Akt Mediates Mechanical Strain-Induced Collagen Production by Mesangial Cells

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Increased glomerular hydrostatic pressure is an important determinant of glomerulosclerosis and can be modeled by in vitro exposure of mesangial cells to cyclic mechanical strain. Stretched mesangial cells increase extracellular matrix protein production, the hallmark of glomerulosclerosis. Recent data indicate that the serine/threonine kinase Akt may be involved in matrix modulation. Thus, Akt activation and matrix synthesis in stretched mesangial cells were studied. Exposure of mesangial cells to 1 Hz cyclic strain led to prompt Akt activation, which was biphasic to 24 h. Activation was dependent on signaling through phosphatidylinositol-3-kinase and required EGF receptor transactivation. Inhibition of signaling through the PDGF receptor, Src kinase, or cytoskeletal disruption failed to prevent strain-induced Akt activation. Collagen type 1A1 transcript expression, promoter activation, and protein secretion were increased by stretch at 24 h and were dependent on phosphatidylinositol-3 kinase. Overexpression of dominant-negative Akt inhibited strain-induced collagen 1A1 production. Conversely, overexpression of constitutively active Akt led to increased collagen 1A1 upregulation and secretion. Finally, Akt activation was observed in the glomeruli of remnant rat kidneys, a model marked by increased intraglomerular pressure. The authors conclude that mechanical strain induces Akt activation in mesangial cells through a mechanism requiring phosphatidylinositol-3-kinase and EGF receptor transactivation. Type 1 collagen production is dependent on Akt and can be induced by Akt overexpression. Akt activation is observed in remnant kidneys in vivo. Thus, the role of Akt in progression of chronic hemodynamic glomerular disease is worthy of further exploration.


Elevated glomerular capillary pressure (Pgc) is an important determinant of progression in chronic renal diseases of diverse causes (1). In the partially nephrectomized (remnant) rat, a well-characterized model of chronic renal failure, an increase in Pgc precedes an increase in glomerular extracellular matrix production, the pathologic hallmark of glomerulosclerosis (2). Experimentally and clinically, interventions that normalize Pgc, such as interruption of the renin-angiotensin system, protect from glomerular injury and matrix expansion (3,4). The mechanisms translating glomerular capillary hypertension to glomerular injury, however, have not been fully elucidated.

Increased Pgc transmits to mesangial cells (MC), which provide architectural support for the glomerular capillary tuft as mechanical strain (5). In vitro, mechanical strain has been studied by culturing cells on plates with deformable bottoms and applying a vacuum to the underlying sealed well to generate cycles of stretch and relaxation. MC subjected to cyclic strain/relaxation increase extracellular matrix protein synthesis (6–8), thus providing a model system to study mechanical strain-induced signaling in MC. Several signaling pathways have been implicated in the strain-induced production of matrix proteins, including RhoA and the mitogen-activated protein kinases (MAPK) Erk, SAPK, and p38 (9–11). Although the profibrogenic cytokines TGF-β and connective tissue growth factor have been shown to play an important role (8,11,12), the regulation of strain-induced matrix protein synthesis in MC is complex and remains poorly understood.

Akt is a serine/threonine kinase important in cell processes including survival, glucose metabolism, cell-cycle progression, protein synthesis, and hypertrophy (13,14). Phosphatidylinositol-3-OH kinase (PI3K) is a well-established upstream mediator of Akt activation, generating phosphorylated lipid second messengers, which recruit Akt to the membrane (15). On translocation, Akt is activated by phosphorylation on T308 and S473, likely by distinct kinases (15,16). It subsequently mediates the inhibition or activation of a number of downstream effectors through phosphorylation of serine/threonine residues (15). Recent data indicate that Akt may also play an important role in matrix elaboration in several settings. Constitutively active Akt or PI3K induced laminin and collagen IV synthesis in embryoid bodies and Chinese hamster ovary cells (17). PI3K was shown to be important in basal production of collagen 1A1 transcript levels in hepatic stellate cells and human lung fibroblasts (18,19). Abnormal production of collagen and laminin, as well as extracellular fibronectin assembly, by keloid fibroblasts...
was dependent on PI3K (20), and this role in fibronectin matrix assembly has also been seen in other cell types (21). The PI3K/Akt pathway has also been shown to mediate TGF-β-induced collagen 1A1 and 1A2 upregulation in lung fibroblasts and MC (19,22).

