The small GTPase Rac-1 is a regulator of mesangial cell morphology and thrombospondin-1 expression

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Giehl K, Graness A, Goppelt-Struebe M. The small GTPase Rac-1 is a regulator of mesangial cell morphology and thrombospondin-1 expression. Am J Physiol Renal Physiol 294: F407–F413, 2008. First published November 28, 2007; doi:10.1152/ajprenal.00093.2007.—Thrombospondin-1 (TSP-1), which is synthesized by mesangial cells, is known for its anti-angiogenic activity and its ability to activate latent TGF-β. TSP-1 is upregulated in renal diseases associated with tissue remodeling. Therefore, we hypothesized that the expression of TSP-1 might be modulated by changes in cell morphology involving proteins of the Rho family. Spreading of mesangial cells after detachment and reseeding was characterized by the formation of lamellipodia and focal adhesions, pointing toward a Rac-1-mediated rearrangement of actin structures. Clustering of focal adhesion proteins was also observed in a model system of nocodazole-induced disruption of microtubules. These morphological alterations were impeded by pharmacological inhibition of Src family kinases, of the small GTPase Rac-1, or by downregulation of Rac-1 by siRNA. Upon cell spreading, TSP-1 was upregulated in the absence and much more prominently in the presence of serum, but also after nocodazole treatment. TSP-1 upregulation was controlled by activation of Src family kinases, ERK 1/2 and Rac-1, whereas activation of RhoA-ROCK signaling was not linked to TSP-1 induction. We thus provide evidence that TSP-1 expression is induced by common signaling pathways, which are activated by morphological alterations of renal mesangial cells or by soluble factors as contained in serum, and these pathways include Src family kinases, ERK 1/2 and Rac-1. Our data suggest that tissue remodeling activates gene expression of pathophysiologically relevant proteins such as TSP-1.

cytoskeleton; Src kinases; Rho proteins

THROMBOSPONDIN-1 (TSP-1) belongs to a family of matricellular glycoproteins, which are involved in the regulation of cellular functions in physiological and pathophysiological settings, including platelet aggregation, inflammation, wound healing, angiogenesis, and tumor growth (7, 10). In terms of renal disease, TSP-1 plays a crucial role as major endogenous activator of transforming growth factor-β (TGF-β) (26). As an activator of TGF-β, TSP-1 has been implicated in the development of different renal pathologies, such as inflammatory glomerular disease (14), tubulointerstitial fibrosis (44), diabetic nephropathy (50), or ischemic kidney failure (47). Additionally, due to its anti-angiogenic properties, increased expression of TSP-1 may contribute to the loss of microvasculature observed in progressive renal failure (29).

TSP-1 is expressed in resident renal cells, tubular epithelial cells, endothelial and mesangial cells. Most data are available with regard to mesangial cells, in which TSP-1 was detected in the eighties (40). However, the molecular mechanisms of the regulation of TSP-1 expression are still incompletely understood. In line with an increased expression of TSP-1 in diabetic nephropathy, elevated levels of glucose induce TSP-1 expression in mesangial cells (39). Glucose-mediated regulation of TSP-1 expression involves activation of protein kinase C (45) and is inhibited by NO-cGMP-dependent signaling (51). TSP-1 is induced by various growth factors, e.g., PDGF (30), insulin (1), TGF-β (e.g., Ref. 19), or ANG II (34). The ANG II-mediated TSP-1 regulation involves the mitogen-activated kinases p38 and JNK (34). Activation of p38 was also implicated in TSP-1 expression induced by TGF-β in pancreatic tumor cells (46) and vascular smooth muscle cells (32), which are closely related to mesangial cells. In vascular smooth muscle cells, mitogen-activated kinases ERK 1/2 also contribute to TSP-1 expression (32).

