Original Paper

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2009;24:577-584

Accepted: August 11, 2009

The Serum and Glucocorticoid-Regulated Kinase 1 in Hypoxic Renal Injury

Krisztina Rusai[1,2], Bettina Wagner[2], Marcel Roos[2], Christoph Schmaderer[2], Matthias Strobl[2], Krishna M. Boini[3], Almut Grenz[4,6], Dietmar Kuhl[5], Uwe Heemann[2], Florian Lang[3] and Jens Lutz[2]

[1]First Department of Pediatrics, Semmelweis University, Budapest, [2]Department of Nephrology, Klinikum rechts der Isar, Munich, [3,4]Departments of [3]Physiology and [4]Pharmacology, University of Tübingen, [5]Department of Biology, Chemistry, and Pharmacy, Free University Berlin, [6]Mucosal Inflammation Program, Department of Anesthesiology and Perioperative Medicine, University of Colorado Health Sciences Center, Denver

Key Words

Apoptosis • Hypoxia • Ischemia/reperfusion • Kidney • SGK1

Abstract

The serum- and glucocorticoid-inducible kinase 1 (SGK1) is a serine threonine protein kinase activated through the phosphatidylinositol 3-kinase (PI3-kinase) pathway and counteracting apoptosis. Protein expression and activation of SGK1 are increased in various models of cell stress. The present study explored the role of SGK1 in renal hypoxia/ischemia induced apoptosis. HEK 293 cells were exposed *in vitro* to hypoxia/reoxygenation (H/R), which increased SGK1 transcript levels, SGK1 protein abundance and SGK1 phosphorylation. H/R injury further enhanced the percentage of apoptotic cells, an effect significantly blunted by prior SGK1 overexpression. *In vivo* renal ischemia/reperfusion (I/R) injury increased SGK1 transcript levels and SGK1 protein abundance. I/R enhanced apoptosis, an effect significantly more pronounced in gene targeted mice lacking SGK1. In conclusion, SGK1 is up-regulated and counteracts apoptosis following H/R *in vitro* and ischemia *in vivo.*

Introduction

Ischemia/reperfusion (I/R) injury is among the leading causes of morbidity and mortality in a variety of disorders such as acute renal failure, heart failure, stroke and graft dysfunction after transplantation [1]. Apoptotic cell death has been recognized as a major mode of cell demise in I/R injury. Thus, the balance between the activation of pro- and anti-apoptotic pathways in the postischemic tissue plays a critical role in organ dysfunction [1].

Apoptosis during ischemia-induced renal injury is opposed by the action of the serine/threonine kinase PKB/Akt [2-4]. PKB/Akt is 45-55% homologous to and shares a variety of targets with the serum- and glucocorticoid-inducible protein kinase 1 (SGK1) [5], a serine/threonine kinase initially identified as a gene under transcriptional control of glucocorticoids and serum [6-9]. SGK1 was later shown to be regulated by a wide variety of hormones and transmitters [10]. Moreover, *in vitro* expression of SGK1 is increased by various types of cell stress, such as hyperosmotic shock, ultraviolet radiation, heat shock, oxidative stress, hyperglycemia and hypoxia [11-13]. SGK1 gene expression *in vivo* has been dem-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015-8987/09/0246-0577$26.00/0

Accessible online at:
www.karger.com/cpb

Prof. Dr. Florian Lang
Physiologisches Institut, Universität Tübingen
Gmelinstr. 5, 72076 Tübingen (Germany)
Tel. +49 7071 29 72194, Fax +49 7071 29 5618
E-Mail florian.lang@uni-tuebingen.de

onstrated to be up-regulated by dehydration [14] and cerebral ischemia [15]. Similar to PKB/Akt, SGK1 is activated through a signalling cascade involving phosphatidylinositol 3-kinase (PI3-kinase) and phosphoinositide-dependent kinase 1 (PDK1) [16]. SGK1 may inhibit apoptosis by phosphorylation of the forkhead transcription factor FKHLR1 or Foxo3a [17, 18] activation of IκB kinase [19], and/or inhibition of B-Raf [20].

Little is known about activation and role of SGK1 in renal hypoxic injury. The present study thus explored the expression and role of SGK1 in hypoxia and renal ischemia.

Materials and Methods

Cell culture

The human embryonic kidney cell line (HEK 293) was purchased from the American Type Culture Collection (ATCC, Rockville, MD). A HEK 293 SGK1 stable cell line was created by neomycin selection after plasmid-mediated transfection with Lipofectamin 2000 (Invitrogen GmBH, Karlsruhe, Germany). All cells were cultured in DMEM supplemented with 10% heat inactive fetal bovine serum (FBS) (PAN Biotech GmbH,) and were propagated at 37°C in a humified air containing 5% CO_2 incubator.

In vitro hypoxia and reoxygenization

HEK 293 cells were seeded out and plated on 10 cm plates at $5x10^5$/ml 24 h before induction of hypoxia. For hypoxia the medium was changed to serum- and glucose-free DMEM saturated with 95% N_2/5% CO_2 and cells were placed in a 37°C airtight box for 16 h. For normoxic conditions, culture medium was changed to serum- and glucose-free DMEM and cells placed in a 37°C/5% CO_2 incubator for 16 h. After 16 h, medium was replaced with normal medium and all cells were placed in a 37°C/5% CO_2 incubator for reoxygenization. After 24 h of reoxygenization cells were analyzed.