Akt has recently been shown to be activated in response to mechanical forces in different cell types including endothelial cells, vascular smooth muscle cells, and pericytes (23–25). Akt activation by mechanical stress has been linked to the generation of the vasodilatory substance nitric oxide in endothelial cells and to proliferation in smooth muscle cells (24,26,27). However, whether strain-induced matrix elaboration involves Akt is unknown. Hence, we studied Akt activation in response to strain in MC and investigated its role in strain-induced upregulation of the matrix protein collagen 1. We also sought evidence of Akt activation in glomeruli of subtotally nephrectomized rats, an animal model of chronic renal failure marked by intraglomerular hypertension.

**Materials and Methods**

**Cell Culture**

Sprague-Dawley primary rat MC were cultured in DMEM supplemented with 20% fetal calf serum (Life Technologies BRL, Inc), streptomycin (100 μg/ml), and penicillin (100 units/ml) at 37°C in 95% air, 5% CO₂. Experiments were performed using cells between passages 6 to 15.

**Application of Strain/Relaxation**

MC were plated onto six-well plates with flexible bottoms coated with bovine type I collagen (Flexcell International Corp). After achieving confluence, cells were rendered quiescent by incubation for 24 h in serum-free medium. Plates were exposed to continuous cycles of strain/relaxation generated by a cyclic vacuum produced by a computer-driven system (Flexercell 4000; Flexcell International Corp), with strain/relaxation being 0.5 s of strain (10%) and 0.5 s of relaxation, for a total of 60 cycles/min.

Pharmacologic inhibitors were added as follows before stretch: Wortmannin (Sigma), 100 nmol for 60 min; LY-294002 (Sigma), 20 μmol for 30 min; AG1478 (Sigma), 5 μmol for 30 min; AG1295 (Sigma), 10 μmol for 30 min; cytochalasin D (Sigma), 200 ng/ml for 60 min; and PP1 (Biomol), 10 μmol for 30 min.

**Protein Extraction and Western Immunoblotting**

Cells were lysed and protein extracted as we have published (9). Briefly, cells were lysed in a buffer containing 20 mmol Tris-HCl (pH 7.5), 150 mmol NaCl, 1% Triton X-100, 1 mmol EDTA, 1 mmol EGTA, 2.5 mmol sodium pyrophosphate, 1 mmol β-glycerophosphate, 2 mmol DTT, 1 mmol sodium vanadate, 1 mmol phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 20 μg/ml aprotinin. After disruption with a rubber policeman, cell lysates were centrifuged at 4°C at 14,000 rpm for 10 min to pellet cell debris. Supernatant (50 μg) was separated on a 2% agarose gel. Primer sequences used were: Rat col 1A1 sense, 5′-TCGTGGATCCTGTTCCTT-3′; and rat col 1A1 antisense, 5′-TGCTGGTAGGGAATC-3′; rat β-actin sense, 5′-AACCCCTAAGGC-CAACCGTGAAAAG-3′; and rat β-actin antisense, 5′-TCATGAGTAGCTGTGCAG-3′. Reactions were performed for 20 to 23 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 60 s, with a 10-min final extension at 72°C using 5 U Taq in PCR buffer (Life Technologies BRL), 1.5 mmol MgCl₂, 0.2 mmol dNTP mix, 0.3 μmol each of sense and antisense primers, and 2 μl of cDNA.

