detection also supplied the similar evidence that the specific XBP-1 shRNA plasmid could lessen effectively the cellular triglyceride as illustrated in Table III.

TABLE II. The Effect of XBP-1 Plasmid on Cellular Triglyceride in HKC Cells (Mean ± SD, mg/ml)

	Triglyceride
Untransfection	70.23 ± 5.83
pcDNA3.1(+)	71.80 ± 6.73
pcDNA3.1(+)-XBP-1s	92.51 ± 4.77*
pcDNA3.1(+)-XBP-1u	71.19 ± 5.31

Compared with untransfection group.
*$P < 0.05$.

PI3K/AKT PATHWAY INVOLVED IN HIGH GLUCOSE-STIMULATED SPLICED XBP-1 UP-REGULATION AND LIPID METABOLISM IN HKC CELLS

PI3K/Akt pathway, a multiple signal transduction pathway, was reported to regulate various cellular functions including proliferation, metabolism, and differentiation. Firstly, we determined the

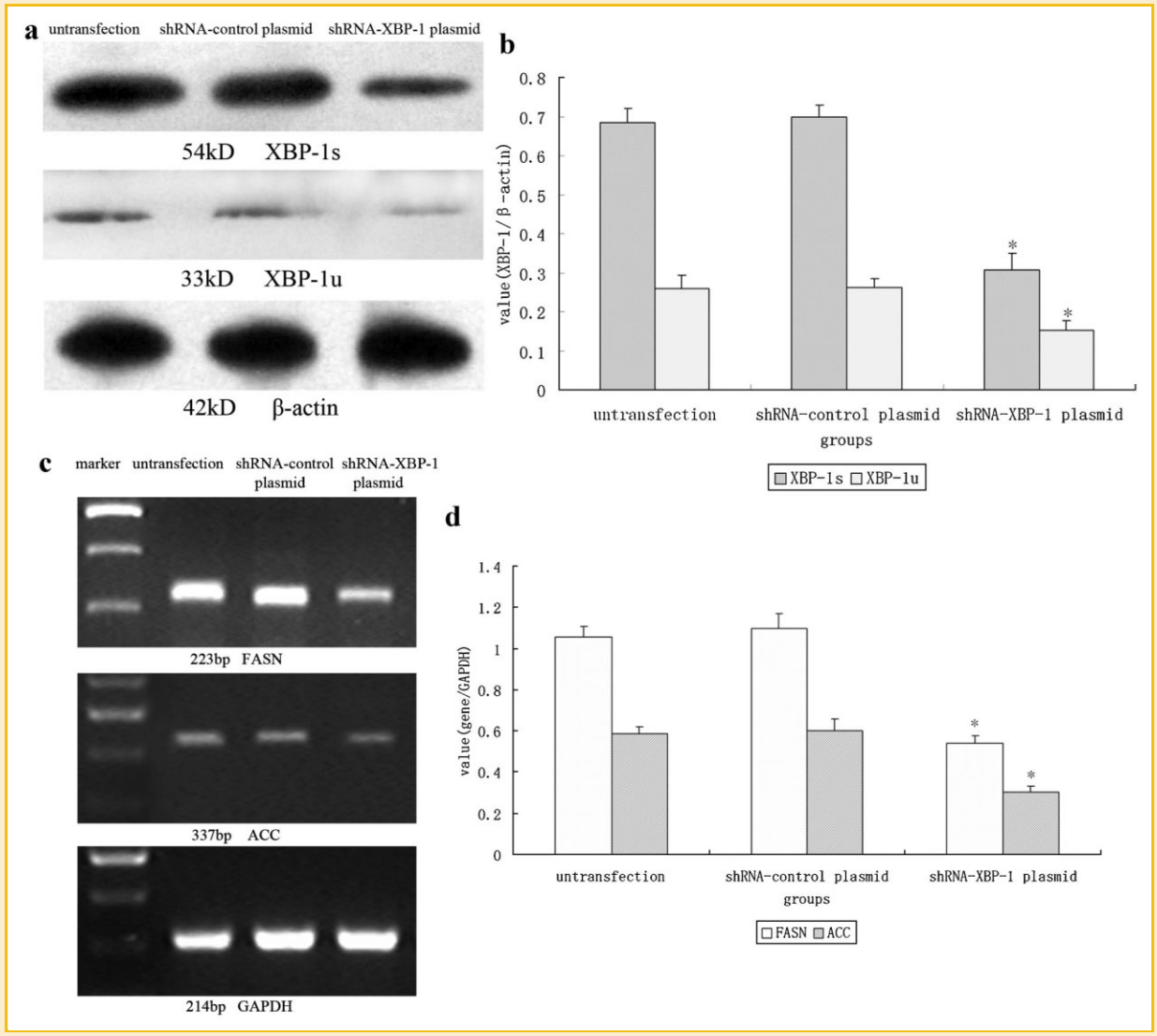

Fig. 4. Specific XBP-1 shRNA expression vector suppressed high glucose-induced FASN and ACC up-regulation in HKC cells. a,b: Expression of XBP-1s and XBP-1u was measured by Western blot analysis and quantified by densitometry. c,d: Normalized by GAPDH, mRNA levels of FASN and ACC were determined by RT-PCR and were quantified by densitometry (mean ± SD, n = 6). $^{*}P < 0.05$ versus untransfection group.

