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Up-Regulation of the Human Serum and Glucocorticoid-Dependent Kinase 1 in Glomerulonephritis

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Key Words
Glomerulonephritis · Cell volume regulation · SGK1

Abstract
Glomerulonephritis is paralleled by excessive formation of transforming growth factor-beta (TGF-β), which participates in the pathophysiology of the disease. Recently, a novel downstream target of TGF-β has been identified, i.e. the human serum and glucocorticoid-dependent kinase 1 (hSGK1), a serine/threonine kinase participating in the regulation of Na^+ transport. The present study was performed to elucidate transcriptional regulation of hSGK1 in glomerulonephritis. To this end, in situ hybridization was performed in biopsies from patients with clinical diagnosis of glomerulonephritis. hSGK1 transcript levels were moderately enhanced in 5 out of 9 patients and strongly enhanced in 4 out of 9 patients. Distal nephron epithelial cell hSGK1 transcript levels were low or absent in 7 of the 9 patients but markedly enhanced in 2 of the 9 patients. In conclusion, glomerulonephritis leads to glomerular and in some cases to epithelial up-regulation of hSGK1 transcription.

Introduction

Ample evidence points to a role of transforming growth factor-beta1 (TGF-β1) in the pathophysiology of glomerulonephritis [1–8]. Accordingly, TGF-β antagonism reversed matrix accumulation by infusing neutralizing TGF-β antibodies [2], soluble TGF-β receptors [9] and decorin, an inhibitor of TGF-β [10, 11].

Recently, a novel downstream target of TGF-β has been identified, the human serum and glucocorticoid-dependent kinase 1 (hSGK1), which is transcriptionally up-regulated by TGF-β in U 937 macrophages [12], HepG2 liver cells [12] and 3T3 fibroblasts [13]. The human hSGK1 has previously been cloned as cell volume sensitive gene [14]. The rat SGK1 was originally cloned from rat mammary tumour cells as serum and glucocorticoid-sensitive kinase [15], but was subsequently shown to be up-regulated by mineralocorticoids [16, 17]. Transcription of the kinase has been demonstrated to be enhanced by excessive extracellular glucose concentrations, an effect blunted by neutralizing anti-TGF-β antibodies [13]. Accordingly, TGF-β at least partially accounts for transcriptional up-regulation of hSGK1 in diabetic nephropathy [13, 18–20].

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Table 1. Clinical details and histopathological grading of the glomerulonephritis patients

Patient	Age years	Sex	Diagnosis	Crea mg/dl	GFR ml/min	Diabetes mellitus	Hypertension >140/90 mm Hg	Proteinuria g/day	SGK grading (0–4) glomerulus	SGK grading (0–4) DT
1	62	f	immune-complex GN	2.5	no data	no	yes	no	3	0-1
2	50	m	perimembranous GN	<1.1	97	no	yes	4.9	0–1	0
3	51	m	perimembranous GN	3.2	no data	no	yes	8.0	0–1	0
4	45	m	mesangio-proliferative GN	1.3	156	no	yes	3.2	2	0–1
5	70	f	immune-complex GN	1.8	no data	no	no	0.8	1	0
6	29	f	mesangio-proliferative GN	1.4	no data	no	no	4.8	3	0–1
7	30	m	rapid progressive GN	9	no data	no	yes	no data	3	4
8	43	f	malignant nephrosclerosis	2.2	no data	no	yes	mild proteinuria	4	4
9	26	m	mesangio-proliferative GN	1.5	no data	no data	no data	3.9	0–1	2

Crea = Creatinine; GFR = glomerular filtration rate; GN = glomerulonephritis; DT = distal tubule.

The present study has been performed to test for deranged transcriptional regulation of hSGK1 in glomerulonephritis.

