Role of Apoptosis Signal-regulating Kinase 1 (ASK1) as an Activator of the GAPDH-Siah1 Stress-Signaling Cascade

Carlos A. Tristan‡††, Adriana Ramos‡†, Neelam Shahani‡†, Francesco E. Emiliani‡†, Hidenori Nakajima‡, Christopher C. Noeh*, Yoshinori Kato*, Tadayoshi Takeuchi*, Takuya Noguchi**, Hisae Kadowaki**, Thomas W. Sedlak†, Koko Ishizuka†, Hidenori Ichijo†**, and Akira Sawa†‡‡

From the Departments of †Psychiatry and ‡Neuroscience, §Radiology and Radiological Science, ¶Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287, the ‡‡Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka 599-8531, and the **Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-8654, Japan

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†‡1 These authors contributed equally to this work.

To whom correspondence should be addressed. Tel.: 410-955-4726; E-mail: asawa1@jhmi.edu.

Background: Apoptosis signal-regulating kinase 1 (ASK1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and seven in absentia homolog 1 (Siah1) are molecules associated with stress-signaling cascades.

Results: Identification of Siah1 as a substrate of ASK1 for activation of the GAPDH-Siah1 signaling cascade.

Conclusion: ASK1 triggers the GAPDH-Siah1 stress-signaling cascade.

Significance: This study provides insight into crosstalk among cell stress-signaling cascades.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) plays roles in both energy maintenance, and stress signaling by forming a protein complex with seven in absentia homolog 1 (Siah1). Mechanisms to coordinate its glycolytic and stress cascades are likely to be very important for survival and homeostatic control of any living organism. Here we report that apoptosis signal-regulating kinase 1 (ASK1), a representative stress kinase, interacts with both GAPDH and Siah1 and is likely able to phosphorylate Siah1 at specific amino acid residues (Thr-70/Thr-74 and Thr-235/Thr-239). Phosphorylation of Siah1 by ASK1 triggers GAPDH-Siah1 stress signaling and activates a key downstream target, p300 acetyltransferase in the nucleus. This novel mechanism, together with the established S-nitrosylation/oxidation of GAPDH at Cys-150, provides evidence of how the stress signaling involving GAPDH is finely regulated. In addition, the present results imply crosstalk between the ASK1 and GAPDH-Siah1 stress cascades.

A major mechanism for supplying cellular energy is glycolysis, in which glucose is catabolized to pyruvic acid via several enzymatic reactions. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)3 is a key enzyme in glycolysis, and so plays a major role in cellular energy supply (9). In addition to this classic concept, GAPDH is involved in many subcellular processes that include; DNA repair (10), membrane fusion, and transport (11), tRNA export (12), and cell death (13–19). The functional diversity of GAPDH is largely regulated by its subcellular localization and post-translational modifications (20). Recently studies have revealed that GAPDH can be oxidized and/or S-nitrosylated under stress conditions. Following this post-translational modification GAPDH is then translocated to the nucleus as a complex with Siah1, which has a strong nuclear localization signal (21). Only about 2% of total GAPDH, and a small pool of Siah1 participate in this mechanism, therefore a gain of function by these two molecules due to the specific posttranslational modifications, instead of their loss of functions, is crucial for this signaling cascade (21). In the nucleus, GAPDH modulates several proteins, in particular stimulating the catalytic activity of acetyltransferase p300/CREB-binding protein (CBP) that regulates transcription of various genes (22). Therefore, GAPDH may modulate homeostatic control by bridging energy supply (glycolytic pathway) to stress response (GAPDH-Siah1 cascade), which is finely regulated by post-translational modification (23–26).

Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase kinase kinase (MAPKKK) family. Although cellular substrates of ASK1 have not yet been fully studied, MAPKK4/7 (a kinase that phosphorylates JNK) and MAPKK3/6 that phosphorylates p38 are well-established substrates (27). ASK1 is activated in response to oxidative stress, endoplasmic reticulum stress, and other forms of cellular stress.
stress (28, 29). In addition, ASK1 plays pivotal roles in a wide variety of cellular responses, which include, but are not limited to, apoptosis (30, 31). Dysregulation of the ASK1 signaling pathway is closely linked to various diseases, such as cancer, cardiovascular diseases, diabetes, and neurodegenerative diseases including polyglutamine-induced neurodegeneration and Parkinson disease (29, 32–37).

The primary focus of the present study is to elucidate regulatory mechanisms of the GAPDH-Siah1 pathway. Here we report that ASK1 phosphorylates Siah1 and critically modulates the GAPDH-Siah1 pathway via direct protein interaction.

EXPERIMENTAL PROCEDURES

Chemicals, Plasmids, and Antibodies—All reagents were purchased from Sigma, unless noted otherwise. cDNA constructs for ASK1 were prepared as previously published (38, 39). GST-tagged-ASK1, GAPDH, and GST only constructs were cloned into the pGEX-4T-1 vector. GST-tagged-Siah1 constructs were cloned into the pGEX-5X-2 vector. HA- and FLAG-tagged-ASK1 constructs were cloned into the pcDNA3 vector. Myc-tagged-Siah1 constructs were cloned into the pRK5 vector. Myc-tagged-Siah1 and its threonine to alanine (T to A) mutants were prepared using the QuickChange site-directed mutagenesis kit according to the manufacturer’s protocol (Stratagene). Single mutants: M1, mutant with T7A and T111A; M2, mutant with T70A and T74A; M3, mutant with T108A and T112A; and M4, mutant with T235A and T239A. Double mutants were M1 + M3 and M2 + M4. Recombinant human GAPDH and human ASK1 (amino acids 649–946) were purchased from Sigma and Cell Sciences, respectively. A pSuper shRNA construct was used to knock down the expression of GAPDH (22). The p38 inhibitor SB203580 and JNK inhibitor SP600125 were purchased from Alexis Biochemicals. Antibodies for GAPDH (clones V18 and 6C5), Siah1 (P-18), ASK1 (F-9 and N-19), Myc (9E10), GADD 153 (B3), pThr (H-2), and p300 (C-20) were from Santa Cruz Biotechnologies; ASK1 (D11C9) and p-c-Jun (S73) were from Cell Signaling Technolo-
gies, HA (16B12) was from Clontech, GST (4C10) was from Biovision, Anti-FLAG antibody were eluted with HA peptide, elutes were subjected to a subsequent co-IP with anti-Myc antibody. The anti-HA antibody were incubated in binding buffer (0.1% Nonidet P-40, 0.5 mM DTT, 10% glycerol, 1 mM PMSF, and 2 μg/ml aprotinin in PBS) for 2 h at 4 °C. For measuring the effects of GAPDH on ASK1-Siah1 binding; 0, 1, and 3 (GAPDH:Siah1) molar concentrations of recombinant GAPDH and Siah1 were incubated in binding buffer, as mentioned above. To obtain recombinant GAPDH and Siah1 without a GST tag, GSH Sepharose-bound protein was released via thrombin digestion, dialyzed and purity analyzed by Western blot. All in vitro binding assays were done by GST pull-down via incubation with GSH-Sepharose beads (50% slurry) for 1 h, the samples were centrifuged at 4000 rpm for 1 min, washed three times in binding buffer, and resuspended in LDS sample buffer (Invitrogen) with 5% β-mercaptoethanol (Sigma) and then heated at 95 °C for 5 min. Western blot analysis of the protein precipitates was done using anti-GAPDH, Siah1, and GST antibodies.

In Vitro Kinase Assay—In vitro phosphorylation assays were performed for 30 min incubation of recombinant Siah1 and GST with or without human recombinant ASK1 (aa 649–946) protein (Cell Sciences) in kinase buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EDTA, 4 mM MgCl₂, 0.05 mM DTT, 40 ng/μl BSA, PIC1 and 2 (Sigma), and 10 mM [γ-32P]ATP). In vitro phosphorylation proteins were subjected to SDS-PAGE and examined by autoradiography.

