REVIEW

The effects of acrolein on the thioredoxin system: Implications for redox-sensitive signaling

Charles R. Myers[1,2], Judith M. Myers[1], Timothy D. Kufahl[1], Rachel Forbes[1] and Adam Szadkowski[1]

[1] Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI, USA
[2] Free Radical Research Center, Medical College of Wisconsin, Milwaukee, WI, USA

The reactive aldehyde acrolein is a ubiquitous environmental pollutant and is also generated endogenously. It is a strong electrophile and reacts rapidly with nucleophiles including thiolates. This review focuses on the effects of acrolein on thioredoxin reductase (TrxR) and thioredoxin (Trx), which are major regulators of intracellular protein thiol redox balance. Acrolein causes irreversible effects on TrxR and Trx, which are consistent with the formation of covalent adducts to selenocysteine and cysteine residues that are key to their activity. TrxR and Trx are more sensitive than some other redox-sensitive proteins, and their prolonged inhibition could disrupt a number of redox-sensitive functions in cells. Among these effects are the oxidation of peroxiredoxins and the activation of apoptosis signal regulating kinase (ASK1). ASK1 promotes MAP kinase activation, and p38 activation contributes to apoptosis and a number of other acrolein-induced stress responses. Overall, the disruption of the TrxR/Trx system by acrolein could be significant early and prolonged events that affect many aspects of redox-sensitive signaling and oxidant stress.

Received: April 1, 2011
Revised: May 31, 2011
Accepted: June 20, 2011

Keywords:
Acrolein / Peroxiredoxin / Thioredoxin / Thioredoxin reductase

1 Introduction

Acrolein (Prop-2-enal), a reactive and volatile α,β-unsaturated aldehyde, is a ubiquitous environmental pollutant that is found in many everyday sources, including cigarette smoke (85–228 μg per cigarette) [1–3], exhaust from internal combustion engines, wood combustion, smoke from other fires, photochemical oxidation of airborne hydrocarbons, and overheated cooking oils [3, 4]. Inhalation is the most likely route of exposure to exogenous acrolein, and occupations with significant exposure include foundries, the manufacture of acrylate polymers, welding coated metals, coffee roasting, printing, rubber vulcanization, and fire fighting [3, 4]. Dangerous levels of acrolein are contained in smoke from rapid combustion (e.g. burning buildings, vegetative fires), and acrolein may be a significant contributor to serious lung damage and death following smoke inhalation [3]. While inhaled acrolein can be absorbed and have systemic effects [5–7], airway epithelial cells would be expected to receive the highest doses from inhaled sources. Adverse respiratory effects are related to the dose and duration/frequency of exposure and include respiratory irritation, airway cell destruction, pulmonary edema, inflammation, chronic obstructive pulmonary disease, lung hemorrhage, and death [1, 8, 9].

Acrolein is also generated in vivo from some drugs (e.g. the anti-cancer agents cyclophosphamide and ifosfamide), and some of their resulting toxicities can be attributed to acrolein [10]. Bladder damage (hemorrhagic cystitis) is the most well-recognized toxicity of the acrolein generated by these drugs, although hepatotoxicity can also occur [11–13]. Thiol compounds (e.g. 2-mercaptoethanesulfonate, amifostine) can protect the bladder by scavenging free acrolein

Correspondence: Dr. Charles R. Myers, Department of Pharmacology and Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA
E-mail: cmyers@mcw.edu
Fax: +1-414-955-6545

Abbreviations: 4-HNE, 4-hydroxynonenal; **ASK,** apoptosis signal regulating kinase; **Cys,** cysteine; **GSH,** reduced glutathione; **GSSG,** glutathione disulfide; **Prx1,** peroxiredoxin-1; **Prx3,** peroxiredoxin-3; **SeCys,** selenocysteine; **Trx1,** thioredoxin-1; **Trx2,** thioredoxin-2; **TrxR,** thioredoxin reductase

[11–13]. Acrolein is the predominant toxin of allylamine, a known cardiac and vascular toxin [14].

