Thioredoxin Promotes ASK1 Ubiquitination and Degradation to Inhibit ASK1-Mediated Apoptosis in a Redox Activity-Independent Manner

Yingmei Liu, Wang Min

Abstract—It has been shown that thioredoxin (Trx) in a reduced form binds to and inhibits apoptosis signal-regulating kinase 1 (ASK1). Apoptotic stimuli such as tumor necrosis factor (TNF) and reactive oxygen species (ROS) activate ASK1 in part by oxidizing Trx (forming intramolecular disulfide between C32 and C35) to release Trx from ASK1. In the present study, we examined if Trx affects ASK1 protein stability and whether the redox activity of Trx is critical in regulating ASK1 activity. First, we showed that overexpression of the wild-type Trx (Trx-WT) in endothelial cells induced ASK1 ubiquitination and degradation. Trx-induced ASK1 ubiquitination/degradation could be blocked by ASK1 activators TNF and TRAF2. We then tested the single-mutation of Trx at the catalytic site C32 or C35 (Trx-C32S or Trx-C35S) and the double-mutation (Trx-CS). The results showed that the single mutants (but not Trx-CS) retained the binding activity for ASK1 and the ability to induce ASK1 ubiquitination/degradation. Unlike Trx-WT, Trx-C32S and Trx-C35S mutants constitutively bind to ASK1 even in the presence of hydrogen peroxide in vitro and TNF in vivo. Finally, we showed that the single mutants (not Trx-WT) significantly (n/H11005 4 and P/H11021 0.05) inhibited ASK1-induced JNK activation, caspase 3 activity, and apoptosis in TNF/ROS-resistant manner. Our data suggest that association of Trx with ASK1 through a single Cysteine (C32 or C35) is necessary and sufficient for Trx activity in inducing ASK1 ubiquitination/degradation leading to inhibition of ASK1-induced apoptosis. (Circ Res. 2002;90:1259-1266.)

Key Words: apoptosis signal-regulating kinase 1 • thioredoxin • apoptosis • ubiquitination • tumor necrosis factor-α
not bind to ASK1. Apoptotic stimuli (TNF, reactive oxygen species [ROS], or serum starvation) activate ASK1 in part by oxidizing Trx to release Trx from ASK1.\textsuperscript{16–18} These data suggest that Trx is a critical mediator in regulating ASK1 activity; however, the mechanism by which Trx inhibits ASK1 activity has not been determined.

In the present study, we examined the mechanism by which Trx inhibits ASK1 apoptotic activity in endothelial cells (ECs). We show that Trx induces ASK1 ubiquitination and degradation to inhibit ASK1-induced apoptosis. Furthermore, inhibition of ASK1 by Trx is not dependent on its redox activity. These results establish, for the first time, that redox activity of Trx is not required for its antiapoptotic function.

**Materials and Methods**

**Plasmids**

Mammalian expression plasmids for poly-Ub were provided by Dr Dirk Bohmann (University of Rochester, NY).\textsuperscript{18} Human thioredoxin cDNA was cloned by RT-PCR using total RNA from human umbilical vein ECs (HUVECs) with a pair of primers: the sense primer was 5'-AACATCATGTTGGAAAGCATGCAG-3' (the HindIII site is underlined), and the antisense primer was 5'-CTTCAGTTAGCTAATATTAT-3' (the XhoI site is underlined). The cDNA was confirmed by DNA sequencing. Mutations of C32S and C35S in Trx were introduced by recombination PCR\textsuperscript{8} and confirmed by DNA sequencing. Expression plasmids for Flag-tagged Trx were constructed into the Flag-vector.\textsuperscript{8}

**Cells and Cytokines**

Bovine aorta endothelial cells (BAECs) and HUVECs were purchased from Clonetics (San Diego, Calif). Human TNF was from R&D Systems (Minneapolis, Minn) and used at 10 ng/mL.

**Transfection**

Transfection of ECs was performed by Lipofectamine\textsuperscript{2000} according to Manufacturer's protocol (Gibco). Cells were cultured at 90% confluency in 6-well plates and were transfected with total 4-7 μg DNA constructs as indicated. Cells were harvested at 36 to 48 hours after transfection, and cell lysates were used for protein assays.