RESULTS

GAPDH and Siah1 Bind to ASK1 and Form a Ternary Complex in Cells—GAPDH-Siah1 and ASK1 have been reported independently to play roles in several pathological brain conditions, and are commonly shown to be key stress mediators (26, 30, 41–43). Thus, we hypothesized that Siah1 and GAPDH might interact with ASK1 at the molecular level. To address this question, we examined mouse brain lysates, and we observed endogenous protein interactions of ASK1-Siah1 and ASK1-GAPDH by co-immunoprecipitation (Fig. 1A). These interactions were also seen and augmented in the presence of H₂O₂ (1 mM, 30 min) in HEK293 cells (Fig. 1B).

To test whether or not ASK1, Siah1 and GAPDH interact, we performed a sequential co-IP on cell lysates from HEK293 cells expressing ASK1, Siah1, and GAPDH: we observed ASK1-Siah1 and ASK1-GAPDH interaction are induced by treatment with H₂O₂ (Fig. 2, upper panel). Previous studies have reported stimulation of ASK1 activity by H₂O₂ (44). To determine if H₂O₂ stimulated ASK1 activity may induce complex formation

nuclear/cytosol extraction kit according to the manufacturer’s instructions.

In Vitro Binding Assays—For ASK1-Siah1 and ASK1-GAPDH in vitro binding assays with equal molar concentrations of GST-tagged-ASK1, Siah1, and His-tagged-GAPDH were incubated in binding buffer (0.1% Nonidet P-40, 0.5 mM DTT, 10% glycerol, 1 mM PMSF, and 2 μg/ml aprotinin in PBS) for 2 h at 4 °C. For measuring the effects of GAPDH on ASK1-Siah1 binding; 0, 1, and 3 (GAPDH:Siah1) molar concentrations of recombinant GAPDH and Siah1 were incubated in binding buffer, as mentioned above. To obtain recombinant GAPDH and Siah1 without a GST tag, GSH Sepharose-bound protein was released via thrombin digestion, dialyzed and purity analyzed by Western blot. All in vitro binding assays were done by GST pull-down via incubation with GSH-Sepharose beads (50% slurry) for 1 h, the samples were centrifuged at 4000 rpm for 1 min, washed three times in binding buffer, and resuspended in LDS sample buffer (Invitrogen) with 5% β-mercaptoethanol (Sigma) and then heated at 95 °C for 5 min. Western blot analysis of the protein precipitates was done using anti-GAPDH, Siah1, and GST antibodies.

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p38/JNK Experiments—HEK293 cells expressing HA-GAPDH, Myc-Siah1, and HA-ASK1 were treated with 10–20 μM p38 inhibitor (SB203580) or JNK inhibitor (SP600125) for 0.5–24 h. Cell lysates were subjected to co-IP followed by Western blot as previously described (21, 22).

Statistical Analysis—Two-group analysis was performed by t test (paired or unpaired as appropriate). A value of p < 0.05 is considered significant. All data were obtained from the results of three or four independent experiments.
we measured ASK1 activity via phosphorylation of ASK1 at Thr-845 and demonstrated that ASK1 activity correlated with ASK1-Siah1-GAPDH complex formation (Fig. 2, lower panel). These results suggest that ASK1, Siah1, and GAPDH form a complex in response to extracellular stressors.