Materials and Methods

Patients

Kidney biopsies from patients with clinical diagnosis of glomerulonephritis were taken for diagnostic reasons. Initially, 13 patients were included in this retrospective study. From those, 4 patients had to be excluded due to initial treatment with steroids or drugs which affect the angiotensin/aldosterone system; the other patients did not receive steroids, immunosuppression or ACE inhibitors at the time of biopsy. From the remaining 9 patients, 3 suffered from mesangio-proliferative glomerulonephritis, 1 from rapid progressive glomerulonephritis, 2 from perimembranous glomerulonephritis, 1 from malignant nephrosclerosis and 2 from immune complex glomerulonephritis after streptococci infection. Six out of the 9 patients were hypertensive (RR > 140/90 mm Hg), no patient was diabetic. Normal renal biopsies from 7 patients were obtained from intact parts of kidneys which had been surgically removed because of renal cancer. For full clinical details, see table 1.

In situ Hybridization

Kidney biopsy tissue was fixed in 4% paraformaldehyde/0.1 *M* sodium phosphate buffer (pH 7.2) for 4 h and embedded in paraffin. Four-micrometer tissue sections were dewaxed and hybridized basically as described [21–23]. Hybridization mixture contained either the ^{35}S-labeled RNA antisense or sense control hSGK1 probe [13] (500 ng/ml) in 10 m*M* Tris HCl, pH 7.4/50% (vol/vol) deionized formamide/600 m*M* NaCl/1 m*M* EDTA/0.02% polyvinylpyrrolidone/0.02% Ficoll/0.05% bovine serum albumin/10% dextrane sulphate/10 m*M* dithiothreitol/denatured sonicated salmon sperm DNA at 200 µg/ml/rabbit liver tRNA at 100 µg/ml [12]. Hybridization with RNA probes proceeded at 42°C for 18 h. The slides were then washed as described [21–23] followed by 1 h at 55°C in 2 × standard saline citrate. Non-hybridized single-stranded RNA probes were digested by RNAse A (20 µg/ml) in 10 m*M* Tris HCl, pH 8.0/0.5 *M* NaCl for 30 min at 37°C. Tissue slide preparations were autoradiographed [23] and stained with haematoxylin/eosin.

Statistical Analysis

Data are expressed as arithmetic means ± SEM. Statistical analysis has been made by the Student t test or ANOVA, where applicable. A p value of <0.05 was considered statistically significant.

Results

As shown in figure 1, expression of hSGK1 mRNA in intact kidneys was restricted to some mesangial cells of the glomeruli and a few epithelial cells in distal convoluted tubules and thick ascending limbs of Henle's loop. In 4 out of the 9 glomerulonephritic kidneys, glomerular hSGK1 transcription was markedly enhanced, in 5 out of the 9 patients, hSGK1 expression remained low or was only moderately enhanced. In 2 of the 9 patients, markedly enhanced transcript levels were observed in epithelial cells from distal tubule and thick ascending limb. Both patients were hypertensive. In addition, high levels of hSGK1 transcripts were observed in interstitial cells, most likely representing macrophages and fibroblasts. Unspecific labelling of kidney cells was excluded by hybridization of tissue sections with the ^{35}S-labeled sense control RNA hSGK1 probe.