**Immunoprecipitation and Immunoblotting**

ECs after various treatments were washed twice with cold PBS and lysed in 1.5 mL of cold lysis buffer (50 mmol/L Tris-HCl, pH 7.6, 150 mmol/L NaCl, 0.1% Triton X-100, 0.75% Brij 96, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 2 mmol/L PMSF, and 1 mmol/L EDTA) for 20 minutes on ice. Protein concentrations were determined with a Bio-Rad kit. For immunoprecipitation to analyze protein interaction in vivo, 400 μg of cell lysate supernatant were incubated with 5 μg of the first protein-specific antiserum (eg, anti-Trx from Medical and Biological Laboratory) for 2 hours with oxidizing Trx from ASK1.\textsuperscript{16–18} These data suggest that Trx is a critical mediator in regulating ASK1 activity; however, the mechanism by which Trx inhibits ASK1 activity has not been determined.

In the present study, we examined the mechanism by which Trx inhibits ASK1 apoptotic activity in endothelial cells (ECs). We show that Trx induces ASK1 ubiquitination and degradation to inhibit ASK1-induced apoptosis. Furthermore, inhibition of ASK1 by Trx is not dependent on its redox activity. These results establish, for the first time, that redox activity of Trx is not required for its antiapoptotic function.

**GST-Trx Pull-Down Assay**

GST fusion protein preparation and GST pull-down assay were performed as described previously.\textsuperscript{8} Briefly, GST-Trx fusion proteins expressed in *Escherichia coli* XL-1 blue were affinity purified on glutathione-Sepharose beads (Pharmacia). Cell lysates (400 μg) expressing HA-tagged ASK1 were incubated with 10 μg of GST-Trx bound to glutathione-Sepharose in the lysis buffer containing either 1 mmol/L DTT or 1 mmol/L H₂O₂. The beads were washed 4 times with the lysis buffer before the addition of boiling Laemmli sample buffer. Bound ASK1 proteins were resolved on SDS-PAGE and detected by Western blot with anti-HA.

**Quantitation of Cell Killing**

Cell killing assay was performed as described previously with a modification.\textsuperscript{20} EC were transfected with a combination of green fluorescent protein (GFP) reporter plasmid and the control vector or experimental expression plasmids for ASK1 and Trx at 1:1 ratio as indicated. GFP-positive cells were visualized under a fluorescence microscope and counted as number of survival cells.

**Caspase 3 Activity Assay**

Caspase 3 activity was measured with a caspase 3 fluorescence kit (Sigma), according to the Manufacturer’s protocol. Briefly, BAECs were harvested in caspase 3 lysis buffer (25 mmol/L HEPES, pH 7.4, 5 mmol/L CHAPS, and 5 mmol/L DTT) and incubated on ice for 10 to 20 minutes followed by a centrifugation at 14 000g for 10 to 15 minutes at 4°C. For each reaction, 5 μL (200 μg) of cell lysate was incubated with 200 μL of 16-μmol/L caspase 3 peptide substrate acetyl-ASP-Glu-Val-Asp-7 amido-4-methylcoumarin (Ac-DEVD-AMC) in the assay buffer (25 mmol/L HEPES, pH 7.4, 5 mmol/L EDTA, 0.1% CHAPS, and 5 mmol/L DTT) in the presence or absence of 100 μmol/L caspase 3 inhibitor (Ac-DEVD-CHO). The reaction was incubated in the dark for 1 to 1.5 hours and fluorescence was measured in a fluorescence plate reader. The measured fluorescence was used as an arbitrary unit.

**Results**

**Trx Induces ASK1 Ubiquitination and Degradation in ECs**

To determine if Trx affects ASK1 protein stability, BAECs were transfected with Trx-expressing plasmid or a control vector (VC) with Lipofectamine\textsuperscript{2000}. Transfection efficiency was determined by transfection of a GFP reporter plasmid and the control vector or experimental expression plasmids for ASK1 and Trx at 1:1 ratio as indicated. GFP-positive cells were visualized under a fluorescence microscope and counted as number of survival cells.