**Direct ASK1-Siah1 Interaction and Modulation by GAPDH in Vitro**—GAPDH and Siah1 are known to bind directly (21). To characterize the interaction of Siah1 and GAPDH with ASK1 we purified recombinant proteins for *in vitro* binding studies. Incubation of recombinant Siah1 together with gluta-
thione S-transferase (GST) or GST-tagged-ASK1 (amino acids 1–940) demonstrated that Siah1 binds directly to the N-terminal region of ASK1 (Fig. 3A). In contrast, incubation of recombinant GAPDH together with GST or GST-tagged-ASK1 failed to demonstrate that GAPDH directly binds to ASK1 in vitro (data not shown). Given that stress has been demonstrated to induce direct binding of GAPDH and Siah1 (21), we hypothesized that GAPDH may augment ASK1-Siah1 binding. To determine if GAPDH modulated ASK1-Siah1 binding we performed in vitro binding assays with recombinant Siah1 and ASK1 (amino acids 1–940) in the presence of increasing amounts of GAPDH. These studies demonstrated that a three molar equivalent of GAPDH augmented ASK1-Siah1 direct binding (Fig. 3B).

**FIGURE 3. ASK1 directly binds to Siah1 and is modulated by GAPDH in vitro.** A, recombinant GST-ASK1 (aa 1–940) or GST were incubated with recombinant Siah1 in vitro and subjected to GST pull-down, followed Western blot with an anti-Siah1 antibody. Input is the starting material for immunoprecipitation (IP). The input lanes were probed as follows: GST-Siah1 with an anti-Siah1 antibody; GST-ASK1 and GST with an anti-GST antibody. The arrow indicates GST-ASK1 (1–940). B, GAPDH facilitates ASK1-Siah1 binding in vitro. ASK1-Siah1 interaction was assessed by incubating recombinant GST-ASK1 (aa 1–940), or GST with recombinant Siah1 and GAPDH protein at 0, 1, or 3 (GAPDH:Siah1) molar concentrations, followed by to GST pull-down. Precipitates were analyzed by Western blot with an anti-Siah1 antibody. Input is the starting material for IP. ASK1-Siah1 binding was quantified by densitometric analyses (t test; *, p < 0.05 versus Siah1).

**ASK1 Phosphorylates Siah1**—Given that Siah1 was determined to directly bind within the kinase domain of ASK1, we hypothesized that Siah1 might be a novel substrate of ASK1 phosphorylation. To investigate whether ASK1 could phosphorylate Siah1, we conducted phosphorylation studies using recombinant proteins in vitro. Phosphorylation of GST-tagged Siah1, but not GST, was observed when Siah1 was incubated with the constitutively active kinase domain of ASK1 (amino acids 649–946) (Fig. 4A). Since ASK1 has been reported to phosphorylate substrates carrying the (S/T)XXS/T) consensus motif (45, 46), we examined the amino acid sequence of Siah1 and identified four potential phosphorylation sites which we designated M1, M2, M3, and M4, respectively (Fig. 4B). To characterize the ASK1 phosphorylation sites on Siah1, we generated mutants of Siah1 with point mutations (threonine to alanine substitution) in each consensus sequence and examined how these mutations affected phosphorylation of Siah1 by ASK1 via in vitro kinase assays (Fig. 4C). Amino acid substitution in M2 led to significant decreases in the phosphorylation of Siah1 by ASK1. However, Siah1 phosphorylation by ASK1 was reduced the most when we introduced mutations at both M2 and M4 (M2/H11001M4) (Fig. 4C). These data indicate that Thr-70/Thr-74, and Thr-235/Thr-239 together are the critical sites in Siah1 phosphorylated by ASK1. We next addressed whether the phosphorylation of Siah1 was induced by ASK1 in cells. To address this question we transfected HEK293 cells with a constitutively kinase activity-positive (CA) ASK1 (amino acids 649–1375) together with WT Siah1 or M2/H11001M4 mutant Siah1 lacking ASK1 phosphorylation sites. The phosphorylation levels of Siah1 were examined by using an anti-Myc antibody after enrichment of proteins with phosphorylation at threonine residues (pThr proteins) by immunoprecipitation with an anti-phosphothreonine antibody. We observed a marked increase in the signal from WT Siah1, but not M2/M4 mutant Siah1, only when co-transfected with CA-ASK1, indicating that Siah1 is phosphorylated at M2/M4 sites by ASK1 (Fig. 4D). At the same time, GAPDH binding with Siah1 was diminished by the
replacement of the key threonine residues (the phosphorylation sites of Siah1 by ASK1) to alanine (Fig. 4E): these results suggest that ASK1 kinase activity is crucial for the binding of GAPDH and Siah1, that is, the activation of the GAPDH-Siah1 stress-signaling cascade.