Expression vector

To analyze the role of SGK1, we investigated the impact of SGK1 overexpression by stable SGK1 transfection on *hypoxic* injury in HEK 293 cells. The plasmid pBK-CMV-sgk1 was a generous gift from Dr. Gary L. Firestone. The expression vector pcDNA3.1(-)sgk1 was constructed by clipping sgk1 from pBK-CMV-sgk1 and inserting it into the EcoRV site in the pcDNA3.1(-) vector after filling up sticky ends with Klenow fragments (Fermentas Life Sciences, St. Leon-Rot, Germany).

Annexin V binding

An annexin V-PI apoptosis kit was used according to the manufacturer's protocol to detect apoptosis *in vitro* (Caltag Laboratories, Karlsruhe, Germany). In brief, cells were washed with phosphate buffered saline and stained with fluorescein labelled annexin V and propidium-iodide (PI) and then analyzed by flow cytometry. This assay takes advantage of the fact that phosphatidylserine (PS) is translocated from the inner (cytoplasmic) layer of the plasma membrane to the outer (cell surface) layer soon after the induction of apoptosis, and that the annexin V protein has a strong, specific affinity for PS. Detection of annexin V binding is a widely used experimental method in the literature to assess apoptotic cell death [20-23].

XTT analysis

To analyze the number of viable cells, a colorimetric XTT Cell Proliferation Kit II assay was employed according to the manufacturer's protocol (Roche GmbH, Mannheim, Germany). In brief, the cleavage of the yellow tetrazolium salt XTT by the viable, metabolically active cells was recorded on an ELISA plate reader by measuring the absorbance.

LDH release

Release of LDH from cells into the supernatant is a marker for an increased permeability of the cell membrane as a sign of cell death [24]. LDH levels were analyzed by an automatic laboratory analyzer (Synchron CX5, Beckman Coulter, Krefeld, Germany).

Animal experiments

In the first series of experiments male Sprague-Dawley rats were subjected to isoflurane anaesthesia and the abdomen was opened through a midline incision and the left kidney prepared together with the renal artery and vein. Left renal artery and vein were clipped by an atraumatic vascular clamp for 45 min, during which right nephrectomy was performed. After 45 min of ischemia, the clamp was removed and the animals were allowed to recover. After 2h, 6h, 24h, 3 days and 7 days of reperfusion (N=7/group), rats were re-anaesthetized and the remnant kidney was removed. Sham-operated right nephrectomized rats served as controls.

In a second series of experiments SGK1 knockout mice ($sgk1^{-/-}$) and their wild-type littermates ($sgk1^{+/+}$) maintained on a 129S1/SvImJ background were generated as described elsewhere [25]. In mice ischemia of the left kidney was induced by clamping of the left renal artery for 30 min using a hanging weight system followed by right nephrectomy as previously described [26]. At the end of surgery, mice received 0.3 ml normal saline intraperitoneally and were allowed to recover for 2 h under a heating lamp. Mice were killed after 24 h of observation, the kidneys were harvested and stored at -80°C until further analysis. Principles of NIH Guide for the Care and Use of Laboratory Animals as well as the German Law on the Protection of Animals were followed. The experimental protocols were approved by the respective authorities.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)

TUNEL analysis [27] was performed on frozen sections fixed in paraformaldehyde 4%. Sections were incubated with Triton 0.1% and sodium citrate 0.1% at 4°C, washed and incubated with TUNEL solution containing terminal deoxynucleotidyltransferase (Boehringer Mannheim, Germany). Sections were washed with stop/wash buffer

Table 1. Primer pairs for RT-PCR analysis.

	primer pairs	annealing temperature (°C)	product length (bp)
SGK1 (human)	Forward: 5'-CTGTGGCACGCCGGAGTATCT-3' Reverse: 5'-GACGCTGGCTGTGACGAGGAC-3'	58	267
SGK1 (rat)	Forward: 5'-TGCTCTATGGCCTGCCTCCGTTCT-3' Reverse: 5'-GTCACTGGGCCCGCTCACATTTG-3'	59	476
GAPDH	Forward: 5'-GGTGAAGGTCGGAGTCAACG-3' Reverse: 5'-CAAAGTTGTCATGGATGACC-3'	56	498

followed by washing and incubation with a rabbit antidigoxygenine antibody (Boehringer Mannheim, Germany). Antibody binding was visualized using fast red chromogene solution. Positive controls were treated with DNAseI and processed as described above. Negative controls were incubated with PBS instead of TUNEL solution. Sections were counterstained with hematoxylin. All positive tubular epithelial cells in each section were counted at a magnification of 40x and related to the number of view fields per section.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from the cells and kidney samples by RNeasy Total RNA Isolation Kit (Qiagen GmbH, Hilden, Germany) according to the instructions of the manufacturer. The quality and quantity of the RNA were photometrically confirmed. Human and rat SGK1, as well as GAPDH mRNA expression in the cells and renal tissue was determined using semi-quantitative RT-PCR as described previously [28]. After initial denaturation at 94ºC, 38 cycles of amplification at the accurate annealing temperature for human, rat SGK1 and GAPDH were performed. The primer sequences and annealing temperatures are shown in Table 1. PCR products were separated on ethidium-bromide stained 2.5% agarose gels, then visualized and photographed under ultraviolet light. Signals were quantified by densitometry and corrected for the GAPDH signal using an image analysis software program (Gel-Pro Analyser 3.1 Software).