Vascular diseases are the most prominent cause of death, and endothelial cell dysfunction is an important initiator of the pathophysiology of atherosclerosis. LDL oxidation, lipid peroxidation, and proinflammatory stimuli are all associated with atherosclerosis. Lipid peroxidation products are common in vascular lesions associated with oxidative stress [15]. Large amounts of reactive aldehydes, including acrolein and 4-hydroxynonenal (4-HNE), are generated during the breakdown of lipid peroxides and during LDL oxidation [15, 16]. Acrolein is an initiator and product of lipid peroxidation. The oxidation of polyunsaturated fatty acids and LDL are major sources of acrolein [1, 17, 18]. LDL oxidation and lipid peroxidation are associated with atherosclerosis, human atherosclerotic lesions contain acrolein adducts, and plasma acrolein levels increase in relation to the extent of atherosclerosis [15, 18]. Vascular disease is common in diabetics, and these individuals have significantly increased acrolein adducts that are in proportion to poor control of blood glucose [7]. Acrolein causes systemic endothelial cell dysfunction [5, 15, 18] and vascular pathogenesis [16, 19–23]. Human endothelial cells are especially sensitive to acrolein, e.g. $\geq 5\,\mu M$ acrolein for 30 min is cytotoxic [24]. This is a realistic exposure given the levels of acrolein adducts in human urine (370–1240 μM) and their levels in plasma (19–62 μM) [25].

Inflammation contributes to vascular disease and atherosclerosis. Acrolein is formed in vivo at significant levels at sites of inflammation [26]. Micromolar levels of prostaglandins are present at sites of inflammation [27], and the J_2 prostaglandins markedly elevate acrolein production [28]. In bovine endothelial cells, acrolein increases monocyte adhesion and the expression of cell adhesion molecules [29]. Neutrophils are activated in atherosclerotic lesions [15], and neutrophil myeloperoxidase converts threonine to acrolein in high yield [26, 30]. Acrolein may play a role in the formation of arterial foam cells [15] which have important roles in various stages of atherosclerosis.

In addition to endogenously generated acrolein, acrolein from cigarette smoke may contribute to vascular effects including atherosclerosis and coronary artery disease [6, 31–33]. Acrolein is a prominent component of the gas phase of cigarette smoke [1–4] and some of this acrolein can be absorbed into the blood [5, 6], e.g. acrolein-protein adducts are significantly increased in the urine of smokers [7]. Several effects of cigarette smoke on the vasculature are consistent with known effects of acrolein, including antioxidant depletion [5, 34], protein oxidation [35], and increased lipid peroxidation [36].

In addition to its toxic effects on endothelial cells [10, 24, 37], acrolein is toxic to other cells types as well, including bronchial epithelium and fibroblasts [1], vascular smooth muscle [14], and neurons [38]. Depending on the cell type, acrolein can be 10–200 times more toxic than formaldehyde [1, 14], and can be more toxic than 4-HNE [38]. Neuronal apoptotic cell death is common in neurodegenerative disorders such as Alzheimer's disease, and acrolein levels are significantly elevated in the brains of Alzheimer's and early Alzheimer's patients, with levels five times those of 4-HNE [38–40].

Apoptosis has an important role in tissue homeostasis, and is thought to be an important contributor to some of the respiratory, cardiovascular, and neurodegenerative pathologies that are associated with elevated acrolein levels/exposure (above). The thioredoxin system is critical for cell survival, and many proteins and signaling events are dependent upon, or regulated by, this system. This review discusses the current understanding of the effects of acrolein on the thioredoxin system, and the implications for several signaling events that are regulated by thioredoxin, including a number that could promote apoptosis. Given the exogenous and endogenous sources of acrolein (as indicated above), its effects on airway epithelium and vascular endothelium are two of the sites that are emphasized.

2 Reactivity of acrolein: potential effects on cellular thiols

Acrolein is a strong electrophile and among the most reactive of the α,β-unsaturated aldehydes, being more reactive with cellular proteins than 4-HNE [1, 15, 41]. Acrolein preferentially reacts with soft nucleophiles, and in biological systems it has been considered to have the highest reactivity with thiols, typically through Michael addition, resulting in acrolein-thiol adducts [16, 41, 42]. While reaction with selenols, such as in selenocysteine (SeCys), has received little consideration, it is likely of great importance (below). Acrolein can also react with amines (e.g. lysine, histidine) by various mechanisms, but these reactions are much slower than the reaction with thiols [1, 42].