**ASK1 Augments GAPDH-Siah1 Binding in Cells**—We next addressed whether ASK1 modulates GAPDH-Siah1 signaling. Thus, we introduced wild-type (WT) ASK1, kinase-dead (KD) ASK1 that was generated with one amino acid substitution at 709 (K709M), and constitutively kinase activity-positive (CA) ASK1 (see above) in cells, respectively (39). We then examined how these distinct forms of ASK1 affected GAPDH-Siah1 binding.

**Replacement of the Key Threonine Residues (Phosphorylation Sites of Siah1 by ASK1) to Alanine**

We previously reported that sodium nitroprusside (a nitric oxide donor) could affect GAPDH-Siah1 binding by S-nitrosylation of GAPDH (21), which was used as a reference of the binding change. Introduction of WT ASK1 dramatically augmented GAPDH-Siah1 binding, which was considerably greater than the change elicited by sodium nitroprusside in total cell lysates (Fig. 5, lower panel). When kinase activity of ASK1 was selectively reduced (KD ASK1), such augmentation was also reduced. Consistent with this observation, introduction of CA ASK1 also dramatically augmented GAPDH-Siah1 binding. In all the conditions, the levels of GAPDH-Siah1 binding were normalized by the levels of Myc-Siah1 (Fig. 5, upper panel).

**FIGURE 4. ASK1 phosphorylates Siah1 in cells and in vitro.**

**A**. Phosphorylation of Siah1 by ASK1 in vitro. In vitro phosphorylation assays were performed by incubation of recombinant GST-Siah1 or GST with constitutively active GST-ASK1 (aa 649–946) in the presence of [γ-32P]ATP. B, four consensus (S/TXXS/T) phosphorylation motifs for ASK1 in Siah1 protein: designated as M1, M2, M3, and M4. C, M2 + M4 mutant Siah1 shows a significant reduction of phosphorylation by ASK1 in vitro. Labeling with [γ-32P]ATP was carried out in vitro with recombinant GST-ASK1 (aa 649–946), along with several mutant (M1, M2, M3, M4, M2 + M4, and M1 + M3) Siah1, followed by detection of Siah1 phosphorylation by autoradiography. Input is GST-ASK1 (aa 649–946) and Siah1 in starting material. Siah1 phosphorylation levels were quantified by densitometric analyses (t test; *, p < 0.05 versus WT Siah1). D, phosphorylation levels of Siah1 by ASK1 in cells. HEK293 cells expressing constitutively kinase activity-positive (CA) ASK1 and Myc-Siah1 (WT-Siah1 or M2 + M4 mutant Siah1). Cell lysates were immunoprecipitated with anti-phosphothreonine antibody, and the immunoprecipitates were analyzed by Western blot with an anti-Myc antibody. Representative Western blots were shown: the levels of Siah1 phosphorylation were calculated after subdividing the band intensity of the Myc-Siah signal in immunoprecipitation (IP) by the intensity in the input [WT, 1; M2 + M4, 0.20 ± 0.17; WT and CA ASK1, 1.63 ± 1.00; M2 + M4 and CA ASK1, 0.40 ± 0.22, mean ± S.D., n = 3]. E, M2 and M2 + M4 Siah1 mutants exhibit reduced binding to GAPDH. HEK293 cells expressing WT-Siah1, M2 or M2 + M4 Siah1 mutants were treated with 1 mM H2O2 for 30 min. Cell lysates were immunoprecipitated with anti-Siah1 antibody and analyzed by Western blot with an anti-GAPDH antibody. Representative Western blots were shown: the levels of GAPDH were calculated after subdividing the band intensity of the GAPDH signal in IP by the intensities of the GAPDH and Siah in the input [WT, 1; M2, 0.89 ± 0.39; M2 + M4, 0.55 ± 0.35, mean ± S.D., n = 3].
FIGURE 5. ASK1 facilitates GAPDH-Siah1 binding in cells. Upper panel, ASK1 augmentS GAPDH-Siah1 binding in a kinase-dependent manner. Cell lysates of HEK293 cells expressing Myc-tagged wild-type (WT) Siah1 together with HA-WT ASK1, kinase-dead (KD) ASK1, or constitutively active (CA) ASK1, were immunoprecipitated with an anti-GAPDH antibody and analyzed by Western blot with an anti-Myc antibody (for Siah1). Input is the total cell lysates. Lower panel, sodium nitroprusside (SNP, a nitric oxide donor) elicits an augmented GAPDH-Siah1 binding. HEK293 cells overexpressing Myc-WT Siah1 were treated with SNP, cell lysates were immunoprecipitated with an anti-GAPDH antibody and immunoprecipitates were analyzed by Western blot with an anti-Myc (Siah1) antibody. Input is Myc-Siah1 in total cell lysates. Western blots were quantified by densitometric analyses. Y axis depicts the level of GAPDH-Siah1 binding that was normalized by the levels of Siah1. (t test; **, p < 0.01 and ***, p < 0.001 versus Mock).