TSP-1 is upregulated in pathological settings of the kidney, which are characterized by extensive reorganization of the structure of the glomerulus. Remodeling implies changes in cell morphology and reorganization of cytoskeletal elements such as the F-actin cytoskeleton, the microtubular network, or cell-matrix adhesion complexes. The binding of extracellular matrix components to integrins leads to clustering and activation of focal adhesion proteins including focal adhesion kinase, paxillin, and associated proteins, e.g., Src family kinases (52). Members of the Rho family of GTPases, RhoA, Rac-1, and Cdc42, are major regulators of the cytoskeletal organization. They are implicated in signaling via focal adhesions leading not only to changes in cell morphology but also to alterations in gene expression. With regard to renal pathology, there is increasing evidence for a functional role of RhoA-ROCK (Rho-associated kinase) signaling (23, 42), whereas comparatively little is known about the role of other members of the Rho protein family. We showed earlier that morphological alterations and activation of RhoA strongly affect the expression of the profibrotic protein connective tissue growth factor (CTGF) in mesangial cells (9, 21). Both CTGF and TSP-1 are matricellular proteins, which are upregulated by TGF-β and implicated in renal pathologies associated with glomerular remodeling (50). Furthermore, CTGF and TSP-1 have been reported to promote the disassembly of focal adhesions and thus seem to be actively involved in tissue remodeling (13, 36). However, in contrast to CTGF, regulation of TSP-1 expression due to changes in cell structure has not yet been investigated. Thus we hypothesized that in addition to soluble factors, the
expression of TSP-1 is regulated by morphological alterations of mesangial cells which implicate the activation of Rho proteins.

To test the hypothesis that glomerular remodeling is not only the consequence of increased levels of soluble growth factors, but also affects the synthesis of regulatory factors such as TSP-1, we investigated the role of Rho proteins and the impact of alterations of the cytoskeleton on TSP-1 expression in rat glomerular mesangial cells.

We provide evidence that Src family kinases and the small GTPase Rac-1 are important mediators in the regulation of TSP-1 expression induced by growth factors as well as by changes in mesangial cell morphology.

**METHODS**

**Materials.** Nocodazole, PD98059, Y27632, LY294002, Rac-1 inhibitor (NSC23766) (17), PP2 and PP3 were from Merck Biosciences (Bad Soden, Germany). Appropriate solvent controls were included in all experiments.

**Cell culture.** Mesangial cells, obtained from Sprague-Dawley rats, were grown in DMEM containing 10% (vol/vol) FCS supplemented with 2 mM L-glutamine, 5 µg/ml insulin, 4.5 g/l glucose, 100 U/ml penicillin, and 100 µg/ml streptomycin.

**Western blot analysis.** TSP-1 and Rac-1 were detected in cellular homogenates by Western blot analysis as described (15) using 8% SDS-polyacrylamide gels for protein separation for TSP-1 and 15% SDS-polyacrylamide gels for Rac-1. The following primary antibodies were used: anti-TSP-1 (Ab-3, Merck Biosciences), anti-Rac-1 (Santa Cruz Biotechnology, Heidelberg, Germany), anti-tubulin (clone E7, developed by M. Klymkowsky, Developmental Studies Hybridoma Bank, developed under the auspices of the NICHD and maintained by the University of Iowa, Dept. of Biological Sciences, Iowa City, IA), and anti-vinculin (Sigma, Munich, Germany). Peroxidase-conjugated anti-mouse secondary antibody was obtained from Amersham Biosciences (Freiburg, Germany), and anti-goat secondary antibody was from Santa Cruz Biotechnology (Santa Cruz Biotechnology, Heidelberg, Germany), anti-tubulin (Sigma, Munich, Germany). Peroxidase-conjugated anti-mouse secondary antibody was obtained from Amersham Biosciences (Freiburg, Germany), and anti-goat secondary antibody was from Santa Cruz Biotechnology. Protein-antibody complexes were visualized by the enhanced chemiluminescence detection system (ECL, Amersham Biosciences). Intensities were quantified using AIDA software (Raytest, Straubenhardt, Germany). All TSP-1 values were related to vinculin or tubulin to control for equal loading and blotting.

