Biochemical and Biophysical Research Communications 434 (2013) 670–675

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Albumin overload induces expression of hypoxia-inducible factor 1α and its target genes in HK-2 human renal proximal tubular cell line

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ARTICLE INFO

*Article history:*
Received 26 March 2013
Available online 12 April 2013

*Keywords:*
HIF-1α
Albumin
Renal proximal tubular cells
HK-2 cells
Proteinuria

ABSTRACT

The aim of this study was to investigate the effect of human serum albumin (HSA) overload on the expression of the transcription factor hypoxia-inducible factor-1α (HIF-1α) in human renal proximal tubular cell line HK-2. First, the cell viability and cytotoxic activity were examined to assess the cellular conditions in HK-2 cells with HSA treatment employed in this study. HSA treatment for 48 h decreased the cell viability and increased the leakage of lactate dehydrogenase (LDH) into the medium in a concentration-dependent manner, but the toxicity was relatively mild. Western Blot analysis revealed that HSA treatment induced the expression of HIF-1α protein in a concentration-dependent manner without a change in β-actin protein expression. Confocal microscopy analysis revealed that HIF-1α protein was predominantly localized in the nucleus but was also observed in the cytoplasm. The HIF-1 target gene mRNAs, glucose transporter 1 and glyceraldehyde 3-phosphate dehydrogenase, were up-regulated by HSA treatment, leading to the increases in the protein expression levels. In addition, the mRNA of HIF-1α was increased by HSA treatment. In conclusion, albumin loading induces HIF-1α in HK-2 cells, resulting in the increases in the expression of proteins of its target genes.



## 1. Introduction

Urinary protein is suggested to play a crucial role in progression of tubulointerstitial damage which is considered to be associated with end-stage renal disease [1–5]. Under normal renal function, a small amount of proteins filtered at the glomerulus are reabsorbed by renal proximal tubular cells [2,4]. On the other hand, in diseased kidneys, highly enhanced glomerular permeability to proteins leads to an increased concentration of proteins in the proximal tubular lumen [5]. When renal proximal tubular cells are exposed to excessive amounts of proteins, a variety of harmful responses are initiated in the proximal tubular cells [3,5,6]. The expression of various genes in the renal proximal tubular cells is reported to be up-regulated or down-regulated in proteinuria [7].

Several transcription factors, especially nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) which are linked to inflammatory and immune responses, are involved in the pathogenesis of tubulointerstitial damage with proteinuria [8–11]. As a consequence of proteinuria, tubular cells become apoptotic, leading to eventual tubulointerstitial fibrosis through some cell signaling pathways. NF-κB is reported to be activated by reactive oxygen and nitrogen species, which are important physiological regulators of redox processes [9]. It has been reported that exposure of proximal tubular cells to albumin increases the cellular hydrogen peroxide content, serving as a signal for the activation of NF-κB [10].

HIF-1, a basic helix–loop–helix transcription factor, is composed of an inducible α-subunit (HIF-1α) and a constitutive β-subunit (HIF-1β) [12,13]. Under normoxic conditions, HIF-1α is hydroxylated by prolyl hydroxylases and binds the von Hippel Lindau protein, leading to HIF-1α ubiquitination and degradation by the 26S proteasome. In hypoxia, the activity of the prolyl hydroxylases decreases, resulting in the prevention of HIF-1α binding to von Hippel Lindau protein. It stabilizes HIF-1α, which is followed by nuclear translocation, dimerization with HIF-1β and binding to hypoxia-response elements in the promoter of target genes such as glucose transporter 1 (GLUT1), erythropoietin and vascular endothelial growth factor (VEGF) [12–14]. Thus, HIF-1α is an important regulatory factor that allows individual cells to adapt to hypoxia. HIF-1 regulates >100 genes encoding metabolic enzymes, growth factors, and factors contributing to modulation of extracellular matrix.

The activation of HIF-1 signaling has been reported to ameliorate renal injury in various animal models of acute kidney injury and chronic kidney disease [15-21]. In contrast, it has been sug-

*Abbreviations:* Ang II, angiotensin II; AP-1, activator protein-1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLUT1, glucose transporter 1; HIF-1α, hypoxia-inducible factor-1α; HSA, human serum albumin; LDH, lactate dehydrogenase; NF-κB, nuclear factor-κB; VEGF, vascular endothelial growth factor.

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http://dx.doi.org/10.1016/j.bbrc.2013.03.140

gested that the increase in HIF-1 expression is associated with the development of tubulointerstitial inflammation and fibrosis, in which proteinuria may elicit pro-inflammatory and pro-fibrotic effects [22,23]. Previously, Katavetin et al. [24] reported that albumin attenuated the hypoxia-induced increase in the expression of VEGF mRNA and protein in immortalized rat proximal tubular cells. However, it remains to be fully clarified whether or not albumin overload modulates the expression of HIF-1α and its target genes. In this study, we investigated the effect of albumin treatment on the expression of HIF-1α and its target genes in HK-2 cells, a human proximal tubular cell line.