Western blotting

To study the involvement of SGK1 in hypoxia and ischemia, we measured the protein expression and activation (phosphorylation) of SGK1 after hypoxia in the cells as well as in kidney tissue after ischemia/reperfusion injury. Cells and kidney tissues were homogenized with a cell disrupter in a lysis buffer containing 1 mg/ml approtinine, 5 mg/ml leupeptin, 1 M Tris, 0.5 M EGTA, 0.25 M NaF, 0.5 M PMSF, 0.5 M Na_3VO_4 and 0.1% Triton X-100. The lysate was centrifuged at 13.000 g for 10 min to remove insoluble debris. Protein concentrations in the supernatant were determined by the Bradford method using BSA as standard. All reagents for PAGE and Western blot were purchased from Sigma Aldrich (Munich, Germany). Lysates were solubilized in a buffer of 12.5 mM Tris-HCl, pH 6.7, containig 4.0% SDS, 1 mM EDTA, 15% glycerol and 0.01% bromphenolblue. 50 µg protein of the samples was separated on 10% SDS-polyacrylamide gel and transferred to nitrocellulose membranes (Amersham, GE Healthcare Europe GmbH,

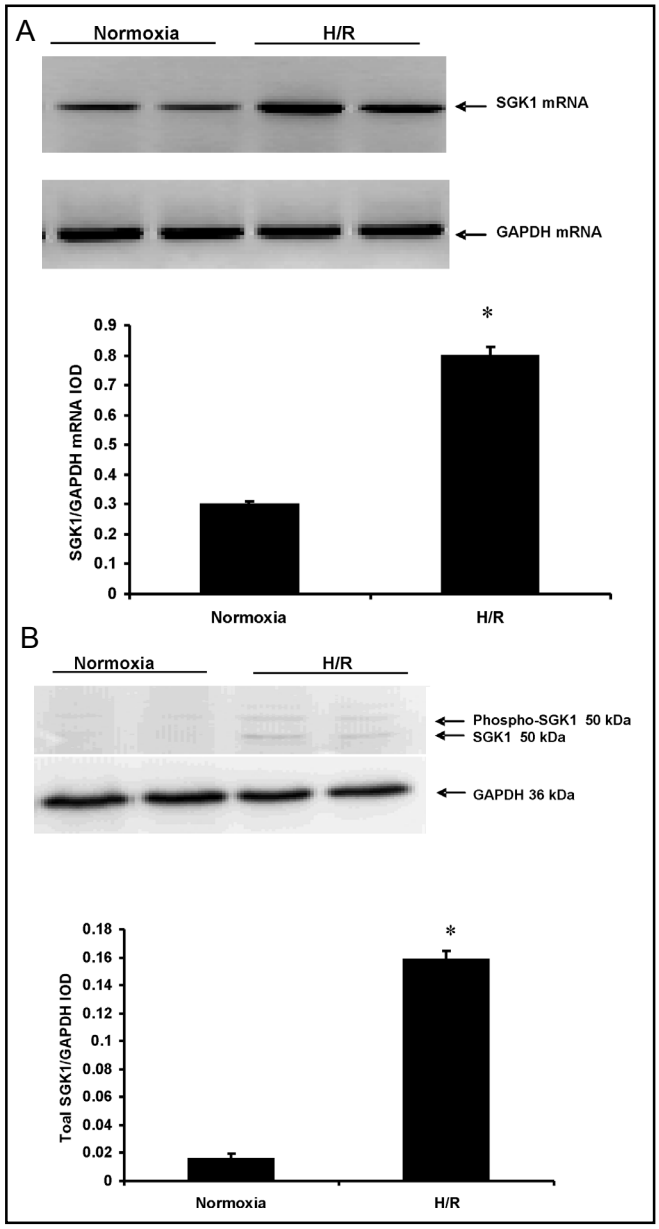

Fig. 1. Increase of SGK1 transcript levels and protein following hypoxia/reoxygenization (H/R). Abundance of SGK1 mRNA (A) and protein (B) expression in normoxia and following H/R injury for 16/24 h in vitro. (*P <0.01 vs. normoxic cells). The original blots are representative for three independent experiments.