**Immunocytochemistry.** F-actin fibers were visualized as described previously using Alexa Fluor 488-conjugated phallolidin (Molecular Probes, Leiden, The Netherlands) (21). Paxillin was detected by indirect immunofluorescence using anti-paxillin (Transduction Laboratories, Lexington, KY) and anti-mouse IgG conjugated with Alexa Fluor 488 as secondary antibody (Molecular Probes, Leiden, The Netherlands) (21). Paxillin was detected by indirect immunofluorescence using anti-paxillin (Transduction Laboratories, Lexington, KY) and anti-mouse IgG conjugated with Alexa Fluor 488 as secondary antibody (Molecular Probes, Leiden, The Netherlands) (21). Paxillin was detected by indirect immunofluorescence using anti-paxillin (Transduction Laboratories, Lexington, KY) and anti-mouse IgG conjugated with Alexa Fluor 488 as secondary antibody (Molecular Probes, Leiden, The Netherlands) (21). Paxillin was detected by indirect immunofluorescence using anti-paxillin (Transduction Laboratories, Lexington, KY) and anti-mouse IgG conjugated with Alexa Fluor 488 as secondary antibody (Molecular Probes, Leiden, The Netherlands) (21).

**Targeting of Rac-1 expression by siRNA.** Upon being seeded, mesangial cells were transfected with 100 nM siRNA using HiPerfect transfection reagent (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. siRNA specific for Rac-1 (5’-CCA CAC CAC UGU CCC AAU A) and the control siRNA for pGL3 luciferase were obtained from Eurogentec (Liege, Belgium). Cells were used for experiments 24 h after transfection. At this time, the amount of Rac-1 was reduced by more than 80% (see Figs. 3 and 5). Transfection of the cells with control siRNA did not significantly alter TSP-1 or Rac-1 protein expression compared with nontransfected cells (see Fig. 5).

**Determination of Rac-1 activity.** The GTP-bound form of Rac-1 was recovered from 600 µg of cell lysate by affinity precipitation using a GST-fusion protein carrying the Rac-1 binding domain of PAK1B as an activation-specific probe for endogenous Rac-1 as described in Ref. 43. Affinity-precipitated Rac-1 was detected by SDS-PAGE and Western blot analysis.

**Statistical analysis.** To compare different experiments, expression of TSP-1 in the absence of inhibitors was set to 100%. To compare multiple conditions, statistical significance was calculated by one-way ANOVA with Bonferroni post hoc tests. Two-sided paired Student’s t-test was used to compare two conditions. A value of P < 0.05 was considered to indicate significance.

**RESULTS**

**Interference with activation of Rac-1 or Src family kinases reduces mesangial cell spreading.** To analyze signal transduction pathways involved in morphological alterations and especially actin cytoskeleton rearrangements of renal mesangial cells, the spreading of cells on fibronectin was used as a model system. Therefore, mesangial cells were detached from the cell culture dish by trypsin treatment and reseeded on fibronectin-coated glass plates. Mesangial cell adherence to fibronectin-coated plates in the presence of 10% FCS was controlled visually by phase contrast analysis and was visualized by staining of filamentous actin (F-actin) with Alexa Fluor 488-conjugated phallolidin. The cells attached as round cells and then formed extended lamellipodia upon spreading within 2 h (Fig. 1A; Co). Spreading was associated with the formation of extended focal adhesions, which were visualized by immunostaining of the focal adhesion protein paxillin (Fig. 1B; Co).