## 2. Materials and methods

### 2.1. Materials

Human serum albumin (HSA) and XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used in the experiments were commercial products of the highest purity available.

### 2.2. Cell culture

HK-2 cells were purchased from American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium/Nutrient Mixture F-12 Ham (Sigma–Aldrich) containing 10% fetal bovine serum (FBS) in an atmosphere of 5% $CO_2$–95% air at 37 °C, and were subcultured every 7 days using 0.02% EDTA and 0.05% trypsin. Fresh medium was replaced every 2 days, and the cells were subcultured every 7 days after seeding.

### 2.3. Trypan blue dye exclusion assay

Trypan blue dye exclusion assay was performed as follows. Briefly, HK-2 cells were cultured in medium containing 10% FBS. At 4 days after seeding, the medium was changed to serum-free medium, and the cells were maintained for further 24 h. Then, the cells were treated with 20 mg/ml HSA and further maintained for 48 h. After the incubation, the cells were detached from the wells by trypsinization, and then the cell suspension was centrifuged. The cell pellets were resupended and stained with trypan blue dye solution. The cell suspension was placed on the Burker–Turk counting chamber. We estimated that the cells that did not take up the dye were viable and those took the dye were non-viable. These viable and nonviable cells were counted under the light microscope.

### 2.4. XTT and LDH assays

In XTT assay, HK-2 cells were cultured in medium containing 10% FBS. At 4 days after seeding, the medium was changed to serum-free medium, and the cells were maintained for further 24 h. Then, the cells were treated with HSA at the indicated concentrations and maintained for 48 h. Then, medium containing HSA was replaced. After the cells were washed twice, 0.2 ml of 250 μM XTT solution containing 10 μM phenazine methosulfate was added to each dish, and then the cells were incubated for 30 min at 37 °C. After transfer of the reaction solution to a microplate well, the amount of orange formazan dye produced was quantitated using a spectrophotometric plate reader to measure absorbance at a wavelength of 490 nm. The reference absorbance (nonspecific readings) was measured at a wavelength of 650 nm.

In LDH assay, HK-2 cells were treated with HSA as described above. At 48 h after the treatment with HSA, LDH activity in the medium and the lysed cells was measured in the Cytotoxicity Detection Kit$^{plus}$ (LDH) (Roche Diagnostics GmbH, Mannheim, Germany) according to the instruction manual. The percentage of LDH released from the cells was calculated by determining the ratio of LDH in the medium compared to that in the lysed cells plus the culture medium.

### 2.5. Western blot analysis

The whole cell lysates from HK-2 cells were used for Western blot analysis, which was performed as described previously [25]. The primary antibodies used in this study were as follows: anti-HIF-1α rabbit antibody (Novus Biologicals, Littleton, CO) (1:250), anti-GLUT1 rabbit antibody (1:100) (Immuno-Biological Laboratories Co., Ltd., Takasaki, Japan), anti-GAPDH rabbit antibody (Sigma–Aldrich) (1:5000) and monoclonal anti-β-actin mouse antibody (Sigma–Aldrich) (1:2000). The corresponding secondary antibodies were as follows: anti-rabbit IgG linked with horseradish peroxidase (GE Healthcare, Buckinghamshire, England) (1:5000) and anti-mouse IgG linked with horseradish peroxidase (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD, USA) (1:5000).

### 2.6. Immunofluorescence

HK-2 cells were cultured in medium containing 10% FBS. At 4 days after seeding, the medium was changed to serum-free medium, and the cells were maintained for 24 h. At 5 days after seeding, the cells were treated without or with HSA and maintained for 48 h. Then, the cells were fixed with 4% paraformaldehyde for 10 min. The cells were washed twice, and then permeabilized with 0.1% Triton X-100 for 3 min. After the cells were blocked with 0.1% non-fat dry milkfor 60 min and washed three times, the cells were incubated with the anti-HIF-1α rabbit antibody (1:100) at room temperature for 60 min. The cells were washed three times, and then polyclonal swine anti-rabbit immunoglobulins/TRITC (Dako, Glostrup, Denmark) (1:30) was added, and the cells were incubated at room temperature for 60 min. After the cells were washed three times, 5 U/ml BODIPY®FL phallacidin (Life Technologies, Foster City, CA) was added, and the cells were incubated at room temperature for 20 min. After the cells were washed three times, 10 μM Hoechst33342 was added, and the cells were incubated at room temperature for 30 min. The cells were washed three times, and then mounted in Vectashield H-1000 (Vector Laboratories Inc., Burlingame, CA, USA), immunofluorescent signals were viewed using a confocal laser scanning fluorescent microscope (LSM510 invert, Carl Zeiss, Jena, Germany).