**ASK1 and JNK Kinase Assays**

ASK1 and JNK assays were performed as described previously\textsuperscript{8,19} using GST-MKK4 and GST-c-Jun (1-80) fusion protein as a substrate, respectively. Briefly, total 400-μg cell lysates were immunoprecipitated with 5 μg of antibody against ASK1 or JNK1 (Santa Cruz). The immunoprecipitates were mixed with 10 μg GST-MKK4 or GST-c-Jun (1-80) suspended in the kinase buffer (20 mmol/L HEPES, pH 7.6, 20 mmol/L MgCl₂, 25 mmol/L β-glycerophosphate, 100 μmol/L sodium orthovanadate, 2 mmol/L DTT, and 20 μmol/L ATP) containing 1 μL (10 μCi) of [γ-32P] ATP. The kinase assay was performed at 25°C for 30 minutes. The reaction was terminated by the addition of Laemmli sample buffer and the phosphorylated GST-MKK4 or GST-c-Jun (1-80) was visualized by autoradiography.

**GSH Regulates ASK1 Activity in BAECs**

We have previously shown that GSH regulates ASK1 activity in BAECs and HUVECs. In the present study, we examined the mechanism by which GSH regulates ASK1 activity.
by Western blot with anti-Ub (Figure 1B). The basal ubiquitination of ASK1 was detected (lane 1 in Figure 1B), and Trx expression significantly increased ASK1 ubiquitination (lane 2 in Figure 1B). The ubiquitination of ASK1 was also confirmed by coexpression of ASK1 and HA-tagged Ub22 (data not shown). These results indicate that Trx overexpression in ECs induced ASK1 ubiquitination and degradation. In contrast, expression of Trx did not induce ubiquitination and degradation of ASK1-DN, a mutant ASK1 lacking the N-terminal Trx-binding domain (Figure 1C). These data suggest that association of Trx with ASK1 (the full-length ASK1) is required for Trx-induced ASK1 ubiquitination and degradation.

**TNF and TRAF2 Block Trx-Induced ASK1 Ubiquitination and Degradation**

TNF through the adaptor protein TRAF2 activates ASK1, in part, by dissociating ASK1 from Trx.17,18 To examine if TNF and TRAF2 reverse Trx-induced ASK1 ubiquitination/degradation, BAECs were either cotransfected with expression constructs for Trx and TRAF2 (both were Flag-tagged), or transfection with Trx followed by treatment with TNF (10 ng/mL for 15 minutes). Expression of Trx and TRAF2 was determined by Western blot with anti-Flag (Figure 2A). Ubiquitination and degradation of endogenous ASK1 in EC were determined as described above. Results showed that TRAF2 expression and TNF treatment blocked Trx-induced ASK1 ubiquitination (increase of high molecular mass) and ASK1 degradation (reduction of 170-kDa ASK1 band) (compare lanes 3 and 4 to lane 2 in Figure 2B). These data further support that Trx regulates ASK1 ubiquitination and degradation.

**Redox Activity of Trx Is Not Required for Induction of ASK1 Ubiquitination and Degradation**

To examine the role Trx redox activity in promoting ASK1 ubiquitination and degradation, we generated a single mutant of Trx at the catalytic site C32 or C35 (Trx-C32S or Trx-C35S) and a double-mutant of C32S and C35S (Trx-CS). Trx-C32S and Trx-C35S, like Trx-CS, are catalytically inactive.13–15 BAECs were transfected with Flag-tagged Trx expression constructs, and Trx protein was determined by Western blot with anti-Flag. Results showed the equal amount of Trx proteins were expressed (Figure 3A). Endogenous ASK1 protein in ECs was determined by Western blot with anti-ASK1. To our surprise, Trx-C32S and Trx-C35S (but not Trx-CS), like Trx-WT, increased ASK1 polyubiquitination with concomitant reduction of ASK1 protein (Figure 3B). These data suggest that Trx redox activity is not required for its ability to induce ASK1 ubiquitination/degradation.
Hydrogen Peroxide Dissociates Wild-Type Trx but not Trx-C32S or Trx-C35S From ASK1 In Vitro