**Luciferase Assay**

MC plated to 85% confluence were transfected with 0.8 μg of a col 1A1-luciferase construct (kindly provided by Dr. V. Falanga, Boston University School of Medicine, Boston, MA) (28) and 0.05 μg pCMV-β-galactosidase (β-gal) (Clontech) using LipofectAMINE (Qiagen). MC were serum-deprived overnight 24 h after transfection, after which they were stretched for 18 h. Cells were then lysed in Reporter Lysis Buffer (Promega) using one freeze–thaw cycle. Lysate was vortexed and cleared by centrifugation, and luciferase and β-gal activities were measured using specific assay kits (Promega) with a Berthold luminometer and a plate reader (420 nm), respectively. β-gal activity was used to adjust for transfection efficiency.

**ELISA for Collagen I**

After MC were stretched for 24 h, media were collected, debris was removed by low-speed centrifugation, and ELISA was performed as described previously (9). Briefly, plates were coated overnight at 4°C with media in a 1:3 dilution with ELISA Coating Buffer (Sigma), blocked, then incubated with a 1:2000 dilution of monoclonal anti-collagen I antibody (Sigma) in blocking buffer. After incubation with alkaline phosphatase-conjugated goat anti-mouse secondary antibody (Sigma), 1:30,000 diluted in blocking buffer, p-nitrophenyl phosphate was added, and reactions were read at 405 nm in a microplate auto-reader. Total cell number was used as a reference. In some studies, blocking rabbit polyclonal anti-TGF-β antibodies or control rabbit IgG (200 ng/ml medium; R&D Systems) were added immediately before the initiation of stretch.

**Infection of MC**

Epitope-tagged constitutively active Akt (HA-AktDD) or dominant-negative Akt (HA-AktAAA, kindly provided by Dr. J. Woodgett, Uni-
versity of Toronto, Toronto, Canada) (29) was cloned into a plasmid compatible with retroviral infection (pLHCX, Clontech). AktAAA was also cloned into pLHCX tagged by the green fluorescence protein (GFP). MC were infected as we have published (9). In brief, competent virus capable of single infection was generated using the vesicular stomatitis virus system (Stratagene), and MC passages 5 to 12 were exposed to virus concentrated by centrifugation in the presence of polybrene. Seventy-two hours after infection, a 2-week antibiotic selection period was begun. Experiments were performed using a population of pooled, stably infected MC.

5/6 Nephrectomy

Experiments were performed with male Sprague-Dawley rats weighing 280 to 300 g (Charlers River, Canada) in accordance with the McMaster University Central Animal Facility and Canadian Council on Animal Care guidelines. Rats were anesthetized with isoflurane and a 5/6 renal ablation performed as we have published (30). Both control (n = 6) and 5/6 (n = 9 per group) rats had free access to regular laboratory chow and water. At 1 wk and 2 wk after renal ablation, rats were anesthetized with isoflurane and the remnant or normal kidney removed. A small cortical section was placed into OCT compound for further immunohistochemical analysis, and the remaining cortical tissue was differentially sieved for glomeruli as previously published (30). Remnant cortex from three rats was pooled for each time point. Protein was extracted from isolated glomeruli (>95% purity by light microscopy) as outlined.

Immunohistochemistry

Renal cortical cryosections (5 μm) were air dried, fixed in ice-cold 100% methanol for 15 min, washed three times in PBS, and then permeabilized with 0.2% Triton X-100 for 15 min. After further washes, sections were blocked with 5% donkey serum/0.2% Triton X-100 in PBS and incubated with primary antibodies overnight at 4°C (rabbit polyclonal anti-pAktS473, 1:50, Cell Signaling; sheep polyclonal anti-von Willebrand factor, 2 μg/ml, Cedarlane). After washing in PBS, FITC-conjugated and rhodamine-conjugated secondary antibodies (1:100; Jackson ImmunoResearch) were applied for 1 h, and slides were then washed again in PBS and mounted in DAPI-containing mounting media (Vectashield). Images were acquired with a fluorescence microscope (Zeiss).

Statistical Analyses

Statistical analyses were performed using one-way ANOVA for experiments that had more than two groups or time points, and Tukey Honestly Significant Difference (HSD) was used for post hoc analysis to determine which groups were significantly different from one another. A t test was used for experiments with only two groups. P < 0.05 (two-tailed) was considered significant. Data are represented as the mean ± SEM. Experiments were repeated multiple times, and the number of repetitions is represented in the figure legends by “n = .” All analyses used the statistical package SPSS for Windows 11.0.

