The Serum- and Glucocorticoid-Inducible Kinase Sgk-1 Is Involved in Pulmonary Vascular Remodeling

Role in Redox-Sensitive Regulation of Tissue Factor by Thrombin

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Abstract—The stress-responsive serum- and glucocorticoid-inducible kinase Sgk-1 is involved in osmoregulation and cell survival and may contribute to fibrosis and hypertension. However, the function of Sgk-1 in vascular remodeling and thrombosis, 2 major determinants of pulmonary hypertension (PH), has not been elucidated. We investigated the role of Sgk-1 in thrombin signaling and tissue factor (TF) expression and activity in pulmonary artery smooth muscle cells (PASMC). Thrombin increased Sgk-1 activity and mRNA and protein expression. 

H$_2$O$_2$ similarly induced Sgk-1 expression. Antioxidants, dominant-negative Rac, and depletion of the NADPH oxidase subunit p22phox diminished thrombin-induced Sgk-1 expression. Inhibition of p38 mitogen-activated protein kinase, phosphatidylinositol 3-kinase 3-kinase, and phosphoinositide-dependent kinase-1 prevented thrombin-induced Sgk-1 expression. Thrombin or Sgk-1 overexpression enhanced TF expression and procoagulant activity, whereas TF upregulation by thrombin was diminished by kinase-deficient Sgk-1 and was not detectable in fibroblasts from mice deficient in sgk1 (-/-). Similarly, dexamethasone treatment failed to induce TF expression and activity in lung tissue from sgk1 (-/-) mice. Transcriptional induction of TF by Sgk-1 was mediated through nuclear factor κB. Finally, Sgk-1 and TF proteins were detected in the media of remodeled pulmonary vessels associated with PH. These data show that thrombin potently induces Sgk-1 involving NADPH oxidases, phosphatidylinositol 3-kinase, p38 mitogen-activated protein kinase, and phosphoinositide-dependent kinase-1, and that activation of nuclear factor κB by Sgk-1 mediates TF expression and activity by thrombin. Because enhanced procoagulant activity can promote pulmonary vascular remodeling, and Sgk-1 and TF were present in the media of remodeled pulmonary vessels, this pathway may play a critical role in vascular remodeling in PH.
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Key Words: sgk-1 ■ thrombin ■ tissue factor ■ NADPH oxidase ■ coagulant activity ■ pulmonary vascular remodeling ■ pulmonary artery smooth muscle cells ■ nuclear factor κB

Pulmonary hypertension (PH) is a complex progressive disorder characterized by remodeling of the lung vasculature with hypertrophy of the media. Moreover, a prothrombotic state is frequently associated with PH, which may be propagated by enhanced thrombogenicity of the injured pulmonary vascular wall, increased thrombin activity, and an imbalance between prothrombotic and antithrombotic factors. The observation that antithrombotic treatment can improve the survival rate in patients with PH points toward the importance of thrombosis in the pathogenesis of this disorder. Thrombin is generated by a series of proteolytic events that initiated when tissue factor (TF) binds to plasma factor VIIa. Interestingly, thrombin has been described to regulate its own generation by promoting TF transcription and TF-dependent procoagulant activity in several cell types including vascular cells by involving mitogen-activated protein kinases (MAPKs), the phosphatidylinositol 3-kinase (PI3K) pathway, and reactive oxygen species (ROS).

The serum- and glucocorticoid-inducible kinase-1, Sgk-1, is a known downstream effector of the PI3K cascade. Sgk-1 belongs to the “AGC” family of serine-threonine kinases and shares approximately 45% to 55% homology with Akt in its catalytic domain. In contrast to Akt, Sgk-1 is also regulated at the transcriptional level in response to various hormones, growth factors, and extracellular stresses in a cell type–dependent manner, allowing Sgk-1 to be available for its targets only when needed. Sgk-1 was originally cloned from murine mammary tumor cells as a glucocorticoid-responsive gene. Human Sgk-1 was subsequently cloned as a cell volume-sensitive gene upregulated by hypertonic cell shrinkage. Increasing evidence
suggests that expression, enzymatic activity, and cellular localization of Sgk-1 are regulated in response to various stimuli controlling not only cell volume and epithelial transport, but also cardiac action potential and cell proliferation, survival, and apoptosis.7,8 Excessive transcription of Sgk-1 has been shown to parallel diabetic nephropathy,10 glomerulonephritis,11 hepatic cirrhosis,12 pulmonary fibrosis,13 and polymorphisms of the Sgk-1 gene correlated with hypertension.14 Despite the wide tissue distribution of Sgk-1 and its sensitivity to various stimuli, the role of Sgk-1 in the cardiovascular and pulmonary system remained ill defined. Because heparin, an inhibitor of thrombin formation, has been shown to decrease Sgk-1 mRNA in aortic smooth muscle cells,15 we hypothesized that Sgk-1 may play a role in thrombin signaling in human pulmonary artery smooth muscle cells (PASMC), the main cell type involved in PH. We found that Sgk-1 is activated and induced by thrombin, that it regulates TF expression and activity in PASMC, and that it is present in remodeled pulmonary vessels with media hypertrophy associated with PH.