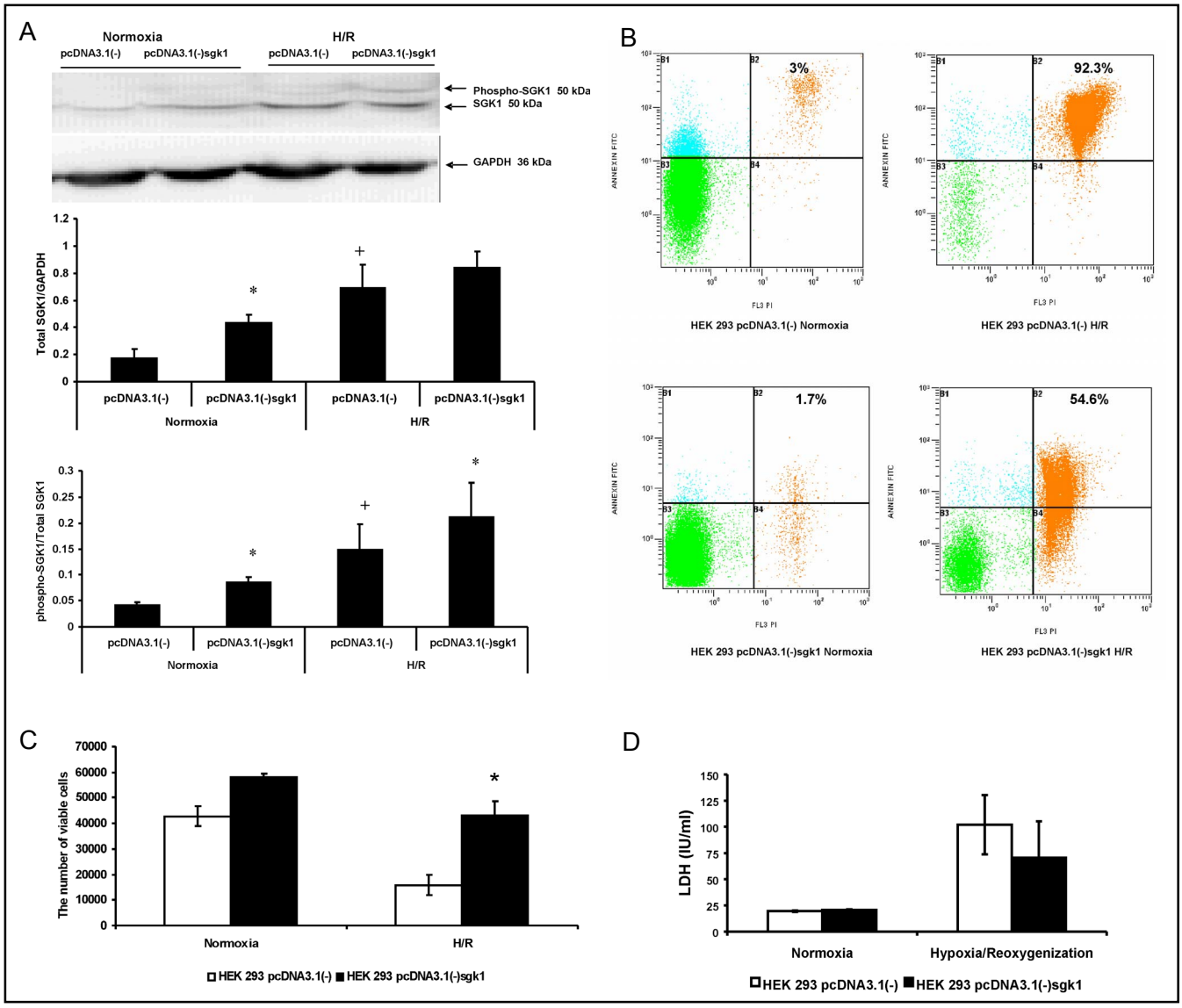

Fig. 2. Influence of SGK1 overexpression on apoptosis following hypoxia/reoxygenization. The effect of SGK1 overexpression in HEK 293 cells on apoptotic cell death induced by H/R (16/24 h) injury. A. SGK1 expression and phosphorylation were measured by Western blot in HEK 293 cells stably transfected with pcDNA3.1(-)sgk1 and control transfected cells with pcDNA3.1(-) under normoxic conditions and after H/R. (*P<0.05 vs. control transfected cells, + P<0.05 vs. normoxia) B. SGK1 overexpression blunted the increase of annexin V staining reflecting apoptosis in HEK 293 cells following H/R injury (*P<0.01 vs. control transfected cells). C. SGK1 overexpression increased overall cell viability measured by XTT analysis after H/R injury (*P<0.05 vs. control transfected cells). D. SGK1 overexpression did not change the release of LDH following H/R injury (P=0.149 vs. control transfected cells).

Munich, Germany). The membranes were blocked with 5% non-fat dry milk for 1 h at room temperature and then probed with rabbit polyclonal anti-SGK1 antibody (1:400, Upstate, Germany) in 5% NFDM overnight at 4°C. The membranes were washed with Tween-phosphate-buffered saline, and incubated with anti-rabbit immunoglobulin antibody, a horseradish peroxidase conjugated from goat (1:5000, Amersham) in 5% non-fat dry milk for 1 h at room temperature. Proteins were detected by enhanced chemiluminescence (ECL, Amersham) by Kodak Image Station 400 D. Prestained standards were used as molecular weight markers (Invitrogen). Signals on Western blots were quantified by densitometry using Gel-Pro Analyser 3.1 Software.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Data were tested using the Chi-Square- or Mann-Whitney U test using the SPSS statistical software package (v. 13.0, SPSS GmbH, Munich, Germany). A P value of <0.05 was considered to be significant.

Results

In vitro experiments

SGK1 expression after in vitro hypoxia/reoxygenization. In HEK 293 cells hypoxia/reoxygenization (H/R) induced both mRNA and protein expression as well as the phosphorylation of SGK1 (Figure 1A-B) (P <0.01 vs. normoxic cells).