Thiolates ($-S^-$) are much more susceptible to attack by oxidants and electrophiles than are thiols (–SH) [43]. The pK_a values of thiols in a typical protein and in reduced glutathione (GSH) are 8.5 and 8.8, respectively [43], so less than 10% of their thiols would be predicted to be thiolates at pH 7.4. Within proteins, however, the amino acids in the immediate vicinity of cysteine (Cys) residues can significantly influence the thiol pK_a such that some Cys residues will largely be in the thiolate form at physiological pH. Prime examples are the active sites of thioredoxin (Trx, pK_a of ca. 6.5) and thioredoxin reductase (TrxR, pK_a estimated at 5.2) [43–45]. Their active site thiols should be largely ionized and therefore very reactive at pH 7.4. The SeCys of the active site of TrxR is even a stronger nucleophile than Cys [46, 47], and this strong nucleophilicity could be a key determinant in the enhanced reactivity of SeCys relative to Cys [47]. Since the SeCys of TrxR is also exposed on the surface of the enzyme [48], its combined properties could render it particularly reactive with acrolein or other strong electrophiles.

Thiol-acrolein adducts can be more stable than those of some other α,β-unsaturated aldehydes [1], and represent a form of protein oxidation by removal of the reductive capacity of the thiol, or of the selenol in the case of SeCys. Such adducts could therefore have significant long-term consequences for proteins with critical Cys or SeCys residues. Such adducts on proteins of the thioredoxin system could cause major disruptions of intracellular thiol redox balance.

3 Acrolein causes irreversible inhibition of TrxR

Mammalian TrxRs are NADPH-dependent homodimers with three known redox-active centers per subunit: one FAD, an N-terminal domain dithiol (C59/C64), and a C-terminal active site containing C497/U498 (U is SeCys) [49, 50]. The C59/C64 dithiol is reduced by the flavin, and in turn is believed to donate electrons to the C497/U498 active site [51]. These redox centers are essential for the catalytic functions of TrxR [49–52]. While cytosolic TrxR1 and mitochondrial TrxR2 are encoded by distinct genes, the three redox-active centers are conserved between these two proteins. A major role of the TrxRs is to keep their respective thioredoxins in the reduced state (Fig. 1). The thioredoxins, in turn, keep many intracellular protein thiols reduced.

The strong nucleophilicity of the active site of TrxR should render it particularly susceptible to electrophilic attack. An initial report showed that 50–75 μM acrolein for 30 min could inhibit TrxR activity by ca. 65% in human lung cancer A549 cells [53]. Another group subsequently noted that 25 μM acrolein for 30 min inhibited 88% of TrxR activity in human umbilical vein endothelial cells [54], but other antioxidant enzymes were not inhibited including glutathione peroxidase, glutathione reductase, catalase, and superoxide dismutase [54]. These data imply that acrolein does not indiscriminately target all antioxidant proteins in cells. More recent studies with normal human bronchial epithelial cells (BEAS-2B) showed that ≥ 2.5 μM acrolein strongly inhibits TrxR activity, and even 1 μM acrolein causes significant inhibition [55]. These activity losses represent inhibition and not protein degradation. Activity was not restored in the cell lysates by removal of free acrolein, or by NADPH (the electron donor for TrxR) which should readily facilitate reduction of its C59/C64 disulfide to the dithiol, and its C497/U498 selenenyl sulfide to selenolthiol. Similarly, TrxR activity does not recover in BEAS-2B cells even 4 hr after acrolein removal [55]. The irreversible effect on TrxR in BEAS-2B cells implies that even intermittent acrolein exposure will inhibit TrxR for at least 4 hr after the acrolein source is removed. The findings in BEAS-2B cells are in contrast to those of A549 cells, in which 70–100% of TrxR activity had recovered at 2–4 hr after acrolein removal [53]. It is not known if the recovery in A549 cells represented new protein synthesis or reactivation of existing enzyme [53]. The differences between the A549 and BEAS-2B cells could reflect their different origins, but are also consistent with the pronounced resistance of A549 cells to other oxidants [56] which is reflected in the much higher levels of acrolein required to inhibit TrxR in A549 [53]. Like many cancers, A549 cells markedly overexpress TrxR [57] which may also contribute to the differences from BEAS-2B cells. While the unsaturated aldehyde 4-HNE is less reactive than acrolein [1, 41], high concentrations of 4-HNE (50 μM for 6 hr) can inhibit ca. 60% of the TrxR activity in HeLa cells [58].