![Image](http://ajprenal.physiology.org/)

**Fig. 1. Regulation of mesangial cell spreading.** Mesangial cells were seeded on fibronectin-coated glass plates for 2 h and then stained for filamentous actin (F-actin). Cells were pretreated with the Rac-1 inhibitor (Rac-IH, 100 µM) for 3 h and with 100 nM siRNA against Rac-1 (Rac-siRNA) overnight. The Src family kinase inhibitor PP2 (2 µM) and its inactive analog PP3 (2 µM) were added at the time of seeding. A: F-actin was detected by Alexa Fluor 488 phallolidin. B: focal adhesions were detected by antibodies directed against paxillin. Arrows indicate extended focal adhesions in control cells. Original magnification 1:40.
Inhibition of Src family kinases, nonreceptor kinases involved in cell-matrix interactions, by 2 μM PP2 prevented the formation of F-actin-rich lamellipodia (Fig. 1A; PP2). As a control for PP2, the cells were treated with PP3, an inactive analog, which did not affect formation of lamellipodia (Fig. 1A; PP3). Higher concentrations of PP2 than 2 μM completely inhibited cell adherence and cell spreading (data not shown). Besides reduced lamellipodia formation, PP2-treated cells exhibited very few focal adhesions (Fig. 1B).

To assess a possible function of Rac-1 in mesangial cell spreading and especially lamellipodia formation, two complementary approaches were used: inhibition of Rac-1 expression by siRNA and inhibition of Rac-1 activation by the inhibitor NSC23766. When the cells were pretreated with the Rac-1 inhibitor (Rac-IH) or with siRNA against Rac-1 (Rac-siRNA), the expanded lamellipodia disappeared or were markedly reduced, respectively (Fig. 1A; Rac-IH and Rac-siRNA), indicating that Rac-1 is essential for mesangial cell spreading. Concomitantly, the formation of focal adhesions was reduced in the presence of the Rac-1 inhibitor (Fig. 1B; Rac-IH). Together, these data pointed toward a role for Rac-1 and Src kinases in mesangial cell spreading as visualized by the formation of lamellipodia and focal adhesions.

**Rac-1 and Src family kinases regulate TSP-1 expression.** Spreading of mesangial cells was associated with a time-dependent upregulation of cell-associated TSP-1 expression, as shown by Western blot analyses (Fig. 2A). To analyze whether activation of Rho family GTPases is involved in this process, activation of Rac-1 and activation of the RhoA effector ROCK were inhibited. Preincubation of the cells with the Rac-1 inhibitor NSC23766 interfered with TSP-1 induction in a concentration-dependent manner (Fig. 2B). In contrast, interference with RhoA-ROCK signaling by the ROCK inhibitor Y27632 barely inhibited TSP-1 expression (Fig. 2B). To further analyze signaling pathways involved in induction of TSP-1 protein expression, which are related to focal adhesion formation upon cell spreading, cells were plated in medium containing different protein kinase inhibitors. A partial inhibition was detected, when activation of the MAP kinases ERK 1/2 was inhibited by the MEK inhibitor PD98059 (Fig. 2C). The used concentration of PD98059 (10 μM) was shown earlier to prevent activation of ERK 1/2 in mesangial cells (18). Furthermore, inhibition of phosphatidylinositol-3 kinase (PI-3 kinase) by LY294002 or inhibition of Src family kinases by PP2 significantly reduced the upregulation of TSP-1 expression by ~50% (Fig. 2C). Inhibition of Src family kinases did not reduce the strong basal Rac-1 activity observed in attached mesangial cells (Fig. 2D). These data indicated that Src family kinases were not located upstream of Rac-1 GTPases.

In the experiments presented in Fig. 2, mesangial cells were exposed to two different stimuli: fresh serum-containing medium and mechanical stimulation due to the process of spreading. To investigate the contribution of these two effects separately, thoroughly washed mesangial cells were plated in medium without serum. Compared with cells seeded in 10% FCS, attachment and spreading were less pronounced with less lamellipodia formation (Fig. 3A). The reduced formation of lamellipodia was indicative of a reduced activation of Rac-1. This was confirmed by determination of Rac-1 activity in cells cultured with or without 10% FCS for 2 and 4 h (Fig. 3B). Next, TSP-1 protein expression was analyzed 4 h after seeding in 0 and 10% serum, respectively. An upregulation of TSP-1 expression was detectable under both conditions but was much more prominent upon spreading in 10% FCS (Fig. 3C).