We hypothesized that the single mutants (Trx-C32S and Trx-C35S) retain ability for ASK1 binding. Furthermore, we reasoned that unlike Trx-WT, Trx-C32S, and Trx-C35S are no longer oxidized to form an intramolecular disulfide bond in response to ROS leading to constitutively association with ASK1. To test these hypotheses, we examined association of ASK1 with various Trx proteins: Trx-WT, Trx-CS, Trx-C32S, or Trx-C35S in the presence of 1 mmol/L DTT or H2O2 in an in vitro GST pull-down assay. Bacteria-expressed GST-Trx proteins were purified and protein concentrations were determined by SDS-PAGE (Figure 2A). Then BAEC lysates containing HA-tagged ASK1-WT8 were used for GST pull-down assay. ASK1 bound to GST-Trx was determined by Western blot with anti-HA. The results showed that in the presence of 1 mmol/L DTT (Trx remains in a reduced form under this condition), ASK1 bound to Trx-WT, Trx-C32S, and Trx-C35S (but not Trx-CS) (lanes 1 to 5 in Figure 2B). As expected, addition of 1 mmol/L H2O2 disrupted the association of Trx-WT with ASK1 by oxidizing Trx-WT (lane 2 in Figure 4A versus lane 7 in Figure 4B). In contrast, Trx-C32S and Trx-C35S retained their associations with ASK1 in the presence of H2O2 (lanes 4 to 5 versus lanes 9 to 10 in Figure 4B). These data suggest that binding of Trx-C32S and Trx-C35 with ASK1 is ROS-resistant, most likely because they cannot form an intramolecular disulfide bond. GST or GST-Trx-CS did not bind to ASK1, indicating that a single Cys residue of Trx (C32 or C35) is necessary and sufficient for ASK1 binding.

TNF Dissociates Wild-Type Trx but not Trx-C32S or Trx-C35S From ASK1 In Vivo

Next, we examined if Trx-C32S and Trx-C35S constitutively bind to ASK1 in vivo. We first examined if regulation of ASK1 by Trx is ROS-dependent in ECs as in other cell types.16–18 BAECs were either untreated or treated with N-acetyl-cysteine (Nac, 1 mmol/L) or vehicle for 60 minutes before TNF-α (10 ng/mL) stimulation for 15 minutes. TNF-induced ASK1 activation was measured by an in vitro kinase assay using GST-MKK4 (JNKK1) fusion protein as a substrate. TNF activated ASK1 in ECs (Figure 5A). Preexposing ECs to Nac significantly inhibited TNF-stimulated ASK1 activity (70% inhibition; n=11005, P<0.01), suggesting that regulation of ASK1 by Trx in EC is ROS-dependent. Association of ASK1 with Trx was easily detected in untreated ECs (Figure 5B, Ctrl). TNF treatment significantly reduced the interaction of ASK1 with Trx, indicating that TNF activates ASK1, in part, by dissociating ASK1 from Trx in ECs. In contrast, Nac pretreatment prevented TNF-induced...
A in vitro ASK1 kinase activity

B in vivo interaction of ASK1 and Trx

C in vivo interaction of ASK1 and Trx

Figure 5. Association of Trx-C32S and Trx-C35S with ASK1 in vivo is resistant to TNF treatment. A and B, TNF activates ASK1 in part by dissociating Trx from ASK1 in a ROS-dependent manner. BAECs were subjected to the following treatment: untreated (Ctrl), TNF-α (10 ng/ml) stimulation for 15 minutes (TNF), N-acetyl-cysteine (1 mmol/L) for 60 minutes (Nac), and Nac for 45 minutes followed by TNF for 15 minutes (Nac+TNF). A, ASK1 activation by TNF is ROS-dependent. Cell lysates were prepared and analyzed for ASK1 activity by an in vitro kinase assay using GST-MKK4 as a substrate. B, TNF induces dissociation of Trx from ASK1. Cell lysates were immunoprecipitated with anti-Flag followed by Western blot with anti-ASK1 (Top). Western blot with anti-Flag indicated equal amounts of Trx protein in the immunoprecipitates was determined with anti-Trx (Bottom). C, Association of Trx-C32S and Trx-C35S with ASK1 in vivo is resistant to TNF treatment. HA-tagged ASK1-WT was cotransfected with Flag-tagged Trx constructs (WT, CS, C32S, or C35S). Twenty-four hours after transfection, cells were either left untreated (Control) or treated with TNF (10 ng/mL) for 15 minutes before harvest. Cell lysates were immunoprecipitated by anti-Flag. ASK1-WT was detected by Western blot with anti-HA and Trx proteins by anti-Flag.