The effect of SGK1 on apoptosis after hypoxia/reoxygenization. We examined the effects of SGK1 overexpression in HEK 293 cells on the apoptosis of cells after H/R. Stable transfection of cells with pcDNA3.1(-)sgk1 led to a significant overexpression of SGK1 (P<0.05 vs. control transfected cells) (Figure 2A.) detected by Western blot analysis. After H/R, the rate of phosphorylation was also significantly higher in the pcDNA3.1(-)sgk1 transfected cells versus control transfected cells (P<0.05).

After H/R apoptotic cell death was determined by annexin V staining, while cell viability was analyzed by XTT analysis and the level of lactate dehydrogenase (LDH) in the culture medium was measured. H/R was followed by a significant increase in apoptotic cell death detected by annexin V and XTT analysis (Figure 2B). Annexin V staining revealed that SGK1 overexpression significantly (P<0.01 vs. control transfected cells) protected the cells from apoptosis after H/R (Figure 2B). These changes correlated well with a reduction in overall cell viability (XTT) (P<0.05 vs. control transfected cells) (Figure 2C). LDH release also tended to be reduced after SGK1 overexpression, a change, however, not reading statistical significance (Figure 2D).

In vivo experiments

SGK1 expression in renal ischemia/reperfusion injury. In order to analyze the expression pattern of SGK1 in renal ischemia/reperfusion (I/R) injury, we measured SGK1 mRNA and protein expression, as well as SGK1 phosphorylation in a rat model of the kindey I/R injury. SGK1 expression could be detected in the kidneys of sham operated animals. SGK1 mRNA expression increased already 2 h after ischemia and was still elevated at 6 h of reperfusion time (P<0.05 vs. sham). Subsequently, the SGK1 transcript levels declined and were not significantly different from control values 24 h, 3 days and 7 days after reperfusion (Figure 3A).

Abundance of total and phosphorylated SGK1 protein increased significantly 6 h after ischemia (P<0.05 vs. sham). Similar to the transcript levels, the abundance of total and phosphorylated SGK1 protein was again close to sham levels 24 h, 3 and 7 days after I/R injury (Figure 3B).

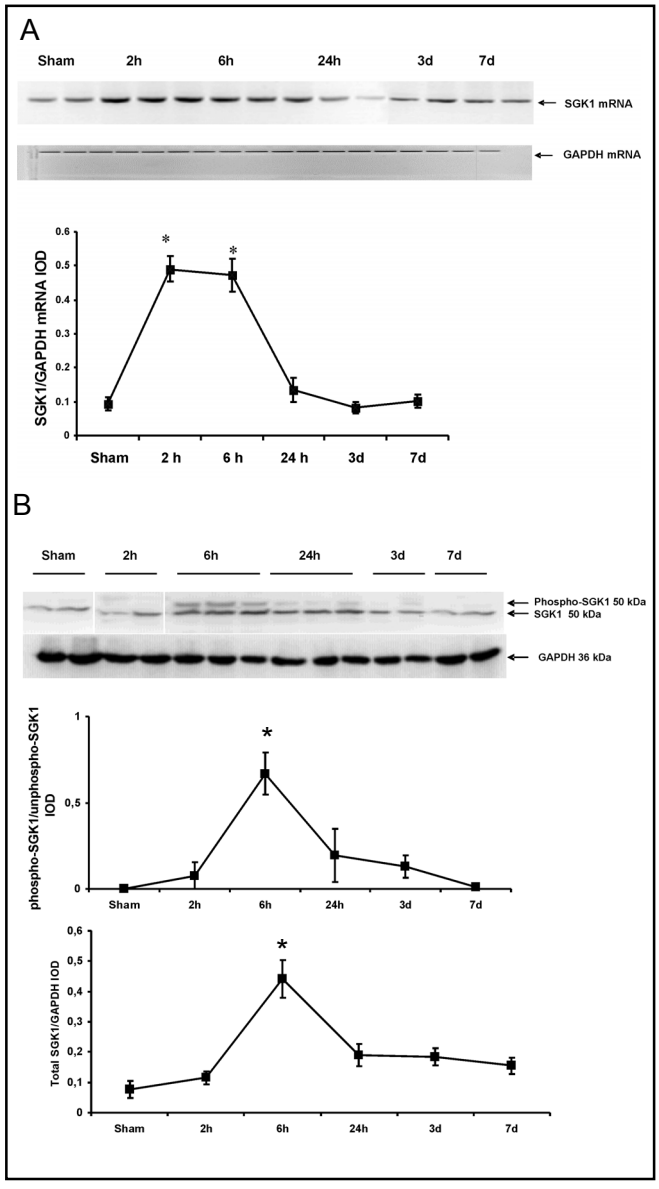

Fig. 3. SGK1 expression and phosphorylation in renal ischemia/reperfusion (I/R) injury. A. SGK1 mRNA expression was up-regulated 2 h after renal reperfusion and decreased to control levels of sham operated animals already 24 h after reperfusion. (*P<0.05 vs. sham) Figure shows representative pictures of RT-PCR analysis. B. SGK1 protein expression and phosphorylation were up-regulated between 2 and 6 h after renal reperfusion and decreased to control levels of sham operated animals 3 days after reperfusion. (*P<0.05 vs. sham). Figure shows representative pictures of Western blot analysis.