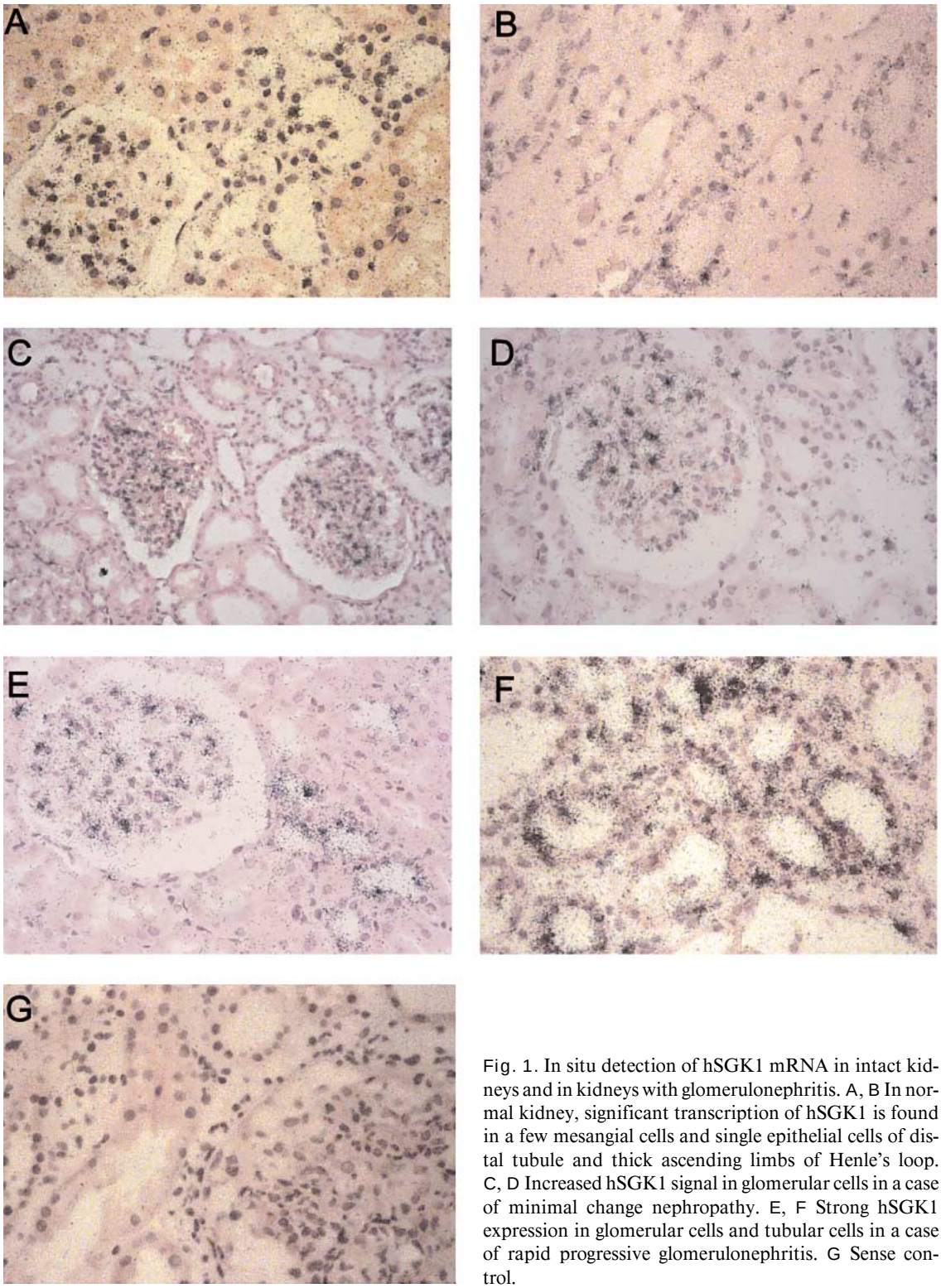

Fig. 1. In situ detection of hSGK1 mRNA in intact kidneys and in kidneys with glomerulonephritis. A, B In normal kidney, significant transcription of hSGK1 is found in a few mesangial cells and single epithelial cells of distal tubule and thick ascending limbs of Henle's loop. C, D Increased hSGK1 signal in glomerular cells in a case of minimal change nephropathy. E, F Strong hSGK1 expression in glomerular cells and tubular cells in a case of rapid progressive glomerulonephritis. G Sense control.

Discussion

The present study demonstrates up-regulation of hSGK1 transcription in kidneys from glomerulonephritic patients. The up-regulation may be expected, as glomerular injury is paralleled by up-regulation of TGF-β [4], which is a strong stimulator of hSGK1 transcription [12, 13] and is thought to damage renal tissue by stimulation of matrix deposition [24]. This does, of course, not rule out the contribution of further cytokines and hormones to the up-regulation of hSGK1 in glomerulonephritic kidneys. The transcription of hSGK1 has proved to be highly sensitive to a wide variety of stimuli [25].