To examine association of Trx mutants with ASK1 in vivo, the Flag-tagged Trx construct (WT, CS, C32S, or C35S) was cotransfected with HA-ASK1 into BAECs and the interaction of these Trx proteins with ASK1 were examined by coimmunoprecipitation assay. As expected, Trx-WT, Trx-C32S, and Trx-Trx-C35S (but not Trx-CS) bound to ASK1 in resting EC (Figure 5C). Trx-WT exists in both reduced form and oxidized form14,15 and showed a weaker binding for ASK1 than Trx-C32S and Trx-C35S (Figure 5C). TNF treatment completely dissociated Trx-WT from ASK1 (Figure 5C, lanes 1 and 5). In contrast, association of Trx-C32S or Trx-C35S with ASK1 was not reduced by TNF treatment, indicating that they remain in a complex with ASK1 (Figure 5C). These data demonstrate that Trx-C32S and Trx-C35S, unlike Trx-WT, bind to ASK1 in a TNF- and ROS-resistant manner.

Trx-C32S and Trx-C35S (but not Trx-WT) Inhibits ASK1-Mediated EC Apoptosis Induced by TNF

To determine the biological consequence of Trx-induced ASK1 ubiquitination/degradation, we examined effects of Trx on ASK1-induced apoptosis. ASK1-induced activation of JNK and caspase 3 has been implicated in cell death.9,23 First, we examined effects of Trx on JNK activation induced by ASK1. BAECs were cotransfected with ASK1 and Trx expression constructs as indicated, and JNK activity was measured by an in vitro kinase assay using GST-c-Jun as a substrate. Results showed that ASK1 expression in ECs activated JNK (compare ASK1/VC to Ctrl in Figure 6A). Coexpression of Trx-WT, Trx-C32S, and Trx-C35S (but not Trx-CS) inhibited ASK1-induced JNK activity (Figure 6A, top). Western blot with anti-Flag indicated equal amounts of Trx proteins were expressed (Figure 6A, bottom). Similar results were obtained in HUVECs (data not shown).

We then examined effects of Trx on ASK1-induced caspase 3 activation, a hallmark of the execution of apoptotic cell death.24,25 Caspase 3 activity was determined by an in vitro assay using peptide substrate acetyl-ASP-Glu-Val-Asp-7 amido-4-methylcoumarin (Ac-DEVD-AMC). Overexpression of ASK1 in BAECs increased caspase 3 activity compared with the control cells (Figure 6B). Caspase 3 activity was specifically inhibited by the presence of the caspase 3 inhibitor (+Ac-DEVD-CHO). Coexpression of Trx-WT, Trx-C32S, or Trx-C35S significantly (n=4 and P<0.05) inhibited ASK1-induced caspase 3 activity (50±2%, 48±4%, and 52±5%, respectively). However, Trx-CS failed to block ASK1-induced caspase 3 activation (Figure 6B).

ASK1-induced EC death was measured by a GFP cotransfection killing assay as previously described with minor modifications.20 Overexpression of ASK1 in ECs (BAECs or HUVECs) induced 60% cell death at 48 hours after transfection, ie, 40% of GFP-positive (survival) ECs compared with the control cells (Figure 6C, white bars). In contrast, Trx did not inhibit ASK1-ΔN–induced apoptosis (data not shown). TNF treatment (to generate ROS and oxidize Trx) specifically diminished the inhibitory effect of Trx-WT on ASK1-induced apoptosis (Figure 6C, striped bars). However, Trx-C32S and Trx-C35S did not respond to TNF treatment and retained their inhibitory effects on ASK1-induced apoptosis (Figure 6C, striped bars).
We finally examined effects of Trx on TNF-induced cell death. TNF alone does not induce EC apoptosis. However, TNF in the presence of protein synthesis inhibitor cycloheximide (CHX) strongly induces ASK1 activation and EC apoptosis.\(^8\) ECs were cotransfected with GFP and Trx, as indicated, followed by TNF and CHX treatment. Effects of Trx expression on TNF+CHX-induced EC death were measured by counting GFP-positive cells. TNF+CHX induced in BAECs induced 75% EC death at 24 hours after treatment, ie, 25% of GFP-positive (survival) ECs compared with the control cells (control as 100% survival; Figure 6D). Consistent with constitutively binding activity for ASK1, Trx-C32S and Trx-C35S (but not Trx-WT) retained the inhibitory effects on TNF+CHX-induced apoptosis (Figure 6D). Trx-C35S showed a slightly stronger inhibitory effect on ASK1 activity (100±12% survival) than Trx-C32S (80±10% survival). Similar results were obtained in HUVECs (data not shown). These data indicate that Trx-C32S and Trx-C35S inhibit ASK1-mediated EC apoptosis in a TNF-resistant manner.