effect of high glucose on Akt and phospho-Akt in HKC cells. The results showed that high glucose markedly increased the phospho-Akt, however, had no significant influence on Akt in HKC cells (Fig. 6). To further study the potential role of PI3K/Akt pathway (activation of Akt) in high glucose-induced XBP-1s up-regulation, we performed a tentative experiment with LY294002, PI3K/Akt pathway chemical inhibitor. As proved in Figure 7, LY294002 reduced the expression of XBP-1s in high glucose-cultured HKC cells accompanied with the decrease of phospho-Akt. Again, the decreased function of high glucose on XBP-1u in HKC cells was reversed with the addition of LY294002 in medium. In addition, compared with high glucose-stimulated HKC cells the corresponding reduction of cellular triglyceride was confirmed in HKC cells treated with LY294002 via triglyceride quantitative detection, which was shown in Table IV.

TABLE III. The Effect of shRNA-XBP-1 Plasmid on Cellular Triglyceride in HKC Cells (Mean ± SD, mg/ml)

	Triglyceride
Untransfection	137.18 ± 5.49
shRNA-control plasmid	134.81 ± 5.94
shRNA-XBP-1 plasmid	109.85 ± 5.61*

Compared with untransfection group.
*$P < 0.05$.

SPECIFIC AKT SHRNA PLASMID EFFECTIVELY AVOIDED INCREASED SPLICED XBP-1 AND LIPID ACCUMULATION IN HKC CELLS UNDER STIMULATION OF HIGH GLUCOSE

RNA interfere is a kind of high efficient technology to silence gene expression and has been extensively applied into various scientific researches. In this present study, the specific Akt shRNA plasmid was