Results

Strain Induces Akt Activation in MC

MC were exposed for various times to cyclic strain as outlined in Materials and Methods, and phosphorylation of Akt on Ser473 was determined by immunoblotting. Phosphorylation on this residue has previously been shown to correlate with Akt activation (31). Akt phosphorylation was seen at 10 min (Figure 1A). This was sustained to 1 h, and longer-term experiments revealed a biphasic pattern to 24 h, with a later peak occurring at 16 h (Figure 1B). Because PI3-K is a well-established upstream mediator of Akt activation in most settings (14,16), we next examined the effects of two inhibitors of this enzyme. Wortmannin irreversibly inhibits PI3-K activity by covalently binding to the p110 catalytic subunit, whereas LY294002 is a reversible inhibitor, binding to the ATP-binding pocket of the catalytic subunit (14,19). Both of these inhibitors effectively blocked Akt phosphorylation at 10 min of strain (Figure 2A). Similar results were observed after 24 h of strain (data not shown). Finally, to confirm that Ser473 phosphorylation indeed correlates with Akt activity in our hands, we performed an Akt activity assay. Strain induced a significant increase in phosphorylation of the substrate GSK-3 by immunoprecipitated to-
tal Akt, indicative of an increase in Akt activity. This was again blocked by wortmannin (Figure 2B).

**EGF Receptor Is an Important Upstream Mediator of Akt Activation**

We next studied potential mechanisms that might subserve PI3-K and Akt activation in stretched MC. We first hypothesized that the cytoskeleton might be an important mediator because we have previously shown that actin reorganization and integrity was critical to activation of MAPK by stretch in MC (9,32). We used cytochalasin D (200 ng/ml, 1 h) to disrupt the actin cytoskeleton, having previously shown this to be effective in MC by rhodamine phalloidin imaging of F-actin (9). However, Akt activation was unaffected by the presence of this inhibitor (Figure 3A). We then hypothesized that the kinase Src might be involved as an upstream mediator of Akt activation because, in some settings, Src is activated by strain and can lie upstream of Akt in sheared endothelia (33). The Src inhibitor PP1 (10 μmol, 30 min) was, however, ineffective in blocking strain-induced Akt activation (Figure 3B). We also sought to study growth factor receptor transactivation. The EGF receptor, in particular, has been shown in other settings to aid in transmitting signals by stimuli other than ligand binding. For instance, angiotensin II uses the EGF receptor as a scaffold to initiate signaling in vascular smooth muscle cells (34). Figure 3C shows that blocking EGF receptor activation with AG1478 (5 μmol, 30 min) (35) inhibited strain-induced Akt activation. However, blockade of the PDGF receptor with AG1295 (10 μmol, 30 min) (36) had no effect. We conclude that neither the actin cytoskeleton nor signaling through Src kinase are essential to strain-induced Akt activation in MC, but transactivation of the EGF receptor plays an important permissive role.

**Akt Activation Mediates Strain-Induced Collagen Production in MC**

Recently, Akt has been implicated in the elaboration of matrix protein in response to the profibrogenic cytokine TGF-β (19,22,37). Overexpression of constitutively active Akt also increased collagen I production in hepatic stellate cells (18) and collagen IV production in embryoid bodies (17). The accumulation of extracellular matrix, including collagen I, is a hallmark of glomerulosclerosis secondary to increased intraglomerular pressure (2,38). We thus hypothesized that strain-induced Akt activation might mediate matrix elaboration in MC. We first investigated the effects of strain on col1A1 transcript levels at 24 h by reverse-transcription PCR. Strain resulted in a two-fold increase in col1A1 transcript, and this was dependent on PI3-K (data not shown). Because Akt has previously been shown to increase col1A1 transcript levels in fibroblasts in response to TGF-β primarily by stabilizing the transcript (19), we investigated how Akt might mediate an increase in transcript levels in stretched MC. We used a construct in which the col1A1 pro-
moter is placed upstream of the luciferase gene to study whether Akt was involved in transcriptional upregulation of collagen I. MC stretched for 18 h showed an increase in col1A1–luciferase activation, and this was inhibited by LY294002, showing dependence on PI3-K signaling (Figure 4A). Strain-induced secretion of collagen I protein, as assessed by ELISA, was also dependent on PI3K (Figure 4B). Hence, the PI3K/Akt signaling pathway is an important mediator in the upregulation and secretion of collagen I in stretched MC.