TrxR in cells is considerably more sensitive to acrolein than are some other proteins with redox-sensitive thiols (Fig. 2). For example, while the active site thiol in glyceraldehyde phosphate dehydrogenase has proven very redox-sensitive [59, 60], the cellular activity of this enzyme is not significantly affected by acrolein (Fig. 2). Glutathione reductase is not inhibited in bronchial cells treated with 2.5 or 5 μM acrolein, and 12.5 μM acrolein only inhibited its activity by ca. 40%, whereas just 2.5 μM acrolein markedly

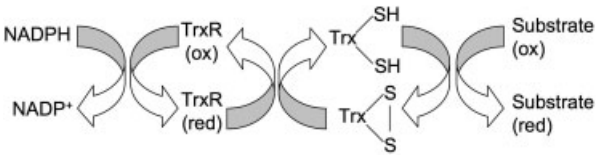

Figure 1. Normal function of the Trx system. TrxR keeps the active site of Trx reduced (–SH, –SH). This applies to the cytosolic (TrxR1, Trx1) or mitochondrial (TrxR2, Trx2) systems. Direct oxidation of Trx, or inhibition of TrxR, can result in oxidized Trx (–S–S–).

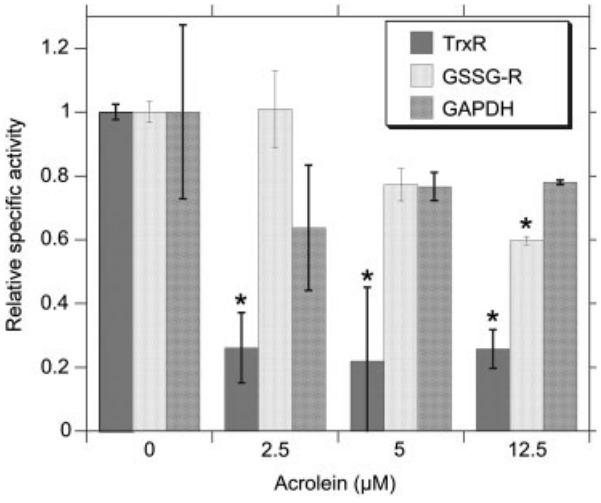

Figure 2. The effects of 30 min acrolein treatments on the specific activities of TrxR, glutathione reductase (GSSG-R), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in normal human bronchial epithelial cells (BEAS-2B). $^{*}p < 0.01$ relative to the cells treated with vehicle alone (no acrolein). Details of the enzymatic assays are described in Supporting Information, methods.

inhibits TrxR activity (Fig. 2). Glutathione reductase in human umbilical vein endothelial cells is unaffected by 25 μM acrolein (30 min), as are other antioxidant enzymes such as glutathione peroxidase and superoxide dismutase [54].

Purified TrxR is very sensitive to acrolein, e.g. 80% irreversible inhibition with 1 μM acrolein [55], implying that direct reaction with acrolein can inhibit the enzyme. The greater sensitivity of purified TrxR relative to that in cells likely reflects the absence of competing reactions with other thiols as might occur in cells. The detailed mechanism by which acrolein causes irreversible inhibition of TrxR remains to be determined, but given its preferential reaction with, and adduction to, soft nucleophiles [42], the Cys and/or SeCys residues are prime candidates. The monomers of rat and human TrxR1 have 14 and 13 Cys residues, respectively, plus one SeCys each; of these, the N-terminal domain dithiol (C59/C64) and the active site Cys-SeCys (C497/U498) are essential for TrxR activity (above). Based on its strong nucleophilicity and exposure on the enzyme surface [46, 48], C497/U498 could be the most susceptible. This site is covalently modified by some other agents (e.g. 2,4-dinitrochlorobenzene, curcumin, and 4-HNE), resulting in irreversible inhibition of TrxR [58, 61–63]. Recent functional studies are consistent with acrolein-SeCys adducts in TrxR, e.g. TrxR1 pre-treated with acrolein behaves like SeCys-minus TrxR in a number of functional assays including inherent NADPH oxidase activity, and redox interactions with some quinones [64].