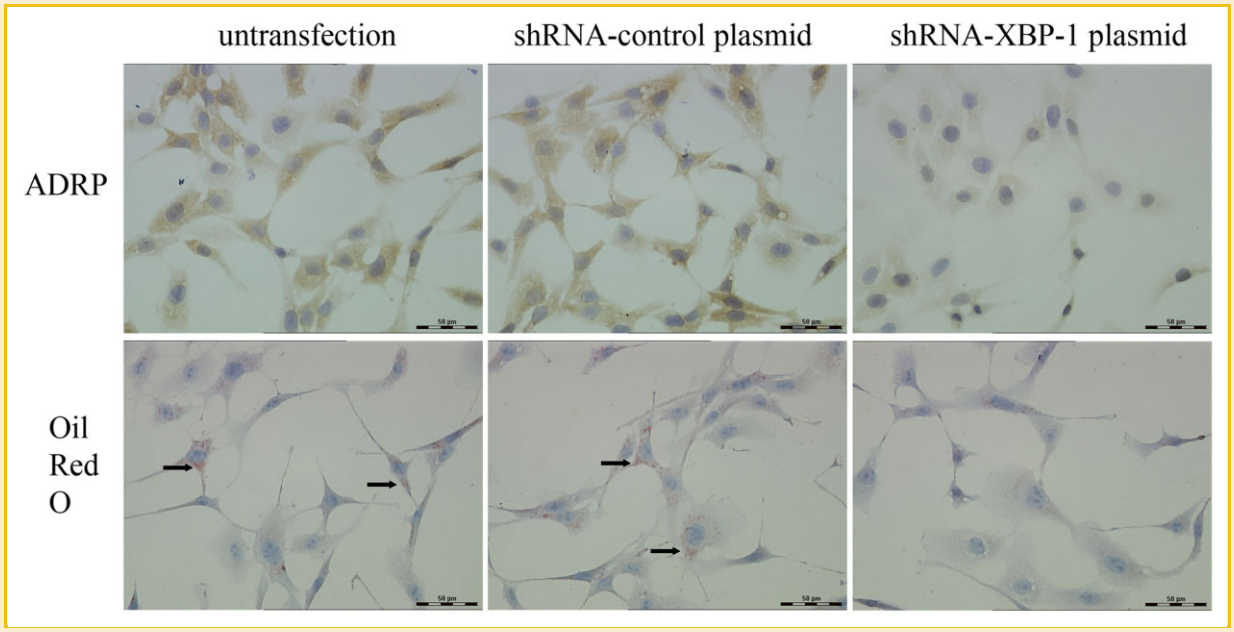

Fig. 5. Specific XBP-1 shRNA vector decreased ADRP expression and lipid droplet formation in high glucose-stimulated HKC cells ×400. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

transfected into HKC cells under the stimulation of high glucose. As seen in Figure 8, in contrast to untransfected HKC cells, there was no obvious variation of Akt, phospho-Akt, and XBP-1s in control sh-Scramble plasmid-transfected HKC cells. However, both Akt and phospho-Akt were significantly inhibited via the transfection of sh-Akt plasmid accompanied with the decrease of XBP-1s. Similar to the application of LY294002, sh-Akt also reversed the effect of high glucose on XBP-1u, that is, up-regulated the expression of XBP-1u in HKC cells. Again, the triglyceride detection also proved the effect of Akt shRNA plasmid on lipid metabolism in HKC cells stimulated by high glucose that could be seen in Table V.

DISCUSSION

The XBP-1 is a transcription factor that could bind to the X-box, a conserved transcriptional element in the promoter of the human leukocyte antigen (HLA) DRα [Liou et al., 1990]. The protein that is

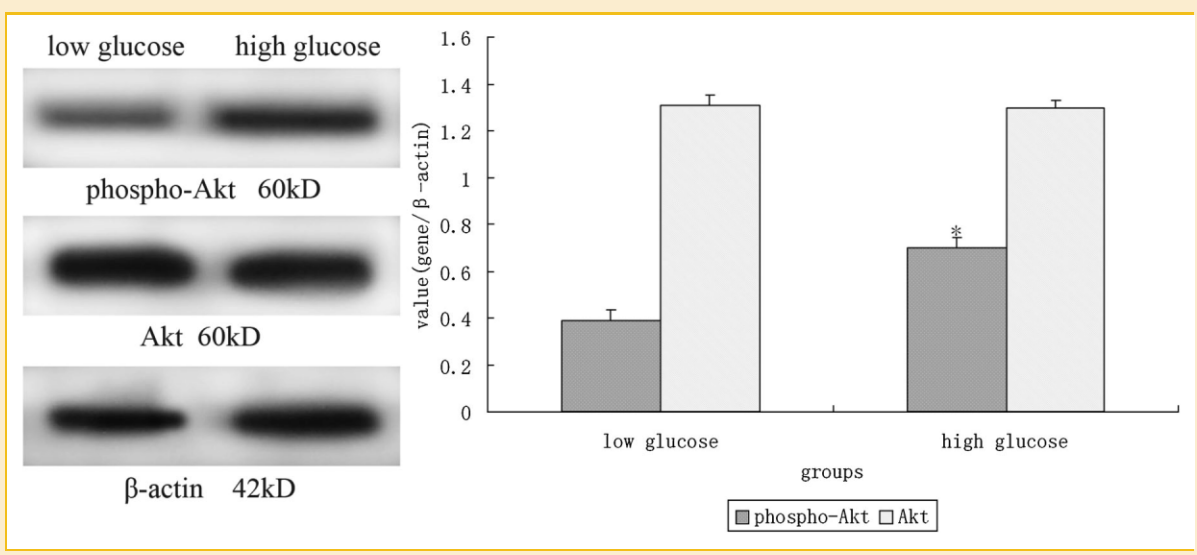

Fig. 6. High glucose increased phospho-Akt in HKC cells. Akt and phospho-Akt were detected in HKC cells respectively treated with high glucose medium or low glucose medium by Western blot and quantified by densitometry (mean ± SD, n = 6). *$P < 0.05$ versus low glucose group.