### 2.7. Real-time PCR

Real-time PCR was performed using SYBR Green Realtime PCR Master Mix (TOYOBO Co., Ltd., Osaka, Japan) as described previously [25]. The primer pair of HIF-1α sense and antisense (sense: 5′-GCTTTAACTTTGCTGGCCCCAGC-3′; antisense: 5′-GCAGGGTCAGCACTACTTCGAAG-3′) was specific for a 221-bp fragment of HIF-1α transcripts. The primer pair of GLUT1 sense and antisense (sense: 5′-ATACTCATGACCATCGCGCTAG-3′; antisense: 5′-AAAGAAGGCCACAAAGCCAAAG-3′) was specific for a 93-bp fragment of GLUT1 transcripts. The primer pair of GAPDH sense and antisense (sense: 5′-CCACCCATGGCAAATTCC-3′; antisense: 5′-TGGGATTTCCATTGATGACAA-3′) was specific for a 69-bp fragment of GAPDH transcripts. The primer pair of β-actin sense and antisense (sense: 5′-CCTGGCACCCAGCACAAT-3′; antisense: 5′-GCCGATCCACACGGAGTACT-3′) was specific for a 70-bp fragment of β-actin transcripts. The PCR conditions consisted of an initial denaturation at 95 °C

for 1 min, followed by amplification for 45 cycles of 5 s at 95 °C, 5 s at 60 °C, and 15 s at 72 °C. The threshold cycle (*Ct*) value for each mRNA was determined using the second derivative maximum method.

### 2.8. Data analysis

Statistically significant differences were determined by Student's *t*-test, or one way or two way analysis of variance with the Tukey–Kramer's test for post hoc analysis. A *P* value less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. Effect of HSA overload on cell viability in HK-2 cells

After HK-2 cells were treated at various concentrations of HSA for 48 h, cell viability and cytotoxicity were estimated. The trypan blue extrusion assay showed that the percentage of non-viable cells in the cells treated with HSA (20 mg/ml, 48 h) (15.9 ± 1.4% of total cells, $n = 4$) was significantly higher than that in control cells (5.6 ± 1.1% of total cells, $n = 4$). XTT assay showed that HSA decreased the cell viability in a concentration-dependent manner (Fig. 1A). A significant decrease in cell viability was detected at HSA concentration of 40 mg/ml, which reduced to 64.0% of control cells. In LDH assay, a significant increase in LDH leakage into the medium was observed at 5 mg/ml HSA or higher, but the increase remained constant over 20 mg/ml HSA (Fig. 1B).

### 3.2. HIF-1α protein expression in HK-2 cells overloaded with HSA

To investigate the effect of HSA overload on HIF-1α protein expression, Western blot analysis was performed. As shown in Fig. 2A and B, HIF-1α protein in HK-2 cell lysate was enhanced 3.6-fold by 20 mg/ml HSA treatment for 48 h, whereas no obvious changes in the expression of β-actin protein were observed. Next we examined the subcellular localization of HIF-1α protein by immunofluorescence with a confocal laser scanning microscope. In control cells, little HIF-1α protein expression was detected (Fig. 2C). When HK-2 cells were treated with 20 mg/ml HSA for 48 h, nuclear localization of HIF-1α protein was clearly observed (Fig. 2D).

### 3.3. Effect of HSA overload on mRNA and protein expression of HIF-1-target genes in HK-2 cells

Next, we investigated whether or not HSA overload induces the expression of mRNA and protein of HIF-1-target genes, GLUT1 and GAPDH. Real-time PCR analysis revealed that HSA treatment for 48 h increased the mRNA expression of GLUT1 and GAPDH in a concentration-dependent manner (Fig. 3A and B). In addition, Western blot analysis showed the increases in protein levels of GLUT1 and GAPDH in the lysates from HK-2 cells treated with HSA for 48 h (Fig. 3C and D).

### 3.4. Effect of HSA overload on HIF-1α mRNA in HK-2 cells

It has been reported that reactive oxygen species such as hydrogen peroxide induce the mRNA of HIF-1α [26]. In addition, albumin overload is shown to generate hydrogen peroxide in HK-2 cells [10]. Therefore, we examined whether or not HIF-1α mRNA level is affected by HSA treatment. As shown in Fig. 4A, HSA increased the mRNA level of HIF-1α in a time-dependent manner. Fig. 4B showed the maximum increase of HIF-1α mRNA at 20 mg/ml HSA when the cells were incubated with HSA at various concentrations for 48 h.