In 2 patients, hSGK1 was not only up-regulated in affected glomeruli but as well in epithelial cells, particularly in distal tubule and thick ascending limb. As hSGK1 has been shown to be a strong stimulator of the renal epithelial Na^+ channel ENaC [16, 13, 17, 26, 27] and the thick ascending limb Na^+, K^+, $2Cl^-$ cotransport [13], the up-regulation of hSGK1 in renal epithelial cells could lead to renal Na^+ retention and thus favour the development of hypertension. However, in the epithelial cells of the remaining 7 patients, the hSGK1 levels remained low even though 4 of those patients developed hypertension. Possibly, hSGK1 is up-regulated in confined nephron segments such as medullary collecting duct outside the available tissue specimen. Nevertheless, widespread epithelial up-regulation of hSGK1 does not appear to be a prerequisite for the development of hypertension in glomerulonephritis.

In conclusion, glomerulonephritis is paralleled by up-regulation of hSGK1 in affected glomeruli and in a subset of patients in renal epithelial cells of thick ascending limb and distal tubule.

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References

1 Bitzer M, Sterzel RB, Bottinger EP: Transforming growth factor-beta in renal disease. Kidney Blood Press Res 1998;21:1–12.

2 Border WA, Okuda S, Languino LR, Sporn MB, Ruoslahti E: Suppression of experimental glomerulonephritis by antiserum against transforming growth factor beta 1. Nature 1990;346: 371–374.

3 Border WA, Noble NA, Ketteler M: TGF-beta: A cytokine mediator of glomerulosclerosis and a target for therapeutic intervention. Kidney Int Suppl 1995;49:S59–S61.

4 Border WA, Noble NA: Cytokines in kidney disease: The role of transforming growth factor-beta. Am J Kidney Dis 1993;22:105–113.

5 Border WA, Noble NA: Transforming growth factor beta in tissue fibrosis. N Engl J Med 1994;331:1286–1292.

6 Border WA, Noble NA: TGF-beta in kidney fibrosis: A target for gene therapy. Kidney Int 1997;51:1388–1396.

7 Ketteler M, Noble NA, Border WA: Transforming growth factor-beta and angiotensin II: The missing link from glomerular hyperfiltration to glomerulosclerosis? Annu Rev Physiol 1995;57:279–295.

8 Yamamoto T, Noble NA, Cohen AH, Nast CC, Hishida A, Gold LI, Border WA: Expression of transforming growth factor-beta isoforms in human glomerular diseases. Kidney Int 1996; 49:461–469.

9 Isaka Y, Akagi Y, Ando Y, Tsujie M, Sudo T, Ohno N, Border WA, Noble NA, Kaneda Y, Hori M, Imai E: Gene therapy by transforming growth factor-beta receptor-IgG Fc chimera suppressed extracellular matrix accumulation in experimental glomerulonephritis. Kidney Int 1999;55:465–475.

10 Border WA, Noble NA, Yamamoto T, Harper JR, Yamaguchi Yu, Pierschbacher MD, Ruoslahti E: Natural inhibitor of transforming growth factor-beta protects against scarring in experimental kidney disease. Nature 1992;360: 361–364.

11 Isaka Y, Brees DK, Ikegaya K, Kaneda Y, Imai E, Noble NA, Border WA: Gene therapy by skeletal muscle expression of decorin prevents fibrotic disease in rat kidney. Nat Med 1996;2: 418–423.

12 Waldegger S, Klingel K, Barth P, Sauter M, Lanzendörfer M, Kandolf R, Lang F: h-SGK serine-threonine protein kinase gene as early transcriptional target of TGF-β in human intestine. Gastroenterology 1999;116:1081–1088.