To confirm the importance of Akt in mediating collagen I production in response to stretch by MC, we studied responses in cells overexpressing dominant-negative Akt (AktAAA). Using retrovirus to introduce AktAAA, we selected a pooled population of MC stably expressing either the HA- or GFP-tagged dominant-negative construct. Overexpression was confirmed by immunoblotting for either HA or GFP, as well as total Akt (Figure 5A). Furthermore, we confirmed that this overexpression was functionally important by using PDGF as a well-established activator of Akt signaling in MC (14). As shown in Figure 5B, dominant-negative Akt successfully blocked phosphorylation of GSK-3, immediately downstream of Akt, by PDGF (10 ng/ml, 5 min). Consistent with a role for Akt in strain-induced matrix elaboration, dominant-negative Akt also successfully blocked strain-induced upregulation of col1A1 transcript levels (Figure 5C) and collagen I protein secretion (Figure 5D).

Conversely, we studied the effects of overexpression of constitutively active Akt (AktDD) on collagen I production in MC. Overexpressed AktDD (Figure 6A) significantly increased col1A1 transcript upregulation as assessed by reverse-transcription PCR (data not shown). This correlated with increased promoter activation, seen as an increase in luciferase activity of the col1A1 promoter-luciferase construct (Figure 6B). Finally, transcript upregulation paralleled collagen I secretion (Figure 6C). Consequently, Akt is important in the baseline and strain-mediated upregulation and secretion of collagen I in MC.

Akt Is Activated in Glomeruli of Remnant Kidneys
A 5/6 nephrectomy in rats results in intraglomerular hypertension and progressive renal failure marked by accumulation of glomerular extracellular matrix and ultimate glomerulosclerosis (2,38). Reversal of increased glomerular hydrostatic pressure by agents such as angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists is protective in this model, preventing the progression of renal failure and limiting extracellular matrix expansion (2–4). Given our observations in vitro that Akt is activated by strain in MC, we sought to correlate these findings in this in vivo model. Surgeries were conducted as described in Materials and Methods. Glomeruli were isolated at 1 wk and 2 wk postoperatively, and phosphorylation of Akt on S473 was assessed by immunoblotting. Figure 7A shows that Akt activation is significantly increased in remnant glomeruli at 1 wk, whereas total levels of Akt remain unchanged (P < 0.05). Similar results were obtained at 2 wk. This is in agreement with our in vitro findings in MC. To further confirm the activation of Akt in remnant glomeruli, we used immunohistochemistry to identify S473-phosphorylated Akt in cortical sections. As can be seen in Figure 7B, increased phosphorylation on S473 is seen at 1 wk and 2 wk after 5/6 nephrectomy (green). Because glomeruli consist of a rich capillary network anchored by the mesangium, and because markers specific to MC are unavailable, we used the endothelial cell marker von Willebrand factor (red) to confirm glomerular localization of pAktS473 immunofluorescence (39).
LY294002 (20 μmol, 30 min pre-incubation). (A) Transcriptional activation of the col 1A1 gene was studied using a reporter construct in which the full-length promoter of the col 1A1 gene is placed upstream of the luciferase gene (28). MC were transfected with col 1A1-luc and pCMV-β-gal as described in Materials and Methods, stretched for 18 h, and luciferase and β-gal activities were assayed as described. β-gal activity was used to adjust for transfection efficiency. Strain induced a 2.4-fold increase in col 1A1 promoter activation (P < 0.01 versus con, n = 6), and this was significantly inhibited by LY294002 (P < 0.04 versus stretch, n = 6). (B) Collagen I protein secretion into the medium was assessed by ELISA after MC were stretched for 24 h. The strain-induced increase (P < 0.001 versus con, n = 6) in collagen I secretion was abrogated by PI3K inhibition (P < 0.001 versus stretch, n = 6).