## 4. Discussion

The present study showed that albumin overload activated the expression of HIF-1α protein, leading to the increases in mRNAs and proteins of its target genes such as GLUT1 and GAPDH. First we examined whether or not albumin induces the cytotoxicity under treatment conditions employed in this study. When HK-2 cells were treated with 20 mg/ml albumin for 48 h, the percentage of dead cells stained with trypan blue was 15.9% of total cells, which was significantly higher than that of non-treated cells (5.6%), but it also showed that more than 80% of the albumin-treated cells were still viable. Shalamanova et al. [27] reported that the cell viability was more than 95% of the cells treated with 30 mg/ml albumin for 48 h. In XTT assay, treatment with 20 mg/ml albumin for 48 h decreased the cell viability by only 15.7%, but significant difference was detected at 40 mg/ml albumin (Fig. 1A). The leakage of LDH from the cells treated with 20 mg/ml albumin for 48 h was significantly higher than that from the cells without albumin, but the albumin-induced increase in LDH leakage remained around 15% over the control (Fig. 1B). Apoptotic DNA laddering was not detected in LLC-PK$_1$ cells treated with 20 mg/ml albumin for 48 h [28]. In Fig. 2D, there was no obvious apoptotic nuclear morphol-

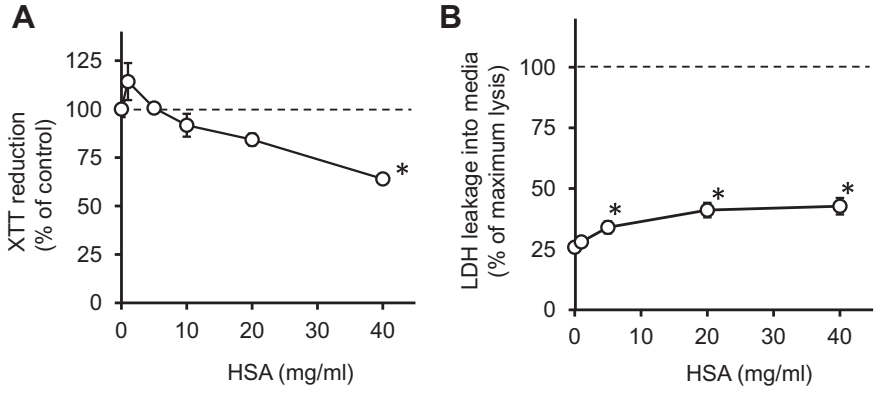


**Fig. 1.** Effect of human serum albumin (HSA) treatment on cell viability and cytotoxicity in HK-2 cells. At 4 days after seeding, HK-2 cells were cultured with serum-free medium for 24 h, and then the cells were treated with various concentrations of HSA for 48 h. Cell viability and cytotoxicity were measured by XTT (A) and LDH (B) assays, respectively. Values are expressed as the means ± SE of three monolayers. $^*p < 0.05$, significantly different compared with the value for the cells without HSA (0 mg/ml).