TrxR1, including the C59/C64 dithiol, shares strong homology with glutathione reductase, except the latter lacks the 16-residue C-terminal SeCys-containing domain found in TrxR [48, 49]. The relative insensitivity of glutathione reductase to acrolein (Fig. 2 and [54]) suggests that the C59/C64 dithiol in TrxR may be less susceptible to acrolein than is C497/U498. Unlike the irreversible inhibition of TrxR [55], GSH levels recover quickly once acrolein is removed (Fig. 3). As noted in Fig. 2, the activity of glutathione reductase, which reduces glutathione disulfide (GSSG) to GSH, is largely intact in acrolein-treated cells (Fig. 2).

The irreversible inhibition of TrxR by acrolein can therefore compromise the maintenance of cellular thiol redox balance for at least 4 hr. It implies diminished capacity to maintain the thioredoxins and thioredoxin-dependent proteins in their reduced (active) state. Inhibition of TrxR can increase the susceptibility of cells to oxidants and promote apoptosis [50], and TrxR1 knockouts do not survive [65]. Putative acrolein-TrxR adducts may have other effects as well. Deletion or inactivation of the SeCys residue may directly promote cell death through mechanisms that are not yet well understood [66, 67]. Such effects are more severe than those caused by siRNA suppression of TrxR [62], which typically does not decrease cell viability in non-stressed conditions. Thus, direct targeting of the SeCys may have some effects that extend beyond those of a decreased ability to support thioredoxin and thioredoxin-dependent processes.

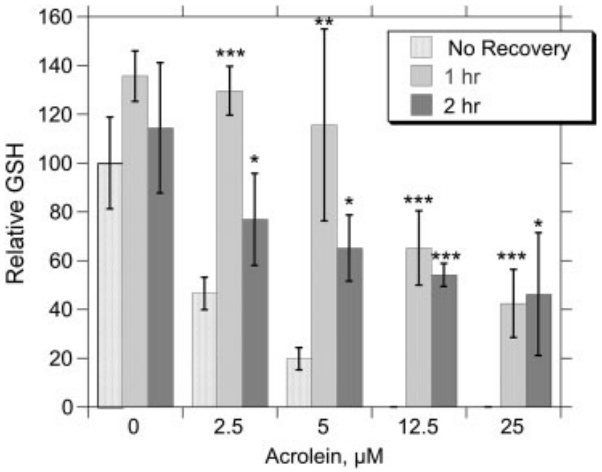

Figure 3. Relative GSH in human endothelial cells (HMEC-1) treated with acrolein for 30 min, washed with HBSS and analyzed immediately (no recovery), or allowed to recover in medium with 10% FBS and supplements for 1 or 2 h. $^{*}p<0.05$, $^{**}p<0.01$, or $^{***}p<0.001$ relative to the corresponding ''No recovery'' samples. GSSG remained below detectable limits in all samples. GSH and GSSG were measured by HPLC [73]; the detailed method is described in Supporting Information, methods.

While it is clear that acrolein causes irreversible inhibition of TrxR, additional experiments are needed to determine the residue(s) within TrxR that are affected by acrolein, and the full extent of the implications of this inhibition. Studies are also need to determine if there are differential effects of acrolein on cytosolic TrxR1 vs. mitochondrial TrxR2.

4 Effects of acrolein on thioredoxins

While both glutathione and the thioredoxins are major players in the maintenance of intracellular thiol redox balance, these systems are not in redox equilibrium with each other [68, 69], and the redox state of the thioredoxins may be more critical to cell survival for some cells [70]. All mammalian cells have cytosolic (Trx1) and mitochondrial (Trx2) thioredoxins [71]. While these 12 kDa proteins are encoded by distinct genes, their conserved active sites (WCGPCK) are cycled between the reduced (dithiol) and oxidized (disulfide) forms [72] as depicted in Fig. 1. Trx2 has no other Cys residues, whereas Trx1 has three additional Cys (C73 and a C62/C69 dithiol) [72] that can influence its activity. The active site in Trx1 (C32/C35) is more readily oxidized than C62/C69 [72].

TrxR1 and TrxR2 normally maintain Trx1 and Trx2 largely in the reduced state in cells [24, 50, 55, 71–73]. A central role of the thioredoxins is to reduce key Cys residues in a number of intracellular proteins including peroxiredoxins, ribonucleotide reductase, protein disulfide

isomerase, methionine sulfoxide reductase, and others [49, 50, 71, 74].