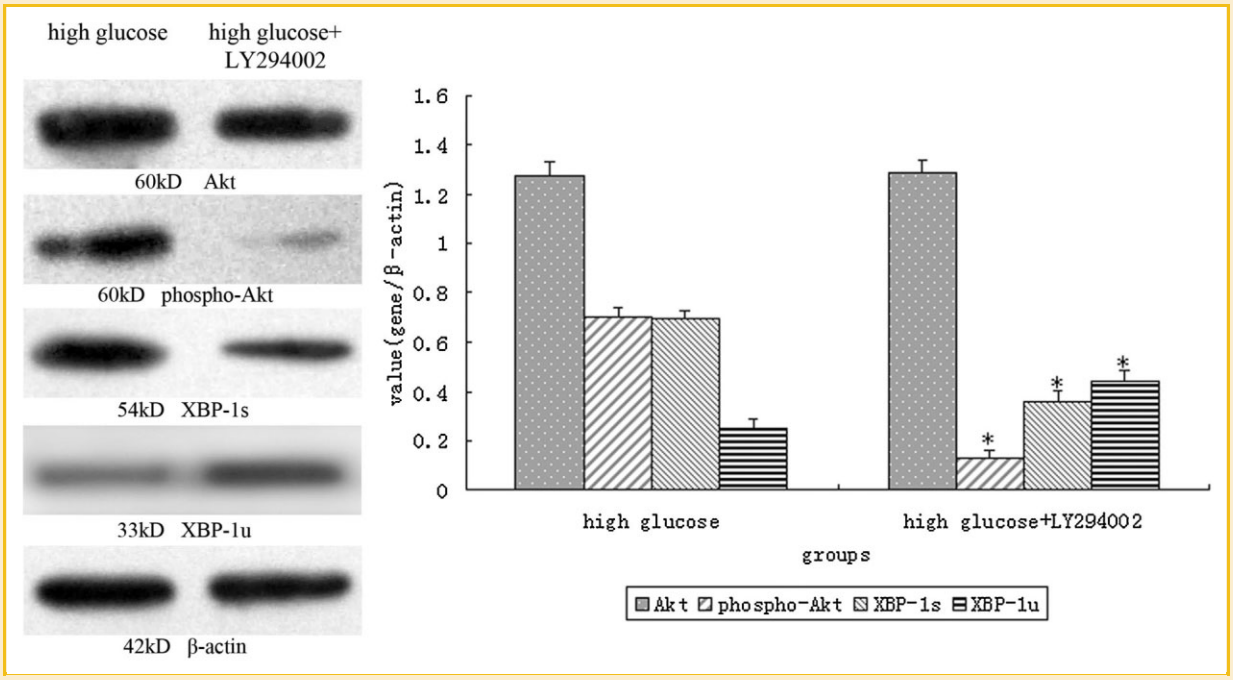

Fig. 7. LY294002 inhibits high glucose-induced XBP-1s increase in HKC cells. Normalized by β-actin, protein levels of Akt, phospho-Akt, XBP-1s, and XBP-1u were determined by Western blot and were quantified by densitometry (mean ± SD, n = 6). $^{*}P < 0.05$ versus high glucose group.

translated from the XBP-1u mRNA, gives rise to a 33 kDa protein, XBP-1u with a functional DNA-binding domain, but no activation domain. Following splicing by IRE1, a frameshift is introduced into the mRNA, resulting in the translation of a 54 kDa protein, XBP-1s containing both DNA-binding and activation domains [Lee et al., 2003].

There is increasing studies that uncovered evidence of the presence of ER stress in renal cells in human diabetic nephropathy [Lindenmeyer et al., 2008]. Stimuli that increase the demand on the ER to synthesize proteins or degrade improperly folded proteins cause this stress. The high glucose was reported to induce ER stress involved in GRP-78 (glucose-regulated protein 78), phosphorylated protein kinase-like ER-associated kinase, p-eIF2α, p-ATF4 (p-activating transcription factor-4), CHOP [Lim et al., 2011], HSPA5 (heat shock 70 kDa protein 5), HYOU1 (hypoxia up-regulated 1), and XBP-1 in renal cells [Lindenmeyer et al., 2008; Cybulsky, 2010]. The present research revealed that high glucose increased XBP-1s expression in HKC cells, which was similar to Lee et al.'s finding in hepatitic cells that high glucose and insulin were positive factors of XBP-1 over-expression. In addition, Zhang et al. [2009] also revealed that prolonged high glucose exposure increased the levels of XBP-1s mRNA in INS-1 832/13 cells. Given these data it can be suggested that high glucose-induced XBP-1s up-regulation might involve in pathophysiological activities of diabetes.