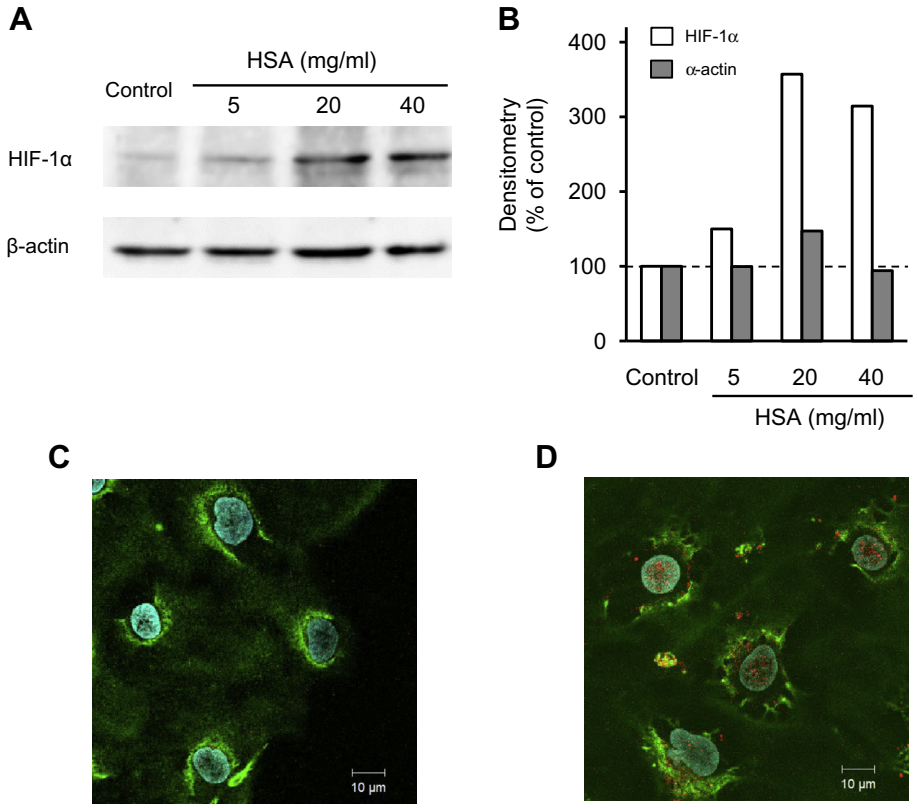


**Fig. 2.** Expression of HIF-1α protein in HK-2 cells treated without or with HSA. (A) Western blot analysis of HIF-1α and β-actin proteins was performed with cell lysates from HK-2 cells treated without (control) or various concentrations of HSA (5, 20 or 40 mg/ml) for 48 h. (B) Percent relative protein expression of HIF-1α (open column) and β-actin (gray column) to each control was calculated by densitometric analysis of bands. The immunofluorescence analysis was performed to examine the subcellular localization of HIF-1α protein in HK-2 cells treated without (C) or with (D) 20 mg/ml HSA for 48 h. Confocal microscopic images show HIF-1α (red), nucleus (Hoechst33342, blue) and F-actin (BODIPY® FL phallacidin, green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ogy such as chromatin condensation and nuclear fragmentation in Hoechst 33342-stained cells treated with 20 mg/ml albumin for 48 h. Thus, it is likely that albumin treatment conditions employed in this study induce relatively mild cytotoxicity.

Under the above-mentioned treatment conditions, we found that albumin treatment enhanced the expression of HIF-1α protein in HK-2 cells. The expression of HIF-1α protein has been reported to be regulated not only in an oxygen-dependent but also in an oxygen-independent manner. Zelzer et al. [29] found that insulin and insulin-like growth factor-I induced the formation of HIF-1α/aryl hydrocarbon receptor nuclear translocator (HIF-1α/HIF-1β) transcription complex in HepG2 cells, followed by the inductions of HIF-1-target genes such as GLUT1 and VEGF. Richard et al. [30] found that angiotensin II (Ang II), thrombin and platelet-derived growth factor increased HIF-1α protein in rat vascular smooth muscle cells, independently of the oxygen environment. In addition, the induction of HIF-1α protein was blocked by reactive oxygen species inhibitors. Interestingly, angiotensin-converting enzyme and angiotensinogen were up-regulated in the kidney of rats with intense proteinuria, showing an increase in Ang II generation in the kidney [31]. In addition, albumin treatment was reported to increase Ang II production in HK-2 cells in a concentration- and time-dependent manner [32]. Therefore, Ang II might be involved in the albumin-induced increase in the expression of HIF-1α protein in HK-2 cells.

Immunofluorescence study revealed that albumin treatment enhanced the expression of HIF-1α protein in the nucleus of HK-2 cells. This observation might indicate the activation of HIF-1-dependent transcriptional activity in the HK-2 cells treated with albumin. Therefore, we examined the changes in the expression of HIF-1-target genes, GLUT1 and GAPDH. As expected, the mRNA levels of these two target genes were increased by albumin treatment in a concentration-dependent manner. Furthermore, Western blot analysis revealed the albumin-concentration-dependent increase in the protein levels of GLUT1 and GAPDH. Though these two proteins play an important role in glycolytic pathway, the reasons why these proteins are enhanced by albumin-overloaded cells are unclear at present. Recently, GAPDH is suggested to exhibit a variety of non-glycolytic functions including regulation of intracellular membrane trafficking, receptor-medicated cell signaling and apoptosis [33,34]. Therefore, the increase in GAPDH protein in albumin-treated HK-2 cells might be responsible for induction of cytotoxicity and/or adaptation to the albumin-overload conditions though further studies are needed.

The levels of not only protein but also mRNA of HIF-1α were increased by albumin treatment in HK-2 cells. This finding might indicate the possibility that the albumin-induced increase in HIF-1α protein is, at least in part, due to a change in its mRNA level. Bonello et al. [26] found that $H_2O_2$ and reactive oxygen species produced by thrombin treatment increased the expression levels of HIF-1α mRNA and protein. Furthermore, the HIF-1α promoter element -197/-188, to which the NF-κB subunits p50 and p60 bind, has been suggested to be responsible for transcriptional activation of the HIF-1α gene by $H_2O_2$ and thrombin [35]. It has been well known that exposure to excess proteins including albumin induces the formation of reactive oxygen species [10,36,37] as well as NF-