Materials and Methods

Reagents
Deoxyctydine 5'-α32P-triphosphate (3000 Ci/mmol) was from Amersham. Human α-thrombin was from Hemochrom Diagnostika. All other chemicals were from Sigma.

Animal Experimentation
Mice deficient in Sgk-1 (sgk1−/−) were generated as described.16 Wild-type (sgk1+/+) and sgk1−/− (6 weeks old) mice were treated with dexamethasone (10 μg/g body weight) for 4 days or vehicle (daily SC injection) and euthanized. Lungs were dissected and snap frozen in liquid nitrogen.

Cell Culture
Human PASMC were from Cambrex and cultured in the medium provided as recommended. PASMC were serum deprived for 24 hours before stimulation with thrombin (3 U/mL).17 Because PASMC do not efficiently express luciferase constructs, A7r5 rat smooth muscle cells (rSMC) were used for reporter gene assays, as described.17 Fibroblasts harvested from the tail of sgk1−/− and sgk1+/+ mice were identified by positive staining for fibronectin and used in experiments as described.16

Plasmids and Transfections
Expression vectors encoding wild-type Sgk-1 (Sgk-1-WT) or kinase-deficient Sgk-1 (Sgk-1-DK) fused to green fluorescent protein (GFP) have been described.17 Expression vectors encoding mitogen-activated protein kinase kinase 3 (MKK3), kinase-deficient MKK3 (MKK3KD), myc-tagged phosphoinositide-dependent kinase-1 (PDK1), kinase-deficient (K111A/D223A) PDK1 (PDKKD), myc-tagged RacT17N or RacG12V, and the TF promoter constructs pTF636 and pTF111 have been described.17,18 PCR-directed mutagenesis was performed on pTF636 to generate pTF636Em with mutations in all AP-1 and EGR-1 sites and on pTF636NFm with a mutated nuclear factor κB (NFκB) site. NFκB-Luc was from Promega. The siSTRIKE U6 Hairpin Cloning System (Promega) was used to create vectors encoding for small interference RNA (siRNA) targeting p22phox or for unspecific, nonsilencing siRNA. Transfection of PASMC or rSMC and luciferase assays were performed as described.17 The transfection efficiency was controlled by fluorescence microscopy and Western blot analysis revealing on average 40%.

Procoagulant Activity Assay
The surface procoagulant activity was evaluated in PASMC transfected with Sgk-1-WT and Sgk-1-DK expression vectors or in extracted lung protein as described.19,20

Immunoprecipitation and Sgk-1 Activity Assay
PASMC were transfected with an Sgk-1 expression vector and stimulated for different time points with 3 U/mL thrombin. Immunoprecipitation was performed using a GFP antibody as described.21 Sgk-1 activity assay (Stressgen) was performed according to the instructions of the manufacturer.

Immunofluorescence
Immunofluorescence was performed as described.22 The p50 antibody was from Santa Cruz Biotechnology, the GFP antibody and the secondary antibodies coupled to Alexa 488 or 594 were from Mobitech.

Immunohistochemistry
Archival lung tissue was obtained from patients with secondary PH with and without signs of pulmonary vascular remodeling or from lung tumor resections lacking apparent pulmonary vascular remodeling. Tissue was formalin fixed under vacuum and paraffin embedded. Immunohistochemistry was performed using the LSAB2 HRP Systems (Dako) and antibodies against Sgk-1 (Upstate), TF (American Diagnostica), and actin (Dako). Counterstaining was performed using Hemalum.