When mesangial cells were cultured for 3 to 4 days in cell culture dishes and then restimulated by addition of 10% FCS or fresh medium containing 10% FCS, cellular TSP-1 was up-regulated within 4 h (Fig. 3D, top). The involvement of Rac-1...
in this growth factor-dependent upregulation of TSP-1 was shown by the inhibitory effect of Rac-1 siRNA (Fig. 3D). Pretreatment of mesangial cells with the specific Rac-1 siRNA (Fig. 3D; R) reduced Rac-1 expression by ~80%, whereas the nonrelated siRNA directed against luciferase (Fig. 3D; L) did not reduce Rac-1 expression. Furthermore, pretreatment of mesangial cells with the inhibitor of Src family kinases PP2 reduced the FCS-mediated upregulation of TSP-1 expression by 80 ± 9% (n = 2, means ± half range; data not shown). These data indicated that the FCS-mediated upregulation of TSP-1 was regulated by signaling molecules, namely Rac-1 and Src family kinases, which were also implicated in the regulation of mesangial cell spreading.

Disruption of microtubules by nocodazole alters mesangial cell morphology and upregulates TSP-1. To further analyze the impact of morphological alterations on TSP-1 expression, mesangial cells were treated with nocodazole, which interferes with microtubule polymerization. Disruption of microtubules by compounds like nocodazole is an efficient method to activate Rho proteins independently of growth factors (24). When mesangial cells were treated with low concentrations of nocodazole (1 μM) for 2 h, the cells altered their morphology. Microtubules disassembled (data not shown) and the cells became elongated forming spike-like cell spanning stress fibers leading to the periphery as visualized by F-actin staining (Fig. 4A; Noco). Immunodetection of paxillin demonstrated that these F-actin fibers terminated in focal adhesions in the cellular periphery (Fig. 4A; Noco). In the presence of the inhibitor of Src family kinases PP2, the rearrangement of stress fibers and focal adhesions was markedly reduced (Fig. 4A; PP2 + Noco), indicating that active Src kinases are necessarily involved in actin and focal adhesion reorganization.

Fig. 3. Influence of FCS on cell spreading and TSP-1 expression. A: mesangial cells were thoroughly washed with PBS and then seeded in the presence or absence of FCS as indicated. F-actin was visualized by staining with Alexa Fluor 488 phalloidin 2 h after seeding. Original magnification 1:40. B: mesangial cells were allowed to adhere in the absence of FCS (0%) or the presence of 10% FCS for 2 and 4 h. Active Rac-1-GTP was determined by binding to GST-PAK. As a control, total Rac-1 was determined by Western blot analysis. C: mesangial cells were allowed to adhere in the absence of FCS (0%) or the presence of 10% FCS for 4 h and TSP-1 expression was analyzed by Western blotting. Control cells (Co) were homogenized before seeding. The blot is representative for 3 experiments with comparable results. D: mesangial cells were transfected with 100 nM siRNA against Rac-1 (R) or against luciferase (L). After 24 h, fresh medium containing 10% FCS was added for the times indicated. Cellular TSP-1 and Rac-1 were detected by Western blot analysis. Data shown are from one blot which was rearranged and are representative for 3 independent experiments.