**Discussion**

In the present study, we show that Trx promotes ASK1 ubiquitination and degradation. The association of Trx with ASK1 is required for Trx-induced ASK1 ubiquitination and degradation. Thus, the single mutation at the redox-active site of Trx (C32S and C35S) retains the binding activity for ASK1 and the ability to induce ASK1 ubiquitination/degradation. Furthermore, we show that Trx-C32S and Trx-C35S constitutively associate with ASK1 and inhibit ASK1 apoptotic activity in a TNF/ROS-resistant manner (Figure 7). Our data suggest a novel mechanism by which Trx inhibits ASK1.

Trx-ASK1 complex preexists in the resting cells and appears to be a target of many extracellular stimuli. Apoptotic stimuli such as ROS and TNF activate ASK1 by disrupting the Trx-ASK1 complex.\(^{16-18}\) In contrast, the antiapoptotic factors such as laminar flow prevent the dissociation of ASK1 from its inhibitors Trx and 14-3-3.\(^8\) Most recently, it is reported that HIV Nef protein inhibits ASK1 activity by preventing Trx release from Trx-ASK1 complex.\(^{26}\) These data suggest that Trx is a critical regulator of ASK1 functions.

The mechanism by which Trx inhibits ASK1 activity is not fully understood. Our data show that Trx promotes ASK1 ubiquitination and degradation in ECs, and this activity of Trx is dependent on its binding activity for ASK1. We conclude that Trx inhibits ASK1 apoptotic activity by inducing ASK1 ubiquitination and degradation. Alternatively, Trx-induced ubiquitination of ASK1 may alter cellular localization of ASK1 or association of ASK1 with signaling complex such as TRAF2 and TRAF6. It has been reported that ubiquitination plays an important regulatory role in stress response pathways,\(^{23}\) including those of TGF-β-activated kinase 1 (TAK1)\(^{27}\) and IkB kinase (IKK).\(^{28}\) Our data suggest that ASK1 is another signaling molecule regulated by ubiquitination. It needs to be further investigated how Trx-ASK1 interaction triggers ASK1 ubiquitination and degradation.
Previously, it has been shown that Trx in a reduced form binds to ASK1, and the double mutant (Trx-CS) fails to bind to ASK1 activity.\textsuperscript{16} Based on this observation, the authors concluded that the redox activity of Trx is essential for Trx inhibitory function on ASK1.\textsuperscript{16} Our data show that the single mutation of Trx at the catalytic site (Trx-C32S or Trx-C35S) can bind to ASK1, suggesting that the redox activity is not required for the association of Trx with ASK1. Although the interacting domain of Trx with ASK1 has not been determined, our results indicate that one of the Cys (C32 or C35) in Trx is essential for the interaction of Trx with ASK1. It has been shown that the single Cys-containing Trx forms a stable complex by intermolecular disulfide bridge with its enzyme Trx reductase (via C32), or its substrate transcription factor NF-\textsuperscript{xB} (via C35).\textsuperscript{29,30} Our data indicate that Trx may form this type of complex with ASK1 via either of the Cys residues. The association of Trx via one of the Cys with ASK1 appears to be necessary and sufficient to promote ASK1 ubiquitination and degradation leading to reduced ASK1 apoptotic activity. The crystal structure of Trx show that only C32 (in our C35S mutant) is exposed to solvent,\textsuperscript{14} suggesting C32 (in Trx-C35S) may be more accessible to ASK1 interaction. This may explain why Trx-C35S has slightly stronger activity than Trx-C32S in ASK1 binding, induction of ASK1 ubiquitination/degradation, and inhibition of ASK1-induced apoptosis. It will be interesting to determine the single-mutant Trx retains binding ability for other proteins such as p53 and NF-\textsuperscript{xB}.

Trx has been shown to exist in many forms—reduced and oxidized, full-length and truncated, intracellular and secreted—and different forms of Trx exhibit different biological activities, such as growth factor, chemokine, antioxidant, transcriptional cofactor, and inhibitor of apoptosis.\textsuperscript{13} Differ-


Thioredoxin Promotes ASK1 Ubiquitination and Degradation to Inhibit ASK1-Mediated Apoptosis in a Redox Activity-Independent Manner
Yingmei Liu and Wang Min

Circ Res. 2002;90:1259-1266; originally published online May 16, 2002; doi: 10.1161/01.RES.0000022160.64355.62
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/90/12/1259