TGF-β1 Is Important in Strain-Induced Collagen I Production

TGF-β plays an important role in stretch-induced matrix expression. Studies have previously shown that neutralizing antibodies to TGF-β or its associated latent TGF-β binding protein inhibit stretch-induced collagen I production, as assessed by transcript levels, in MC (8,40). Furthermore, recent studies have shown that TGF-β is able to activate the PI3K–Akt signaling pathway in various cell lines including hepatocellular carcinoma cells, MC, and mesenchymal cells (41–43). We thus performed experiments to assess whether strain-induced collagen I protein secretion also requires the autocrine action of TGF-β. MC were stretched for 24 h in the presence or absence of anti-TGF-β1 neutralizing antibody or control IgG, and collagen I secretion into the medium was assessed by ELISA. Figure 8 shows that strain-induced collagen I secretion was inhibited by neutralization of TGF-β1, whereas control rabbit IgG had no effect. Autocrine TGF-β1 production is thus an important factor in the secretion of collagen I protein in response to mechanical strain.

Discussion

Elevated intraglomerular hydrostatic pressure has been shown, both experimentally and clinically, to be an important hemodynamic determinant of progression of glomerular sclerosis and renal failure (1–3). Using an in vitro approach to model the effects of intraglomerular hypertension on MC, we showed that mechanical strain induced a rapid and sustained activation of Akt and that this was dependent on PI3K. Early events leading to Akt activation included transactivation of the EGF receptor, but neither Src kinase activation nor actin cytoskeletal integrity were required. PI3K/Akt signaling played a crucial role in the upregulation and secretion of the matrix protein collagen I. We confirmed that the effects of strain on Akt activation are relevant in vivo in a model of renal failure in the setting of intraglomerular hypertension, showing that Akt is activated in the glomeruli of remnant rats at early time points preceding the occurrence of glomerulosclerosis.

The activation of Akt by tyrosine kinase and G-protein coupled receptors in response to diverse stimuli is well-defined (44). Evidence is emerging that mechanical forces may also lead to Akt activation. In vitro, Akt is phosphorylated in endothelial cells by fluid shear stress (23) and by cyclic stretch in pericytes, osteoblasts, and aortic and bladder smooth muscle cells (24,25,27,45). In vivo, myocardial pressure overload and aortic stent implantation stimulate activation of Akt (46,47). The events leading to PI3K/Akt activation by mechanical strain, however, remain largely unexplored. We first investigated the role of the actin cytoskeleton in this regard given its importance in the stretch-induced activation of MAPK pathways, as we and others have published (9,32,48,49). Disruption of the actin cytoskeleton with cytochalasin D, however, did not inhibit strain-induced Akt activation. Similar results were seen with the Rho-kinase inhibitor Y-27632, which also disrupts F-actin cytoskeletal organization (data not shown). We have previously shown that both of these inhibitors block strain-induced Erk activation, and that Y-27632 prevents subsequent fibronectin upregulation and secretion (9). Taken together, these results demonstrate the existence of specific and parallel mechanisms whereby mechanical signals are transmitted in MC.