Northern Blot Analysis
Total RNA was isolated as described.17 RNA (10 μg) was subjected to Northern blot analysis, and hybridizations were performed with 32P-labeled DNA fragments for human Sgk-1 (1338 bp), human TF (1820 bp),17,19 or 18S.

Western Blot Analysis
Western blot analysis was performed as described.17 The antibody against human TF (3D1) was provided by Dr T. Zooncheck (Genentech). The antibody against mouse TF was from American Diagnostica. The antibody against pSer422-Sgk-1 was provided by Sir P. Cohen (Dundee University). The antibody against pThr256-Sgk-1 was from Santa Cruz Biotechnology. The antibody against p22phox was provided by Dr D. Roos (University of Amsterdam, Sanquin Research, and Landsteiner Laboratory, Academic Medical Center, Amsterdam, The Netherlands). The antibodies against actin or the c-myc epitope were from Sigma.

Statistical Analysis
Values are presented as means±SD. Results were compared by ANOVA for repeated measurements followed by Student–Newman–Keuls t test. P<0.05 was considered statistically significant.

Results
Thrombin Stimulates Expression and Activity of Sgk-1
To investigate whether Sgk-1 is regulated by thrombin, PASMC were stimulated with thrombin and Sgk-1 mRNA and protein levels were determined. Sgk-1 mRNA levels were peaking after 0.5 hour of exposure (Figure 1A and 1B) and Sgk-1 protein levels peaked after 2 hour stimulation (Figure 1A and 1B). Pretreatment with cycloheximide or actinomycin D decreased Sgk-1 protein induction indicating that thrombin regulates Sgk-1 at the transcriptional level and via de novo synthesis (Figure 1C). To evaluate whether thrombin is also able to activate Sgk-1, PASMC were transfected with a vector encoding Sgk-1 and stimulated with thrombin for increasing time periods. Sgk-1 was immunoprecipitated and Western
blot analyses were performed to detect phosphorylation of Sgk-1 at serine 422 and threonine 256, which are required for full Sgk-1 activity. In both cases, thrombin rapidly stimulated the phosphorylation of Sgk-1 after only 1 minute of exposure (Figure 1D). Similarly, thrombin enhanced Sgk-1 enzyme activity within 1 minute (data not shown).

ROS Are Involved in Thrombin-Stimulated Sgk-1 Expression

ROS have been described as important signaling molecules in the response to thrombin. To determine whether Sgk-1 expression is mediated by ROS, PASMC were exposed to H2O2. Sgk-1 protein levels were maximally increased at a dose of 50 μmol/L (Figure 2A) and an exposure time of 2 hours (Figure 2B). Pretreatment with the antioxidants vitamin C and N-acetyl cysteine (NAC) or the nonspecific NADPH oxidase inhibitor diphenyleneiodonium (DPI) prevented induction of Sgk-1 by thrombin (Figure 2C). To further delineate the role of NADPH oxidases in this response, constitutively active (RacG12V) or dominant-negative (RacT17N) mutants of Rac, which is required for ROS production by NADPH oxidases, were expressed in PASMC. Active Rac further enhanced thrombin-stimulated Sgk-1 protein, whereas dominant-negative Rac inhibited Sgk-1 upregulation by thrombin (Figure 2D). Furthermore, transfection of siRNA targeting the NADPH oxidase subunit p22phox downregulated thrombin-stimulated Sgk-1 protein levels (Figure 2E).

Thrombin-Stimulated Sgk-1 Expression Involves p38 MAPK, PI3K, and PDK1

Because ROS and NADPH oxidases are known to activate MAPKs,5,19,23 we determined their involvement in the regulation of Sgk-1. Pretreatment of PASMC with the p38 MAPK (p38MAPK) inhibitor SB220025 (5 to 20 μmol/L) diminished thrombin-induced Sgk-1 (data not shown) with a maximal effect at 10 μmol/L (Figure 3A), a concentration that blocked p38MAPK phosphorylation by thrombin (data not shown). In addition, overexpression of MKK3, an upstream kinase of p38MAPK, enhanced Sgk-1 protein levels, whereas kinase-deficient MKK3 decreased thrombin-induced Sgk-1 protein (Figure 3B). In contrast, application of the c-jun N-terminal kinase (JNK) inhibitor SP600125 (5 to 20 μmol/L) did not significantly inhibit thrombin-induced Sgk-1 protein (Figure 3A). In addition, exposure to different doses of the PI3K inhibitor LY294002 (10 to 50 μmol/L) abolished Sgk-1 expression at a dose of 20 μmol/L (Figure 3A). Because the PI3K downstream target PDK1 has been shown to phosphorylate Sgk-1,24 we explored whether PDK1 is also involved in the control of Sgk-1 expression by thrombin. Thrombin rapidly phosphorylated PDK1, peaking at 2.5 minutes (data not shown). Overexpression of PDK1 increased Sgk-1 protein levels, whereas expression of kinase-deficient PDK1 blunted the effect of thrombin on Sgk-1 expression (Figure 3C). Furthermore, kinase-deficient PDK1 prevented induction of Sgk-1 by MKK3, suggesting that p38MAPK acts upstream of PDK1 in regulating Sgk-1 levels (Figure 3D).
Sgk-1 Is Involved in Tissue Factor Expression and Activity by Thrombin