FIGURE 6. GAPDH-Siah1 binding is independent of p38 and JNK signaling. GAPDH-Siah1-ASK1 interactions occur independent of p38 and JNK activation. A representative figure in which GAPDH-Siah1-ASK1 interactions were assessed in lysates from HEK293 cells treated with 10 μM p38-specific inhibitor (SB203580) followed by the immunoprecipitation (IP) and Western blot with indicated antibodies. Input is expression of HA-ASK1, HA-GAPDH and Myc-Siah1 in total cell lysates. B, representative figure in which GAPDH-Siah1-ASK1 interaction assessed in HEK293 cells treated with 10 μM JNK-specific inhibitor (SP600125) followed by the IP and Western blot with indicated antibodies. Input is expression of HA-ASK1, HA-GAPDH, and Myc-Siah1 in total cell lysates.

ASK1 as an Activator of GAPDH-Siah1 Signaling

ASK1-induced GAPDH-Siah1 Binding in Cells Is Independent of p38 and JNK Signaling—We considered the possibility that increased GAPDH-Siah1 binding could be affected by JNK and p38, two key kinases downstream of ASK1 (27). To test this idea, we used specific kinase inhibitors (SB203580 specific for p38 and SP600125 specific for JNK) and examined the effects on GAPDH-Siah1 binding in the presence of exogenous WT ASK1. Neither SB203580 nor SP600125 affected ASK1-GAPDH, ASK1-Siah1, and GAPDH-Siah1 interactions (Fig. 6, A and B), suggesting that ASK1-induced GAPDH-Siah1 binding occurs independent of p38/JNK signaling.

ASK1 Augments Nuclear Translocation of GAPDH and p300 Acetylation in Cells—The major event of activated GAPDH-Siah1 stress signaling is translocation of GAPDH (21). Thus, we questioned whether ASK1 might facilitate the nuclear translocation, and tested the effects of WT or KD ASK1. We observed robust levels of nuclear translocation of GAPDH in the presence of exogenous WT ASK1, whereas introduction of KD ASK1 did not elicit significant levels of GAPDH translocation (Fig. 7A). We further tested whether this translocation by ASK1 was related to phosphorylation of Siah1. In the presence of ASK1 the complex of GAPDH and mutant Siah1 (M2+M4 mutant; lacking ASK1 phosphorylation sites) displayed significantly reduced nuclear translocation compared with the complex of WT Siah1 and GAPDH (Fig. 7B). We then questioned whether these Siah1 mutations (M2+M4) critically affect nuclear functions in ASK1-triggered GAPDH-Siah1 stress signaling. Thus, we examined GAPDH-p300 binding and acetylation of p300, which have been established as good functional indicators in the nucleus (22). Western blot analysis revealed a significant decrease in p300-

GAPDH binding and acetylation of p300, which can be interpreted as a key consequence of reduction in nuclear translocation of GAPDH with the mutant Siah1 (Fig. 7C).