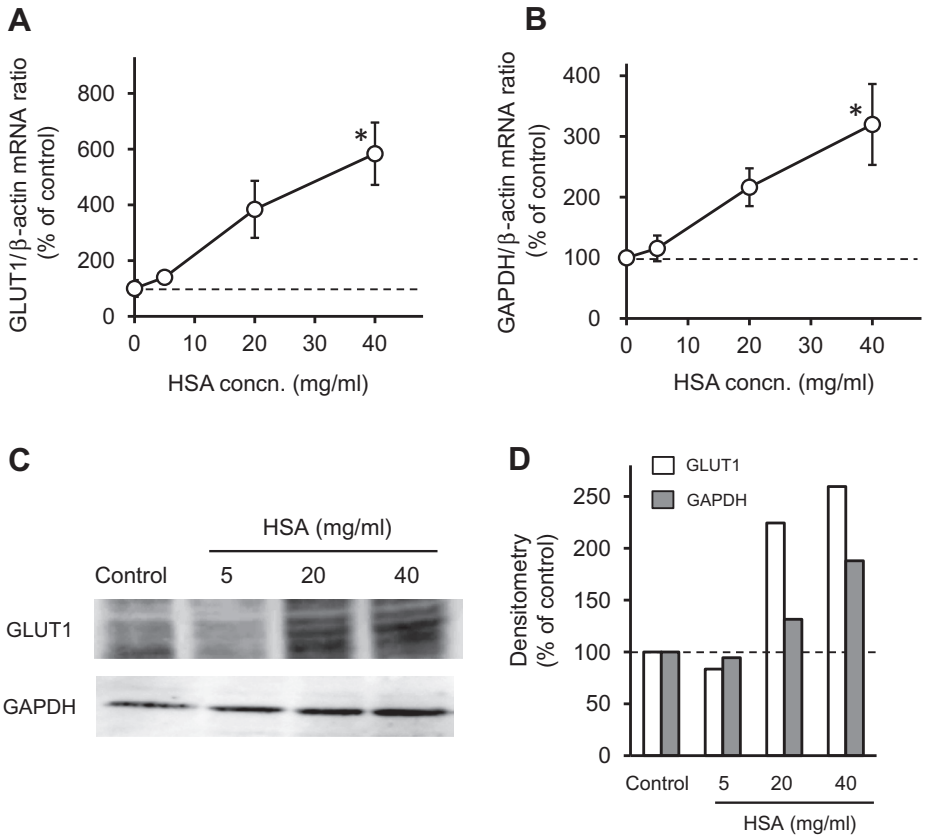


**Fig. 3.** Expression of mRNAs and proteins of HIF-1 target genes GLUT1 and GAPDH in HK-2 cells treated without or with HSA. GLUT1 (A) and GAPDH (B) mRNA levels were measured by real-time PCR analysis in HK-2 cells treated without or with various concentrations of HSA (5, 20 or 40 mg/ml) for 48 h. Relative level of each target gene mRNA was determined after normalization to β-actin mRNA. The mRNA levels without HSA (0 mg/ml) were set to 100%. Values are expressed as the means ± SE of three monolayers. $^{*}p < 0.05$, significantly different compared with the value for the cells without HSA (0 mg/ml). (C) Western blot analysis of GLUT1 and GAPDH proteins was performed with cell lysates from HK-2 cells treated without (0 mg/ml) or various concentrations of HSA (5, 20 or 40 mg/ml) for 48 h. (D) Percent relative protein expression of GLUT1 (open column) or GAPDH (gray column) to each control was calculated by densitometric analysis of bands.