Because thrombin has been shown to upregulate TF mRNA and protein in PASMC, we investigated the role of Sgk-1 in thrombin-stimulated TF expression and activity. Sgk-1 overexpression enhanced TF mRNA and protein levels and TF activity. In contrast, kinase-deficient Sgk-1 prevented thrombin-induced TF (Figure 4A through 4C). Furthermore, TF protein was not detected in fibroblasts from Sgk-1 knockout (sgk1−/−) mice stimulated with thrombin for 8 hours but was upregulated in sgk1+/+ cells (Figure 4D). To assess the involvement of Sgk-1 in vivo, TF expression and activity were determined in lungs from sgk1−/− or sgk1+/+ mice. Because thrombin stimulation induces multiple side effects and is thus not advisable for in vivo treatment, mice were treated with dexamethasone, known to induce Sgk-1 and TF. TF protein levels were enhanced in lung tissue derived from dexamethasone-treated sgk-1+/+ but not from sgk1−/− mice (Figure 4E). Similarly, TF activity in lung extracts derived from sgk1−/−, but not from sgk1+/− mice, was significantly enhanced by dexamethasone (Figure 4F).

NFκB Mediates Sgk-1–Dependent Tissue Factor Expression

To investigate the role of Sgk-1 in the transcriptional regulation of TF by thrombin, reporter gene assays were performed in the presence of Sgk-1-WT or Sgk-1-DK using luciferase constructs containing 2 fragments of the TF promoter: pTF111 contains the promoter sequence −111 to +121 bp including a proximal enhancer with EGR-1 and Sp1 consensus sites. pTF636 contains in addition a distal enhancer 121 bp including a proximal enhancer with EGR-1 and Sp1 consensus sites. pTF636 contains in addition a distal enhancer.

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activity was observed between pTF636 and pTF636AEm, whereas pTF636NFm-driven luciferase activity was significantly diminished (Figure 5H).

**Sgk-1 Is Expressed in Remodeled Pulmonary Vessels**

Finally, we assessed whether Sgk-1 is also associated with pulmonary vascular remodeling. To this end, lung tissue was obtained from patients with PH (n=5) or lung carcinoma (n=1) and evaluated for media hypertrophy as sign of pulmonary vascular disease. Immunohistochemistry revealed that Sgk-1 was expressed in the bronchial epithelium (Figure 6A), as described. In addition, in all tissue samples with apparent vascular disease (n=3), strong expression of Sgk-1 was found in the media of small vessels (Figure 6A). In contrast, in tissue samples lacking vascular remodeling (n=3), Sgk-1 protein was almost undetectable in the smooth muscle cell layer (Figure 6B). Similarly, Sgk-1 could not be detected in airway smooth muscle (Figure 6A), suggesting that Sgk-1 is specifically expressed in remodeled pulmonary vessels. In addition, TF protein colocalized with Sgk-1 in the media of small vessels (Figure 6C), further emphasizing a role of Sgk-1 in pulmonary vascular remodeling.