To address functional outcome downstream of P300-GAPDH, we applied H2O2 (1 mM, 30 min) to HEK293 cells and examined expression of PUMA, which is known as a representative target of P300-GAPDH activation (22). Under these conditions we observed a significant reduction of PUMA levels in the cells that expressed M2+M4 mutant Siah1 deficient in ASK1 phosphorylation sites, compared with the cells with wild-type Siah1 (Fig. 8). These results suggest that phosphorylation of Siah1 by ASK1 is likely to play a key role in the GAPDH-Siah1 signaling cascade and subsequent functional effects in the nucleus.

DISCUSSION

Cellular signaling in response to stressors is crucial in homeostatic control and survival of all organisms. Thus, crosstalk among signaling cascades and their finely tuned regulation are expected. In the present study, we show that ASK1, a representative stress kinase, regulates GAPDH-Siah1 signaling. We demonstrate that ASK1 binds with GAPDH and Siah1:
these bindings are augmented under oxidative stress and are likely to be the basis of this signal network. We have identified Siah1 is a novel substrate of ASK1, with Thr-70/Thr-74 and Thr-235/Thr-239, as the critical phosphorylation sites on Siah1. Phosphorylation by ASK1 was found to increase GAPDH-Siah1 binding, its nuclear translocation, and subsequent acetylation of nuclear P300 and PUMA expression. Thus far, a specific post-translational modification of GAPDH (S-nitrosylation/oxidation at Cys-150) had been established as an initial trigger of GAPDH-Siah1 signaling (21). In the present study we show that specific post-translational modifications of Siah1, the other partner of this complex, can also be a trigger of this cascade (Fig. 9). ASK1 is activated in response to various stressors, such as oxidative stress and endoplasmic reticulum stress, it is likely that Siah1 is subsequently phosphorylated and mediates stress signaling by forming a complex with GAPDH. Thus, this study establishes the notion that the GAPDH-Siah1 cascade is activated by more than one mechanism in the presence of various cellular stressors. This cascade is also inhibited by at least two mechanisms: both interaction with a cytosolic protein GOSPEL (47) and a set of deprenyl-related compounds (40). It is likely that the GAPDH-Siah1 cascade, which is crucial for homeostatic control, has multiple
mechanisms that regulate its initiation in both positive and negative ways. It will be interesting to clarify how these two distinct post-translational modifications (oxidation of GAPDH and phosphorylation of Siah1) are coordinated under different types of stress. Given that both ASK1 and GAPDH-Siah1 cascades are major routes of stress signaling, an important future question is to understand how GAPDH-Siah1 can also influence ASK1.

Here we demonstrate that ASK1 and GAPDH-Siah1 co-mediate stress signaling and up-regulate p300. It is known that p300 has multiple functions in different contexts: for example, in the heart p300 activation can lead to heart hypertrophy mediated by myocyte enhancer factor-2 (MEF2) (48), whereas in the brain p300 affects memory function via cAMP response element-binding (CREB) (49). Roles for stressors in these conditions are appreciated, but further molecular mechanisms remain to be elucidated. Thus, new generation of conditional knock-out mice or inducible transgenic models targeting these molecules will be crucial to test context-dependent crosstalk of ASK1 and GAPDH-Siah1. As far as we are aware, such mice are not available at present. Better understanding of the crosstalk between these two stress cascades (ASK1 and GAPDH-Siah1) may provide a more integrated and comprehensive picture of how our body responds to stressors in a context-dependent fashion and how disturbances of such mechanisms may lead to pathological conditions.

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FIGURE 9. Scheme to represent the mechanisms through GAPDH-Siah1 pathway is activated under stress.
ASK1 as an Activator of GAPDH-Siah1 Signaling

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