The effect of SGK1 on the apoptosis of tubular cells during renal ischemia/reperfusion injury. To determine whether SGK1 conferred protection of

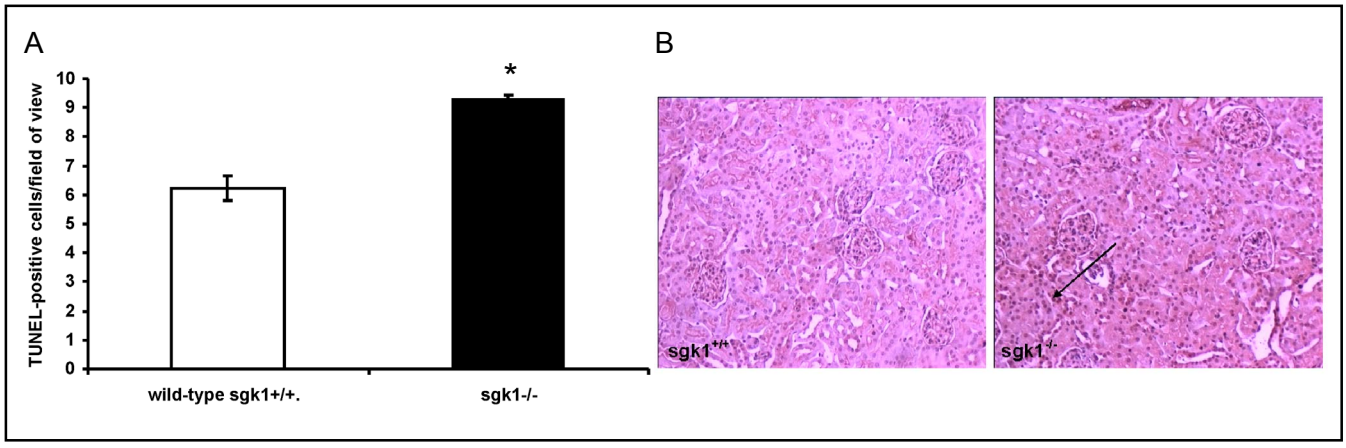

Fig. 4. SGK1 protects the cells from apoptosis in ischemic renal injury. A. Arithmetic means ± SEM (n = 6 each) of TUNEL-positive tubular cells per field of view (*P<0.01 vs. $sgk1^{+/+}$). B. Representative pictures of TUNEL staining. The arrow points to a representative TUNEL-positive tubular cell.

ischemic kidneys, unilateral I/R experiments were again performed in SGK1 knockout mice ($sgk1^{-/-}$) and their wild-type littermates ($sgk1^{+/+}$). The mice were subjected to unilateral left renal I/R injury with a 30 min of ischemia and 24 h reperfusion time. The TUNEL staining revealed that the number of apoptotic tubular cells was significantly higher in $sgk1^{-/-}$ mice than in $sgk1^{+/+}$ mice (P<0.01 vs. $sgk1^{+/+}$, Figure 4A-B).

Discussion

The present study reveals that SGK1 expression is modified by H/R of cultured renal cells and following renal ischemia. More importantly, the present studies disclose a functional role of SGK1 in renal ischemia.

The transcript levels of SGK1 were increased as early as 2 hours after initiation of ischemia, the SGK1 protein abundance increased as early as 6 hours after ischemia. As shown previously [29], SGK1 is an early gene up-regulated and downregulated at the genomic level within less than 30 minutes. The up-regulation of SGK1 is transient and is reversed within 24 hours. The present observations do, however, not rule out that SGK1 expression remains high during sustained ischemia.

The functional role of SGK1 in H/R injury is evidenced by significantly blunted apoptosis in SGK1-overexpressing HEK 293 cells exposed to H/R injury. The functional significance of SGK1 expression following a 30 min period of renal ischemia *in vivo* is illustrated by the significantly higher percentage of apoptotic cells in gene targeted mice lacking functional SGK1 ($sgk1^{-/-}$) than in their wild-type littermates ($sgk1^{+/+}$).

The present paper did not address the mechanisms involved in SGK1 dependent protection of cells during H/R injury or ischemia. SGK1 has previously been shown to phosphorylate the forkhead transcription factor Foxo3a [30-35]. Downregulation of Foxo3a fosters cell survival [36-38], an effect at least partially due to the expression of the Bcl2-interacting mediator BIM, which stimulates apoptosis and inhibits cell proliferation [39, 40]. Moreover, SGK1 could participate in the regulation of cell survival by phosphorylation and activation of IκB kinase [41] and/or by inhibition of B-Raf [42]. Clearly, additional effort will be necessary to elucidate SGK1 dependent mechanisms conferring cell survival in ischemic tissue.

In conclusion, SGK1 is heavily up-regulated by H/R *in vitro* and ischemia *in vivo*. The kinase participates in the machinery fostering cell survival under those stress conditions.

Acknowledgements

The excellent technical assistance of Tanja Dravits, Sandra Haderer and Marcel Konhäuser is gratefully acknowledged. The authors would also like to thank Dr. Christine Bayer and Dr. Daniela Schilling for their kindly help by establishing the *in vitro* hypoxia model.