Because we have previously also observed a lack of cytoskeletal dependence on PDGF signaling to Erk (9), and because PDGF is a potent activator of Akt in MC (50), we postulated that transactivation of growth factor receptors might play a role in strain-induced Akt activation. We first tested the role of the PDGF receptor but found that its inhibition by AG1295 had no effect on Akt activation. EGF receptor transactivation has been shown to mediate signaling in response to stretch and other stimuli such as endothelin I (51–53), and it played a role in arginine vasopressin-induced PI3K activation and proliferation in MC (54). We next evaluated its potential role in strain-induced Akt activation. The
EGF receptor inhibitor AG1478 completely blocked Akt activation in stretched MC. How the EGF receptor might function to transmit mechanical signals, however, is not understood. Its activation is dependent on phosphorylation of different tyrosine residues, through autophosphorylation and through phosphorylation of distinct residues by Src kinase (55,56). Src is known to be activated by mechanical stress in endothelial cells, fibroblasts, cardiomyocytes, and fetal rat lung cells (57–61). Furthermore, arginine vasopressin-induced EGF receptor transactivation was mediated by the activation of c-Src and its association with the receptor (54). This led us to investigate the role of Src in strain-induced Akt activation. Using the Src inhibitor PP1, we found no significant inhibitory effect, raising the possibility that strain itself induces a conformational change in the EGF receptor, leading to its autophosphorylation and thus enabling it to act as a scaffold for the assembly of downstream signaling molecules. The EGF receptor has recently been shown to interact with PI3K in some settings (16,62). Further studies will need to be performed to elucidate the mechanism whereby mechanical strain leads to EGF receptor-dependent PI3K/Akt activation.

Akt signaling is involved in diverse cellular processes, including cell cycle progression, hypertrophy, and apoptosis (14,63,64). More recently, Akt has also been implicated in signaling matrix protein elaboration in some settings. In embryoid bodies, overex-

Figure 5. Dominant-negative Akt inhibits strain-induced collagen I production. (A) Using a retroviral method of infection, a heterogeneous population of MC stably expressing either GFP- or HA-tagged dominant-negative Akt (AktAAA) was created. Expression of AktAAA was confirmed by immunoblotting for GFP or HA and for total Akt. MC infected with empty vector served as controls. (B) MC expressing AktAAA or empty vector were treated for 5 min with PDGF (10 ng/ml) and phosphorylation of endogenous GSK-3, a downstream substrate of activated Akt, was assessed by immunoblot. Functional inhibition of Akt signaling by AktAAA was confirmed by its ability to block PDGF-induced GSK-3 phosphorylation. (C) MC infected with empty vector or AktAAA were stretched for 24 h. Increased collagen 1A1 transcript production, as assessed by reverse-transcription PCR, was inhibited by dominant-negative Akt (*P = 0.03 versus con, #P < 0.04 versus stretch, n = 3). (D) Collagen I protein secretion into the medium after 24 h of stretch, as measured by ELISA, was also inhibited in cells infected with AktAAA (*P < 0.01 versus con, #P < 0.04 versus stretch, n = 6).
pH 7.4. The patient's platelet count was normal, indicating no thrombocytopenia.

In conclusion, the patient's clinical symptoms, laboratory findings, and response to treatment support the diagnosis of a vasculitis associated with SLE.

References


in vivo by using a well-established model of chronic kidney disease marked by intraglomerular hypertension. In the 5/6 nephrectomized rat, increased Akt activation in glomeruli was clearly seen at time points that precede the usual appearance of significant glomerular injury (78). These results strongly support a role for Akt in the progression of chronic hemodynamic glomerular disease.

Figure 7. Akt activation is observed in remnant glomeruli. In the remnant model of chronic renal failure marked by intraglomerular hypertension, rats underwent 5/6 nephrectomy as described in Materials and Methods. Glomeruli were isolated from control and nephrectomized rats after 1 wk and 2 wk, and Akt phosphorylation on S473 assessed by immunoblotting or immunohistochemistry. (A) Remnant glomeruli showed a significant increase in Akt phosphorylation at both time points (*P < 0.05 versus control). (B) This was confirmed by immunohistochemistry, in which glomeruli were identified by endothelial cell staining for von Willebrand factor (red). Significantly increased Akt S473 phosphorylation was observed at 1 wk and 2 wk (green).

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tion was inhibited by TGF-β1. MC were stretched for 24 h in the presence or absence of a neutralizing TGF-β1 antibody or control rabbit IgG (200 ng/ml). Collagen I secretion into the medium was assessed by ELISA. Strain-induced collagen I secretion was inhibited by TGF-β1 neutralization, whereas the control Ig had no effect (*P < 0.04 versus control, #P < 0.05 versus stretch ± rabbit IgG, n = 3).

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