TABLE IV. The Effect of LY294002 on Cellular Triglyceride in HKC Cells Stimulated by High Glucose (Mean ± SD, mg/ml)

	Triglyceride
High glucose	135.33 ± 5.00
High glucose + LY294002	72.59 ± 6.59*

Compared with high glucose group.
*$P < 0.05$.

It has been proved that XBP-1 has multiple functions, such as plasma cell differentiation [Hu et al., 2009], viral replication, and endoplasmic reticulum stress response [Iwakoshi et al., 2003]. However, in a recent research a new function of XBP-1 was determined to increase serum lipid by affecting liver lipid metabolism. Again, in our previous study, we have revealed that high glucose could enhance the expression of SREBP-1, a key transcription factor involved in lipid metabolism in renal tubular cells. To some extent, gene silence of SREBP-1 could avoid high glucose-induced lipid accumulation in renal tubular cells. These above data gave us such a result that high glucose up-regulated the lipogenesis in renal tubular cells via SREBP-1, at the same time presented a suggestion that other genes also were involved in high glucose-mediated cellular lipogenesis [Jun et al., 2009]. Considering the role of XBP-1s in liver lipid metabolism and the effect of high glucose on XBP-1s, we speculated that XBP-1s might be a candidate regulating high glucose-induced lipid droplet formation in renal tubular cells.

ADRP, firstly cloned by Ginette et al. [Jiang and Serrero, 1992], is a kind of lipid droplet protein existing in all cells presenting lipid accumulation [Brasaemle et al., 1997; Heid et al., 1998]. Although it has been found for about 20 years it is the last several years that people began to further realize its function on regulating lipid dynamic metabolism in various tissues, including liver [Chang et al., 2006], foam cells [Wang et al., 1999], and muscle and breast gland [Phillips et al., 2005; Robenek et al., 2006]. ADRP is also revealed to

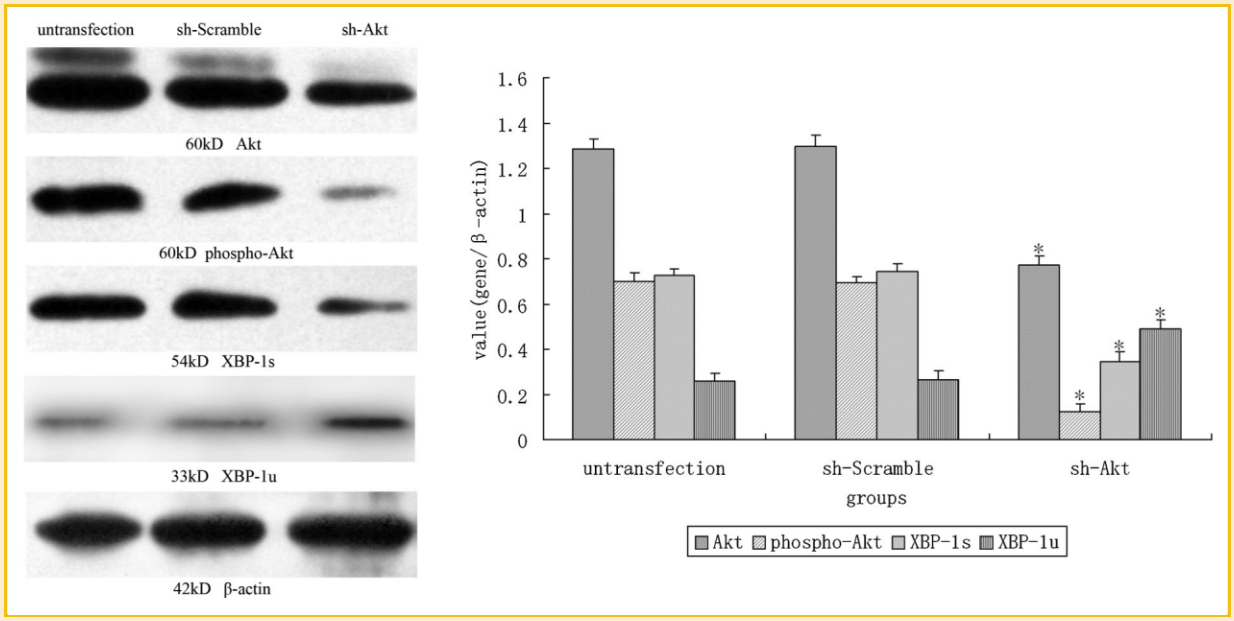

Fig. 8. Specific sh-Akt vector effectively prevented high glucose-induced increase of XBP-1s in HKC cells. Expression of Akt, phospho-Akt, XBP-1s, and XBP-1u were determined by Western blot analysis and quantified by densitometry (mean ± SD, n = 6). $^{*}P < 0.05$ versus sh-Scramble group.

locate in not only the surface of lipid droplet, but also external region of lipid droplet [Chen et al., 2001]. Recently, ADRP has been considered to be the sensitive marker of lipid droplet.