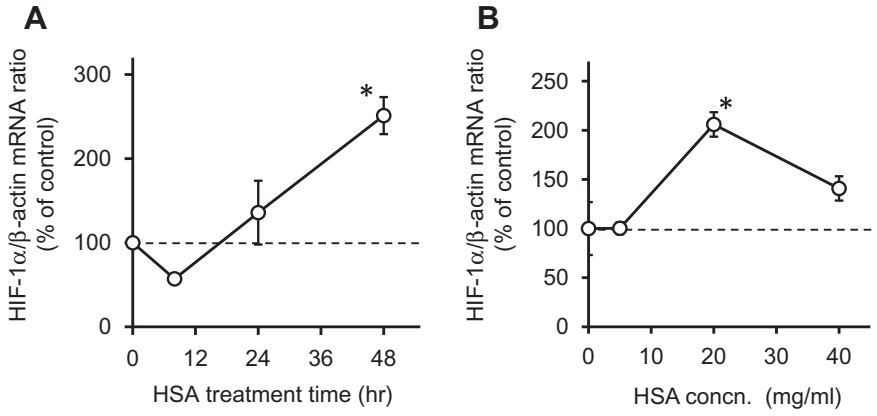


**Fig. 4.** Expression of HIF-1α mRNA in HK-2 cells treated without or with HSA. (A) HIF-1α mRNA level was measured by real-time PCR analysis in HK-2 cells treated with 20 mg/ml HSA for different incubation periods (0, 8, 24 or 48 h). The mRNA levels treated without HSA (0 h) were set to 100%. (B) HIF-1α mRNA level was measured by real-time PCR analysis in HK-2 cells treated without (0 mg/ml) or with various concentrations of HSA (5, 20 or 40 mg/ml) for 48 h. Relative level of HIF-1α mRNA was determined after normalization to β-actin mRNA. The mRNA levels without HSA (0 mg/ml) were set to 100%. Values are expressed as the means ± SE of three monolayers. $^{*}p < 0.05$, significantly different compared with the value for the cells without HSA.

κB activation [8,10] in renal proximal tubular cells. Therefore, reactive oxygen species-induced NF-κB activation might, at least in part, contribute to the increase in HIF-1α mRNA in HK-2 cells.

In conclusion, we found that albumin overload induced the expression of HIF-1α mRNA and protein. In addition, the HIF-1 target genes such as GLUT1 and GAPDH mRNAs were enhanced by albumin overload, followed by increases in the protein levels. These observations might indicate that HIF-1α plays an important role in changes in various genes in renal proximal tubular cells which are observed under proteinuria.

## Acknowledgments

We thank the Analysis Center of Life Science, Natural Science Center for Basic Research and Development, Hiroshima University, for the use of their facilities. This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