References

1 De Broe ME: Apoptosis in acute renal failure. Nephrol Dial Transplant 2001;16 Suppl 6:23-26.

2 Andreucci M, Michael A, Kramers C, Park KM, Chen A, Matthaeus T, Alessandrini A, Haq S, Force T, Bonventre JV: Renal ischemia/reperfusion and ATP depletion/repletion in LLC-PK(1) cells result in phosphorylation of FKHR and FKHRL1. Kidney Int 2003;64:1189-1198.

3 Kwon DS, Kwon CH, Kim JH, Woo JS, Jung JS, Kim YK: Signal transduction of MEK/ERK and PI3K/Akt activation by hypoxia/reoxygenation in renal epithelial cells. Eur J Cell Biol 2006;85:1189-1199.

4 Zhuang S, Yan Y, Daubert RA, Han J, Schnellmann RG: ERK promotes hydrogen peroxide-induced apoptosis through caspase-3 activation and inhibition of Akt in renal epithelial cells. Am J Physiol Renal Physiol 2007;292:F440-F447.

5 Lang F, Bohmer C, Palmada M, Seebohm G, Strutz-Seebohm N, Vallon V: (Patho)physiological significance of the serum- and glucocorticoid-inducible kinase isoforms. Physiol Rev 2006;86:1151-1178.

6 Firestone GL, Giampaolo JR, O'Keeffe BA: Stimulus-dependent regulation of serum and glucocorticoid inducible protein kinase (SGK) transcription, subcellular localization and enzymatic activity. Cell Physiol Biochem 2003;13:1-12.

7 Loffing J, Flores SY, Staub O: Sgk kinases and their role in epithelial transport. Annu Rev Physiol 2006;68:461-490.

8 Pearce D, Kleyman TR: Salt, sodium channels, and SGK1. J Clin Invest 2007;117:592-595.

9 Webster MK, Goya L, Firestone GL: Immediate-early transcriptional regulation and rapid mRNA turnover of a putative serine/threonine protein kinase. J Biol Chem 1993;268:11482-11485.

10 Lang F, Bohmer C, Palmada M, Seebohm G, Strutz-Seebohm N, Vallon V: (Patho)physiological significance of the serum- and glucocorticoid-inducible kinase isoforms. Physiol Rev 2006;86:1151-1178.

11 Lang F, Bohmer C, Palmada M, Seebohm G, Strutz-Seebohm N, Vallon V: (Patho)physiological significance of the serum- and glucocorticoid-inducible kinase isoforms. Physiol Rev 2006;86:1151-1178.

12 Simon P, Schneck M, Hochstetter T, Koutsouki E, Mittelbronn M, Merseburger A, Weigert C, Niess A, Lang F: Differential regulation of serum- and glucocorticoid-inducible kinase 1 (SGK1) splice variants based on alternative initiation of transcription. Cell Physiol Biochem 2007;20:715-728.

13 Waldegger S, Barth P, Raber G, Lang F: Cloning and characterization of a putative human serine/threonine protein kinase transcriptionally modified during anisotonic and isotonic alterations of cell volume. Proc Natl Acad Sci U S A 1997;94:4440-4445.

14 Waerntges S, Klingel K, Weigert C, Fillon S, Buck M, Schleicher E, Rodemann HP, Knabbe C, Kandolf R, Lang F: Excessive transcription of the human serum and glucocorticoid dependent kinase hSGK1 in lung fibrosis. Cell Physiol Biochem 2002;12:135-142.

15 Nishida Y, Nagata T, Takahashi Y, Sugahara-Kobayashi M, Murata A, Asai S: Alteration of serum/glucocorticoid regulated kinase-1 (sgk-1) gene expression in rat hippocampus after transient global ischemia. Brain Res Mol Brain Res 2004;123:121-125.

16 Alessi DR, Deak M, Casamayor A, Caudwell FB, Morrice N, Norman DG, Gaffney P, Reese CB, MacDougall CN, Harbison D, Ashworth A, Bownes M: 3-Phosphoinositide-dependent protein kinase-1 (PDK1): structural and functional homology with the Drosophila DSTPK61 kinase. Curr Biol 1997;7:776-789.

17 Brunet A, Park J, Tran H, Hu LS, Hemmings BA, Greenberg ME: Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHRL1 (FOXO3a). Mol Cell Biol 2001;21:952-965.

18 Dehner M, Hadjihannas M, Weiske J, Huber O, Behrens J: Wnt signaling inhibits Forkhead box O3a-induced transcription and apoptosis through up-regulation of serum- and glucocorticoid-inducible kinase 1. J Biol Chem 2008;283:19201-19210.

19 Zhang L, Cui R, Cheng X, Du J: Antiapoptotic effect of serum and glucocorticoid-inducible protein kinase is mediated by novel mechanism activating I{kappa}B kinase. Cancer Res 2005;65:457-464.

20 Brumatti G, Sheridan C, Martin SJ: Expression and purification of recombinant annexin V for the detection of membrane alterations on apoptotic cells. Methods 2008;44(3):235-40.