In this present study, we explored the relationship between XBP-1s and ADRP expression and lipid accumulation in high glucose-treated HKC cells. Finally, we confirmed the correlation between XBP-1s up-regulation and the expression of ADRP, cellular lipid deposition. Again, high glucose-caused lipid metabolism abnormality was reversed via suppression of XBP-1 splicing. This was similar to Lee et al.'s [2008] study that presented firstly an unexpected function of XBP-1, regulating hepatitic lipid metabolism. The specific deletion of hepatitic XBP-1 resulted into hypo-lipid through affecting hepatitic lipogenesis and transport. Additionally, a recent study demonstrated that the IRE1α–XBP-1 UPR pathway is indispensable for adipogenesis. XBP-1-deficient mouse embryonic fibroblasts and 3T3-L1 cells with XBP-1 or IRE1α knockdown exhibit profound defects in adipogenesis [Sha et al., 2009]. These results suggested that high glucose might partly induce cellular lipid droplets formation via XBP-1 splicing.

TABLE V. The Effect of sh-Akt on Cellular Triglyceride in HKC Cells Stimulated by High Glucose (Mean ± SD, mg/ml)

	Triglyceride
Untransfection	136.43 ± 7.24
sh-Scramble	134.34 ± 6.05
sh-Akt	76.64 ± 5.86*

Compared with untransfection group.
*$P < 0.05$.

PI3K/Akt is a multiple functional signal pathway that involved in cell proliferation, differentiation [Kim et al., 2010], motility [Jeong and Kim, 2004], and lipid metabolism [Hao et al., 2011a]. Park et al. [2010] revealed that the regulatory subunits of PI3K, p85α, and p85β form heterodimers that were disrupted by insulin treatment. This disruption of heterodimerization allows the resulting monomers of p85 to interact with the XBP-1s and increase the nuclear translocation. Another research showed a report that melanoma cells under ER stress were more resistant to apoptosis induced by the microtubule-targeting chemotherapeutic drugs, docetaxel and vincristine. And this is, at least in part, due to activation of the PI3K/Akt pathway mediated by the XBP-1 axis of the UPR [Jiang et al., 2009]. Although the exact mechanism involved in the relationship between PI3K/Akt pathway and XBP-1 splicing is still not fully elucidated. However, these past studies have surely shown that the regulation between PI3K/Akt pathway and XBP-1s really existed. Therefore, to determine whether PI3K/Akt pathway mediates high glucose-induced XBP-1s expression and cellular lipid accumulation, this pathway was inhibited by chemical inhibitor LY294002 and it can be detected that high glucose-induced XBP-1 expression was lowered and cellular lipid droplets decreased.

To further confirm the involvement of PI3K/Akt pathway in high glucose-regulated XBP-1 expression we silenced this pathway by shRNA plasmid aimed at Akt. It was expectedly found that blockade of PI3K/Akt pathway effectively prevented increased XBP-1, ADRP, and lipid droplet formation in HKC cells under stimulation of high glucose. These results further confirmed that PI3K/Akt pathway mediated high glucose-caused XBP-1s expression. However, the exact mechanism involved in phospho-Akt-caused XBP-1 splicing in high glucose-stimulated HKC cells is still elusive. Kurata et al.

[2011] found that interleukin-3 stimulation of hematopoietic cell line, BaF3 cells, enhanced XBP-1 promoter activity, and induced phosphorylation of the endoplasmic reticulum stress sensor protein IRE1, resulting in the increase in XBP-1s that activates UPR. When downstream signaling from interleukin-3 was blocked by LY294002, XBP-1s expression was down-regulated and IRE1 phosphorylation was suppressed. Considering IRE-1 is a key factor in regulating the splice of XBP-1, here, we speculate that PI3K/Akt pathway might mediate high glucose-induced XBP-1 splicing by affecting IRE-1 in HKC cells, however, the exact mechanism involved in the process needs further study.

In the end, the conclusion can be drawn that high glucose-induced lipid deposition in diabetic nephropathy was partly regulated by XBP-1s up-regulation. Furthermore, increase in XBP-1s was resulted from activation of PI3K/Akt pathway in high glucose-stimulated HKC cells.

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