**Discussion**

In this study, we identified Sgk-1 as a novel element in the regulation of TF expression and activity by thrombin which required activation of NFκB, whereby thrombin induced Sgk-1 by stimulating NADPH oxidases, PI3K, p38MAPK, and PDK1. This was based on the following findings. (1) Thrombin stimulated phosphorylation as well as de novo synthesis of Sgk-1. (2) Sgk-1 induction by thrombin was redox sensitive and diminished by dominant-negative Rac and depletion of p22phox. (3) Inhibition of PI3K, MKK3/p38MAPK, and PDK1 prevented Sgk-1 induction by thrombin. (4) Sgk-1 overexpression increased TF expression and activity, whereas TF expression and activity were diminished in PASMC expressing kinase-deficient Sgk-1, in cultivated sgk1−/− cells or in lungs derived from sgk1−/− mice treated with dexamethasone. (5) Sgk-1 and thrombin enhanced degradation of IkB and promoted NFκB nuclear translocation and activity. (6) Sgk-1−/− induced TF promoter activity was diminished by dominant-negative IkBα or a mutation in its NFκB binding site. Because Sgk-1 and TF proteins were detected in the media of vessels with pulmonary vascular disease these pathways may be involved in pulmonary vascular remodeling in PH.

**Thrombin Stimulates Sgk-1 Activity and Expression**

Pulmonary vascular remodeling is a common complication of PH and is associated with in situ thrombosis and increased procoagulant activity. The coagulation factors thrombin and TF have been shown to interact with the vascular wall and to activate signaling pathways and gene expression thus being key players in promoting vascular remodeling processes.

In this study, we identified Sgk-1 as a target for thrombin. In PASMC, thrombin rapidly phosphorylated overexpressed
as well as endogenous (data not shown) Sgk-1 at serine 422 and threonine 256, which is required for activation of Sgk-1.24 In addition, thrombin transiently enhanced Sgk-1 mRNA and protein levels, consistent with previous findings that Sgk-1 is regulated at the level of activity and expression.7,8

NADPH Oxidases and ROS Mediate Sgk-1 Upregulation by Thrombin

Our results further indicate that ROS are critically involved in the upregulation of Sgk-1 because H2O2 time- and dose-dependently enhanced Sgk-1 levels similar to previous observations in murine mammary glandular cells.28 Consistently, the antioxidants vitamin C and NAC as well as the unspecific NADPH oxidase inhibitor DPI diminished thrombin-induced upregulation of Sgk-1 expression in PASMC. In addition, depletion of the NADPH oxidase subunit p22phox or dominant-negative Rac, known to inhibit thrombin-induced ROS formation,17,19,22 prevented upregulation of Sgk-1 by thrombin, indicating that NADPH oxidases are the source of ROS promoting induction of Sgk-1. Similarly, RacG12V activated Sgk-1 in MDCK cells, although RacT17N failed to prevent activation of Sgk-1 in response to integrins.29

Thrombin-Induced Sgk-1 Is Controlled by PI3K, PDK1, and p38MAPK

Our studies further showed that MKK3 and p38MAPK contribute to upregulation of Sgk-1 by thrombin. Similarly, p38MAPK was involved in Sgk-1 upregulation by hyperosmotic stress and transforming growth factor-β in different cell types7,8,10,12,13,30 and by interleukin 6 in cholangiocarcinoma.31 Because p38MAPK can be activated by thrombin involving ROS and NADPH oxidases,19,23 stimulation of NADPH oxidases by thrombin may activate p38MAPK and subsequently induce Sgk-1. Although inhibition of ERK1/2 also reduced Sgk-1 consistent with previous findings,32 relatively high doses of PD98059 were required to downregulate Sgk-1 (data not shown). Together with the observation that JNK inhibition did not affect Sgk-1 induction by thrombin, these data further emphasize the role of p38MAPK in regulating Sgk-1 expression by thrombin.

In addition, inhibition of PI3K blunted Sgk-1 upregulation by thrombin, consistent with previous observations.21,30 Furthermore, PDK1, a downstream kinase of PI3K, which can phosphorylate Sgk-1,23 was also involved in thrombin induction of Sgk-1. Thus, in addition to PI3K and p38MAPK, which have been shown to both activate and induce Sgk-1,7,8,21,24,31,33 our data indicate that PDK1 is able to activate and upregulate Sgk-1. Because activation of PI3K and PDK1 was shown to be redox sensitive,33,34 MKK3-stimulated Sgk-1 expression was inhibited by kinase-deficient PDK1 in PASMC, and LY294002 inhibited phosphorylation of PDK1, but not of p38MAPK, in PASMC (data not shown), our results suggest a model whereby thrombin induces Sgk-1 via stimulation of NADPH oxidases and activation of PI3K or MKK3/p38MAPK followed by PDK1 activation (Figure 7). This model is further supported by our previous observations.
that inhibition of MKK3 prevents thrombin-stimulated PDK1 phosphorylation.\textsuperscript{18}