Fig. 4. Interference of PP2 with nocodazole-induced signaling. A: mesangial cells were seeded for 24 h on glass plates and then treated with nocodazole (1 μM) for 2 h with or without pretreatment with PP2 (2 μM) for 30 min. F-actin was detected by Alexa Fluor 488 phalloidin and paxillin was detected by indirect immunofluorescence. Original magnification 1:100. B: mesangial cells were allowed to adhere in the absence of FCS (0%) or the presence of 10% FCS for 2 and 4 h. Active Rac-1-GTP was determined by binding to GST-PAK. As a control, total Rac-1 was determined by Western blot analysis. C: expression of TSP-1, as detected by Western blot analysis, was quantified by densitometry. Expression of TSP-1 in nocodazole-treated cells was set to 100%. The graph summarizes data ± SD of several experiments performed as outlined in B. In addition, it shows the effect of PD98059 on nocodazole-induced TSP-1 expression. **P < 0.01, nocodazole (Noco)/PP2 vs. Noco/PP3, n = 5. ***P < 0.001, Noco/PD98059 vs. Noco, n = 3 (PD, 10 μM).
Biochemical analysis revealed that nocodazole induced a rapid upregulation of TSP-1 protein expression within 2 h (Fig. 4B). Preincubation of mesangial cells with PP2 effectively inhibited TSP-1 expression (Fig. 4, B and C). However, the inactive compound PP3, which does not interfere with the activity of Src family kinases (3), also inhibited TSP-1 expression, although to a lesser extent. The molecular basis of this interference is not known, but was not due to solvent effects, as control cells were treated with appropriate solvent concentrations. Inhibition of ERK 1/2 by PD98059 reduced TSP-1 induction to a similar extent as did PP2 (Fig. 4C). These results provide evidence for active Src and ERK signaling pathways in the induction of TSP-1 by nocodazole.

**Rac-1 is critical for nocodazole-mediated upregulation of TSP-1.** Disruption of microtubules by nocodazole has been shown to activate RhoA-ROCK signaling (4, 20). In mesangial cells, inhibition of this pathway by the ROCK inhibitor Y27632 did not affect TSP-1 induction as shown in Fig. 5A. By contrast, TSP-1 expression was significantly reduced by 50% by 100 μM of the Rac-1 inhibitor NSC23766. A comparable reduction in TSP-1 expression was obtained when Rac-1 expression was downregulated by siRNA (Fig. 5B), confirming a role for Rac-1 in the regulation of TSP-1 expression. Transfection of the cells with nonrelated siRNA directed against luciferase did not affect nocodazole-mediated TSP-1 expression. Treatment of mesangial cells with nocodazole, however, did not affect the activity of Rac-1 as determined by pull down assays (data not shown), suggesting that the high basal Rac-1 activity significantly contributed to the induction of TSP-1 by nocodazole.

**DISCUSSION**

Structural remodeling of the glomerulus is characteristic of renal failure based on complex cell-matrix interactions. In mesangial cells bidirectional signaling occurs between adhesion molecules at the plasma membrane and the actin cytoskeleton (8). We could show in this report that activation of the small GTPase Rac-1 and of nonreceptor tyrosine kinases of the Src family is not only essential for mesangial cellular remodeling but at the same time regulates the synthesis of the anti-angiogenic factor TSP-1, a protein implicated in nephropathy as an endogenous activator of latent TGF-β.

Spreading of mesangial cells is a very dynamic process characterized by interactions between the extracellular matrix, integrins, and cytoskeletal components and is modulated by soluble factors. This was evident in our study when spreading of mesangial cells on fibronectin-coated glass plates was analyzed in the presence and absence of serum. Serum components strongly supported the formation of lamellipodia and the organization of integrin-associated focal adhesions. Clustering of integrins recruits adaptor and signaling molecules such as nonreceptor tyrosine kinases of the Src family (31, 38) to focal adhesions. In mesangial cells, inhibition of Src family kinases impaired the formation of focal adhesion as well as the extension of lamellipodia, pointing toward an tight and coordinated regulation of these morphological alterations. Furthermore, our study demonstrates that interference with Rac-1 activity affects not only the actin cytoskeleton but also the formation of cell-matrix adhesions in mesangial cells. The functional role of Rac-1 in mesangial cells was addressed by the use of a specific inhibitor of Rac-1 and an siRNA approach. Overexpression of dominant negative Rac-1 N17 was not applicable in the experimental set-up used here, as the mesangial cells overexpressing Rac-1 N17 were no longer able to spread (data not shown). Basal Rac-1 activity is thus indispensable for mesangial cell survival.

The same signaling pathways shown to regulate mesangial cell morphology were also involved in the regulation of TSP-1 synthesis. Thus far, TSP-1 expression has been shown to be induced by growth factors (7, 10). This was confirmed in our study by the upregulation of TSP-1 in FCS-treated mesangial cells. In addition, we could demonstrate that serum-induced expression of TSP-1 was dependent on the activity of Src family kinases and of Rac-1.