## References

[1] M. Abbate, C. Zoja, G. Remuzzi, How does proteinuria cause progressive renal damage?, J Am. Soc. Nephrol. 17 (2006) 2974–2984.
[2] E.I. Christensen, J. Gburek, Protein reabsorption in renal proximal tubule-function and dysfunction in kidney pathophysiology, Pediatr. Nephrol. 19 (2004) 714–721.
[3] A.A. Eddy, Proteinuria and interstitial injury, Nephrol. Dial. Transplant. 19 (2004) 277–281.
[4] H. Birn, E.I. Christensen, Renal albumin absorption in physiology and pathology, Kidney Int. 69 (2006) 440–449.
[5] R. Nielsen, E.I. Christensen, Proteinuria and events beyond the slit, Pediatr. Nephrol. 25 (2010) 813–822.
[6] R.J. Baines, N.J. Brunskill, Tubular toxicity of proteinuria, Nat. Rev. Nephrol. 7 (2011) 177–180.
[7] H. Nakajima, M. Takenaka, J.Y. Kaimori, et al., Gene expression profile of renal proximal tubules regulated by proteinuria, Kidney Int. 61 (2002) 1577–1587.
[8] C. Zoja, R. Donadelli, S. Colleoni, et al., Protein overload stimulates RANTES production by proximal tubular cells depending on NF-kappa B activation, Kidney Int. 53 (1998) 1608–1615.
[9] C. Guijarro, J. Egido, Transcription factor-κB (NF-κB) and renal disease, Kidney Int. 59 (2001) 415–424.
[10] M. Morigi, D. Macconi, C. Zoja, et al., Protein overload-induced NF-κB activation in proximal tubular cells requires $H_2O_2$ through a PKC-dependent pathway, J. Am. Soc. Nephrol. 13 (2002) 1179–1189.
[11] J.E. Baek, W.S. Yang, J.W. Chang, et al., Fatty acid-bearing albumin induces VCAM-1 expression through c-Src kinase-AP-1/NF-κB pathways: effect of L-carnitine, Kidney Blood Press Res. 33 (2010) 72–84.
[12] G.L. Semenza, Oxygen sensing, homeostasis, and disease, N. Engl. J. Med. 365 (2011) 537–547.
[13] Q. Ke, M. Costa, Hypoxia-inducible factor-1 (HIF-1), Mol. Pharmacol. 70 (2006) 1469–1480.
[14] V.H. Haase, Hypoxia-inducible factors in the kidney, Am. J. Physiol. Renal Physiol. 291 (2006) F271–F281.
[15] A. Weidemann, W.M. Bernhardt, B. Klanke, et al., HIF activation protects from acute kidney injury, J. Am. Soc. Nephrol. 19 (2008) 486–494.
[16] S. Ohtomo, M. Nangaku, Y. Izuhara, et al., Cobalt ameliorates renal injury in an obese, hypertensive type 2 diabetes rat model, Nephrol. Dial. Transplant. 23 (2008) 1166–1172.
[17] P. Hill, D. Shukla, M.G. Tran, et al., Inhibition of hypoxia inducible factor hydroxylases protects against renal ischemia-reperfusion injury, J. Am. Soc. Nephrol. 19 (2008) 39–46.
[18] Y.R. Song, S.J. You, Y.M. Lee, et al., Activation of hypoxia-inducible factor attenuates renal injury in rat remnant kidney, Nephrol. Dial. Transplant. 25 (2010) 77–85.
[19] A. Deng, M.A. Arndt, J. Satriano, et al., Renal protection in chronic kidney disease: hypoxia-inducible factor activation vs. angiotensin II blockade, Am. J. Physiol. Renal Physiol. 299 (2010) F1365–F1373.
[20] H. Oka, H. Goto, K. Koizumi, et al., Effect of hachimijiogan against renal dysfunction and involvement of hypoxia-inducible factor-1α in the remnant kidney model, Evid. Based Complement. Alternat. Med. 2011 (2011) 348686.
[21] J.M. Ahn, S.J. You, Y.M. Lee, et al., Hypoxia-inducible factor activation protects the kidney from gentamicin-induced acute injury, PLoS One 7 (2012) e48952.
[22] D.F. Higgins, M.P. Biju, Y. Akai, et al., Hypoxic induction of Ctgf is directly mediated by Hif-1, Am. J. Physiol. Renal Physiol. 287 (2004) F1223–F1232.
[23] K. Kimura, M. Iwano, D.F. Higgins, et al., Stable expression of HIF-1alpha in tubular epithelial cells promotes interstitial fibrosis, Am. J. Physiol. Renal Physiol. 295 (2008) F1023–F1029.
[24] P. Katavetin, R. Inagi, T. Miyata, et al., Albumin suppresses vascular endothelial growth factor via alteration of hypoxia-inducible factor/hypoxia-responsive element pathway, Biochem. Biophys. Res. Commun. 367 (2008) 305–310.
[25] T. Sawada, J. Nagai, Y. Okada, et al., Gadolinium modulates gentamicin uptake via an endocytosis-independent pathway in HK-2 human renal proximal tubular cell line, Eur. J. Pharmacol. 684 (2012) 146–153.
[26] S. Bonello, C. Zähringer, R.S. BelAiba, et al., Reactive oxygen species activate the HIF-1α promoter via a functional NFκB site, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 755–761.
[27] L. Shalamanova, F. McArdle, A.B. Amara, et al., Albumin overload induces adaptive responses in human proximal tubular cells through oxidative stress but not via angiotensin II type 1 receptor, Am. J. Physiol. Renal Physiol. 292 (2007) F1846–F1857.
[28] E. Erkan, M. De Leon, P. Devarajan, Albumin overload induces apoptosis in LLC-$PK_1$ cells, Am. J. Physiol. Renal Physiol. 280 (2001) F1107–F1114.
[29] E. Zelzer, Y. Levy, C. Kahana, et al., Insulin induces transcription of target genes through the hypoxia-inducible factor HIF-1alpha/ARNT, EMBO J. 17 (1998) 5085–5094.
[30] D.E. Richard, E. Berra, J. Pouyssegur, Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1alpha in vascular smooth muscle cells, J. Biol. Chem. 275 (2000) 26765–26771.
[31] R. Largo, D. Gómez-Garre, K. Soto, et al., Angiotensin-converting enzyme is upregulated in the proximal tubules of rats with intense proteinuria, Hypertension 33 (1999) 732–739.
[32] B.C. Liu, J. Gao, Q. Li, et al., Albumin caused the increasing production of angiotensin II due to the dysregulation of ACE/ACE2 expression in HK2 cells, Clin. Chim. Acta 403 (2009) 23–30.
[33] A.V. Bryksin, P.P. Laktionov, Role of glyceraldehyde-3-phosphate dehydrogenase in vesicular transport from golgi apparatus to endoplasmic reticulum, Biochemistry (Moscow) 73 (2008) 619–625.
[34] M.A. Sirover, On the functional diversity of glyceraldehyde-3-phosphate dehydrogenase: biochemical mechanisms and regulatory control, Biochim. Biophys. Acta 2011 (1810) 741–751.
[35] R.S. Belaiba, S. Bonello, C. Zähringer, et al., Hypoxia up-regulates hypoxia-inducible factor-1α transcription by involving phosphatidylinositol 3-kinase and nuclear factor κB in pulmonary artery smooth muscle cells, Mol. Biol. Cell. 18 (2007) 4691–4697.
[36] S. Tang, J.C. Leung, K. Abe, et al., Albumin stimulates interleukin-8 expression in proximal tubular epithelial cells in vitro and in vivo, J. Clin. Invest. 111 (2003) 515–527.
[37] H. Nakajima, M. Takenaka, J.Y. Kaimori, et al., Activation of the signal transducer and activator of transcription signaling pathway in renal proximal tubular cells by albumin, J. Am. Soc. Nephrol. 15 (2004) 276–285.