13 Lang F, Klingel K, Wagner CA, Stegen C, Friedrich B, Wärntges S, Lanzendörfer M, Melzig J, Moschen I, Steuer S, Waldegger S, Sauter M, Paulmichl M, Gerke V, Risler T, Gamba G, Capasso G, Kandolf R, Hebert SC, Massry SG, Bröer S: Deranged transcriptional regulation of cell-volume-sensitive kinase hSGK1 in diabetic nephropathy. Proc Natl Acad Sci USA 2000; 94:8157–8162.

14 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 USA 1997;94:4440–4445.

15 Webster MK, Goya L, Ge Y, Maiyar AC, Firestone GL: Characterization of SGK, a novel member of the serine/threonine protein kinase gene family which is transcriptionally induced by glucocorticoids and serum. Mol Cell Biol 1993;13:2031–2040.

16 Chen SY, Bhargava A, Mastroberardino L, Meijer OC, Wang J, Buse P, Firestone GL, Verrey F, Pearce D: Epithelial sodium channel regulated by aldosterone-induced protein SGK. Proc Natl Acad Sci USA 1999;96:2514–2519.

17 Naray-Fejes-Toth A, Canessa C, Cleaveland ES, Aldrich G, Fejes-Toth G: SGK is an aldosterone-induced kinase in the renal collecting duct. Effects on epithelial Na^+ channels. J Biol Chem 1999;274:16973–16978.

18 Kumar JM, Brooks DP, Olson BA, Laping NJ: SGK, a putative serine/threonine kinase, is differentially expressed in the kidney of diabetic mice and humans. J Am Soc Nephrol 1999;10: 2488–2494.
19 Reeves WB, Andreoli TE: Transforming growth factor-β contributes to progressive diabetic nephropathy. Proc Natl Acad Sci USA 2000;97:7667–7669.
20 Ziyadeh FN, Hoffman BB, Han DC, Iglesias-de la Cruz MC, Hong SW, Isono M, Chen S, McGowan TA, Sharma K: Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-α antibody in db/db diabetic mice. Proc Natl Acad Sci USA 2000;97:8015–8020.
21 Hohenadl C, Klingel K, Mertsching J, Hofschneider PH, Kandolf R: Strand-specific detection of enteroviral RNA in myocardial tissue by in situ hybridization. Mol Cell Probes 1991;5:11–20.
22 Kandolf R, Ameis D, Kirschner P, Canu A, Hofschneider PH: In situ detection of enteroviral genomes in myocardial cells by nucleic acid hybridization: An approach to the diagnosis of viral heart disease. Proc Natl Acad Sci USA 1987;84:6272–6276.
23 Klingel K, Hohenadl C, Canu A, Albrecht M, Seemann M, Mall G, Kandolf R: Ongoing enterovirus-induced myocarditis is associated with persistent heart muscle infection: Quantitative analysis of virus replication, tissue damage, and inflammation. Proc Natl Acad Sci USA 1992;89:314–318.
24 Nakamura T, Miller D, Ruoslahti E, Border WA: Production of extracellular matrix by glomerular epithelial cells is regulated by transforming growth factor-beta 1. Kidney Int 1992; 41:1213–1221.
25 Lang F, Cohen P: Regulation and physiological roles of serum- and glucocorticoid-induced protein kinase isoforms. Sci STKE 2001;2001: RE17.
26 Böhmer C, Wagner CA, Beck S, Moschen I, Melzig J, Werner A, Lin J-T, Lang F, Wehner F: The shrinkage-activated Na^+ conductance of rat hepatocytes and its possible correlation to rENaC. Cell Phys Biochem 2000;10:187–194.
27 Alvarez de la Rosa D, Zhang P, Naray-Fejes-Toth A, Fejes-Toth G, Canessa CM: The serum and glucocorticoid kinase SGK increases the abundance of epithelial sodium channels in the plasma membrane of Xenopus oocytes. J Biol Chem 1999;274:37834–37839.