Disruption of microtubules by nocodazole activates small GTPases of the Rho family. Activation of RhoA was observed within minutes after nocodazole treatment (37) and is associated with formation of stress fibers as also observed in mesangial cells in this study. Signaling via RhoA-associated kinase (ROCK) and a shift in the ratio of G-actin and F-actin lead to the induction of gene expression as analyzed in detail in terms of regulation of CTGF expression (24, 25, 33). Activation of...
ROCK, however, was not involved in the regulation of TSP-1 expression as shown by the use of the ROCK inhibitor Y27632, which did not reduce TSP-1 induction. Recent reports show that activation of RhoA targets c-Src to focal adhesion (48) and that the RhoA binding proteins mDia1 and mDia2 couple RhoA and c-Src activation (41, 49). Thus nocardazole-induced TSP-1 expression most likely activates a RhoA-c-Src pathway and this signal transduction might explain that inhibition of Src family kinases by PP2 reduced nocardazole-induced TSP-1 expression in mesangial cells. Furthermore, nocardazole has been shown to activate the Rac-p21-activated kinase (PAK) module, which was linked to the activation of the Raf1-ERK signaling pathway (53). In our experiments, we did not observe activation of Rac-1 by nocardazole. Activation may have been obscured by the high basal Rac-1 activity in mesangial cells under standard cell culture conditions. Alternatively, the basal activity was already sufficient to contribute to nocardazole-mediated induction of TSP-1, which was evident by the effect of Rac-1 siRNA or pharmacological inhibition of Rac-1.

The MAP kinases ERK 1/2 are central molecules in multiple signaling networks, including Src kinases and Rac-1-dependent signaling, and are functionally related to gene expression as well as changes in morphology (e.g., Refs. 6, 28). In our study, inhibition of ERK 1/2 activation by PD98059 inhibited nocardazole- as well as FCS-mediated induction of TSP-1 expression, supporting the notion that common signaling pathways are induced by mechanical and soluble stimuli. The partial inhibition obtained in mesangial cells treated with PD98059 is in line with previous data obtained by Nakagawa et al. (35) related to TGF-β-induced TSP-1 expression. Moreover, it has been shown that Rac-1 or Src-ERK1/2 signaling activates the transcription of genes containing a serum response element (SRE) (22). An SRE was also characterized in the promoter of TSP-1 (16). In the context of our data, this suggests a role for serum response factor in TSP-1 regulation in mesangial cells. As a multifunctional matricellular protein, TSP-1 interacts with integrins and integrin-associated proteins and thereby activates different signal transduction pathways (10). It is interesting to note that TSP-1 has been shown to activate Rac-1 in fibroblasts (2). This pathway needs to be confirmed in mesangial cells, but might implicate a positive feedback loop.

Within the glomerulus, mesangial cells are exposed to combinations of mechanical forces and soluble mediators such as growth factors and inflammatory mediators as shown in various animal models (e.g., Refs. 12, 27). These extracellular constraints most likely affect mesangial cell morphology, and together with soluble stimuli contribute to the synthesis of functionally important proteins such as TSP-1. Our study is the first one to show common signaling pathways activated by soluble stimuli/factors present in serum and changes in cell morphology leading to the induction of TSP-1. Furthermore, Rac-1, which is a major regulator of the actin cytoskeleton in cell adhesion and migration, is essential for TSP-1 activation. This link between Rac-1 and TSP-1 provides a molecular explanation for previous reports, which showed that statins, which reduce the posttranslational modification of proteins of the Rho family, inhibited TSP-1 expression (5, 11). TSP-1 contributes to glomerular scarring by its anti-angiogenic action and by its activation of TGF-β, and at the same time its own expression may be influenced by the process of glomerular remodeling. It is assumed that this mutual interrelationship will also be relevant for other proteins involved in renal pathological conditions.

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