21 Flemmig J, Lessig J, Reibetanz U, Dautel P, Arnhold J: Non-vital polymorphonuclear leukocytes express myeloperoxidase on their surface. Cell Physiol Biochem 2008;21(4):287-96.

22 Korngold EC, Jaffer FA, Weissleder R, Sosnovik DE: Noninvasive imaging of apoptosis in cardiovascular disease. Heart Fail Rev 2008;13(2):163-73.

23 Lang F, Gulbins E, Lerche H, Huber SM, Kempe DS, Foller M: Eryptosis, a window to systemic disease. Cell Physiol Biochem 2008;22(5-6):373-80.

24 Zhang BH, Tang ED, Zhu T, Greenberg ME, Vojtek AB, Guan KL: Serum- and glucocorticoid-inducible kinase SGK phosphorylates and negatively regulates B-Raf. J Biol Chem 2001;276:31620-31626.

25 Wulff P, Vallon V, Huang DY, Volkl H, Yu F, Richter K, Jansen M, Schlunz M, Klingel K, Loffing J, Kauselmann G, Bosl MR, Lang F, Kuhl D: Impaired renal Na(+) retention in the sgk1-knockout mouse. J Clin Invest 2002;110:1263-1268.

26 Grenz A, Eckle T, Zhang H, Huang DY, Wehrmann M, Kohle C, Unertl K, Osswald H, Eltzschig HK: Use of a hanging-weight system for isolated renal artery occlusion during ischemic preconditioning in mice. Am J Physiol Renal Physiol 2007;292:F475-F485.

27 Muppidi J, Porter M, Siegel RM: Measurement of apoptosis and other forms of cell death. Curr Protoc Immunol 2004;Chapter 3:Unit 3.17.

28 Heemann U, Szabo A, Hamar P, Muller V, Witzke O, Lutz J, Philipp T: Lipopolysaccharide pretreatment protects from renal ischemia/reperfusion injury : possible connection to an interleukin-6-dependent pathway. Am J Pathol 2000;156:287-293.

29 Waldegger S, Barth P, Raber G, Lang F: Cloning and characterization of a putative human serine/threonine protein kinase transcriptionally modified during anisotonic and isotonic alterations of cell volume. Proc Natl Acad Sci U S A 1997;94:4440-4445.

30 Brunet A, Park J, Tran H, Hu LS, Hemmings BA, Greenberg ME: Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHRL1 (FOXO3a). Mol Cell Biol 2001;21:952-965.

31 Dehner M, Hadjihannas M, Weiske J, Huber O, Behrens J: Wnt signaling inhibits Forkhead box O3a-induced transcription and apoptosis through up-regulation of serum- and glucocorticoid-inducible kinase 1. J Biol Chem 2008;283:19201-19210.

32 Huang H, Tindall DJ: Dynamic FoxO transcription factors. J Cell Sci 2007;120:2479-2487.
33 Liu D, Yang X, Songyang Z: Identification of CISK, a new member of the SGK kinase family that promotes IL-3-dependent survival. Curr Biol 2000;10:1233-1236.
34 Shelly C, Herrera R: Activation of SGK1 by HGF, Rac1 and integrin-mediated cell adhesion in MDCK cells: PI-3K-dependent and -independent pathways. J Cell Sci 2002;115:1985-1993.
35 Xu J, Liu D, Gill G, Songyang Z: Regulation of cytokine-independent survival kinase (CISK) by the Phox homology domain and phosphoinositides. J Cell Biol 2001;154:699-705.
36 Kandel ES, Hay N: The regulation and activities of the multifunctional serine/threonine kinase Akt/PKB. Exp Cell Res 1999;253:210-229.
37 Mikosz CA, Brickley DR, Sharkey MS, Moran TW, Conzen SD: Glucocorticoid receptor-mediated protection from apoptosis is associated with induction of the serine/threonine survival kinase gene, sgk-1. J Biol Chem 2001;276:16649-16654.
38 You H, Jang Y, You T, Okada H, Liepa J, Wakeham A, Zaugg K, Mak TW: p53-dependent inhibition of FKHRL1 in response to DNA damage through protein kinase SGK1. Proc Natl Acad Sci U S A 2004;101:14057-14062.
39 Dijkers PF, Medema RH, Pals C, Banerji L, Thomas NS, Lam EW, Burgering BM, Raaijmakers JA, Lammers JW, Koenderman L, Coffer PJ: Forkhead transcription factor FKHR-L1 modulates cytokine-dependent transcriptional regulation of p27(KIP1). Mol Cell Biol 2000;20:9138-9148.
40 Dijkers PF, Medema RH, Lammers JW, Koenderman L, Coffer PJ: Expression of the pro-apoptotic Bcl-2 family member Bim is regulated by the forkhead transcription factor FKHR-L1. Curr Biol 2000;10:1201-1204.
41 Zhang L, Cui R, Cheng X, Du J: Antiapoptotic effect of serum and glucocorticoid-inducible protein kinase is mediated by novel mechanism activating I{kappa}B kinase. Cancer Res 2005;65:457-464.
42 Zhang BH, Tang ED, Zhu T, Greenberg ME, Vojtek AB, Guan KL: Serum- and glucocorticoid-inducible kinase SGK phosphorylates and negatively regulates B-Raf. J Biol Chem 2001;276:31620-31626.