Although to date only limited data exist with regard to the transcriptional machinery regulating Sgk-1,\textsuperscript{7} it has been suggested that p38MAPK either directly or indirectly activates a Sp1 transcription factor protein complex on the Sgk-1 promoter.\textsuperscript{30} Because Sp1 is redox sensitive and can be activated by thrombin\textsuperscript{35} and PDK1,\textsuperscript{36} one might thus speculate that such a pathway also contributes to Sgk-1 regulation by thrombin in PASMC. However, further studies, which are beyond the scope of this study, are required to dissect the transcription factors involved in Sgk-1 regulation by thrombin.

**Tissue Factor Expression Is Mediated by Sgk-1 and Involves NF-κB**

We further showed that Sgk-1 is involved in the regulation of TF expression and activity by thrombin in PASMC. More-
over, TF expression was not detectable in 

fibroblasts

stimulated with thrombin or in lung tissue from 

mice

treated with dexamethasone, known to stimulate TF expression. The importance of Sgk-1 for thrombin-induced TF expression is further supported by our previous findings that TF mRNA and protein expression peak at 4 and 8 hours of stimulation with thrombin in PASMC, respectively, thus clearly following the upregulation of Sgk-1. In addition, treatment with the antioxidants vitamin C or NAC (data not shown), expression of dominant-negative Rac or depletion of p22phox prevented thrombin-induced TF expression in PASMC and other smooth muscle cells.17,19 Moreover, similar concentrations of PI3K or p38MAPK inhibitors were required to prevent TF upregulation by thrombin, whereas inhibition of ERK1/2 was not effective,18,19 and MKK3 and PDK1 have been shown to regulate TF expression by thrombin in PASMC.18

The importance of Sgk-1 for controlling TF expression was confirmed by our findings that Sgk-1 activates the NFκB pathway in PASMC by promoting IκκB degradation, thus allowing translocation of NFκB to the nucleus and that NFκB is required for Sgk-1–mediated activation of the TF promoter. Consistently, NFκB activation is sensitive to ROS and thrombin stimulation17 and contributes to TF expression. Recently it has been shown that Sgk-1 interacts with and activates IκK in MDA231 cells by phosphorylating serine 181, thus allowing the degradation of IκκB.37 Together with the findings that Rac is able to activate NFκB and to control TF expression,17 this suggests that activation of NADPH oxidases, PI3K, p38MAPK, and PDK1 is required for induction of Sgk-1, which then phosphorylates IκK, allowing degradation of IκκB, nuclear translocation of NFκB, and transcriptional activation of TF (Figure 7).

**Figure 7.** Proposed model of regulation of Sgk-1 and TF by thrombin. Thrombin stimulates ROS production by NADPH oxidases, leading via activation of PI3K, p38MAPK, and PDK1 to the induction of Sgk-1, resulting in activation of NFκB and increased TF expression and activity, which may lead to the formation of thrombin (dashed line) and thus a thrombogenic cycle.

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**Sgk-1 Is Associated With Pulmonary Vascular Remodeling**

In addition to our findings in cultivated PASMC, Sgk-1 protein was detected in the media of small vessels in lung tissue with vascular remodeling and PH. In contrast, Sgk-1 was not expressed in airway smooth muscle cells or in lung vessels without signs of pulmonary disease, suggesting the specific upregulation of Sgk-1 in remodeled vessels. Interestingly, Sgk-1 has been implicated in vasculogenesis in mouse development during embryonic days 8.5 to 12.5.26 Moreover, although Sgk-1 was increased in fibrotic lungs in particular in bronchi, lung epithelial cells or lung fibroblasts, Sgk-1 was barely detectable in nondiseased lung vessels.13 In addition, TF protein colocalized with Sgk-1 in the media of small vessels, further indicating that Sgk-1 and TF are involved in pulmonary vascular remodeling in PH.

Taken together, our data provide evidence that thrombin activates NADPH oxidases to generate ROS, thus leading to activation of PI3K or p38MAPK followed by activation of PDK1 and subsequent induction of Sgk-1. Furthermore, Sgk-1 is involved in upregulation of TF by activating NFκB. Because activated TF increases the formation of thrombin, and Sgk-1 and TF proteins were present in pulmonary vessels with vascular disease, this pathway may contribute to the prothrombotic state and vascular remodeling in PH.

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