Trx1 and Trx2 are not in redox equilibrium with each other, so their redox states can be used to differentially assess the effects of oxidants and electrophiles on the thiol redox status of the cytosolic and mitochondrial compartments [69, 75]. An initial report showed that acrolein can inhibit the activity of Trx in A549 cancer cells, with maximal effects at $\geq 15\,\mu M$ [53]. Surprisingly, however, Trx was considerably more sensitive to acrolein than was TrxR activity in the A549 cells [53]. This is in contrast to normal human bronchial cells for which TrxR and Trx displayed similar sensitivity to acrolein [55]. Redox blot studies with human endothelial and bronchial epithelial cells show that 5 μM acrolein can eliminate the reduced forms of both Trx1 and Trx2, although Trx1 was partially affected by even lower concentrations [24, 55]. The enhanced sensitivity of Trx1 vs. Trx2 to acrolein is in contrast to the effects of some other agents (e.g. *t*-butyl hydroperoxide, diamide, hexavalent chromium, or triapine) for which Trx2 is more susceptible [69, 73, 76, 77].

While the exact mechanism(s) by which acrolein inactivates the Trx1 and Trx2 thiols in cells remains to be determined, the overall data to date are consistent with the formation of Trx-S-acrolein adducts as a significant mechanism. However, a portion of Trx could be converted to disulfides under some conditions. Trx-S-acrolein adducts would prevent Trx redox function by blocking one or more thiols important for activity, and the adducted proteins would run coincident with oxidized Trx in redox western blots. The progressive transition of Trx1 through three redox states (running coincident with fully reduced, partially reduced, and fully oxidized Trx) with increasing acrolein in human endothelial and bronchial cells [24, 55] implies that first one, and then additional thiols are compromised. Multiple redox states of Trx1 are also seen in redox blots of acrolein-treated bovine cells [29]. While Trx2 has only one dithiol, three redox states were also implied from the blots [24, 55], suggesting that first one, and then both of its thiols are compromised as acrolein is increased.

Trx-S-acrolein adducts would inhibit Trx activity, and acrolein-thiol adducts are not easily reversed [1]. Consistent with the formation of Trx-acrolein adducts, high concentrations of the potent disulfide reductants dithiothreitol and Tris(2-carboxyethyl)phosphine hydrochloride were unable to restore Trx1 or Trx2 to their reduced states in cell lysates from human bronchial cells that had been treated for 30 min with 12.5 μM acrolein [55]. Similarly, dithiothreitol was unable to restore reduced Trx1 to acrolein-treated bovine endothelial cells [29]. Overall, these data are consistent with Trx-S-acrolein adducts as an important mechanism of Trx inactivation. Following a 1-hr in vitro incubation of equal amounts of human Trx1 and acrolein, acrolein adducts on C73 were observed coincident with a large decline in Trx activity [29]. Other modifications at C62/C69 of Trx1 can also block activity [78], so acrolein adducts on any of its thiols could potentially inhibit normal function. Acrolein adducts on C32/C35 have been noted for Trx1 in which the other Cys residues were replaced by serine [29]. In addition to Trx-S-acrolein adducts, it is possible that other as yet unrecognized changes to Trx in acrolein-treated cells may contribute to the inability of disulfide reductants to restore the reduced form.

In human bronchial epithelial cells, there is a strong relationship between the acrolein-mediated inhibition of TrxR and the inactivation of Trx1 [55]. While this might suggest that Trx1 oxidation results from the inability of TrxR to maintain Trx1 in a reduced state, such effects on Trx1 should be reversible by disulfide reductants, which proved not to be true [55]. Thus, it seems more likely that a majority of the Trx inactivation was the result of acrolein-Trx adducts, or other effects that are not easily reversed.

While acrolein adducts may largely account for Trx inactivation in cells, there is modest recovery of the redox state of Trx1 in human endothelial cells after a 4-hr acrolein-free period [24]. Protein synthesis is not required for this modest recovery following low acrolein concentrations (2.5 and 5 μM), but protein synthesis is required for any recovery from 12.5 μM [24]. These data imply that, with low acrolein concentrations, there may be a mix of acrolein-Trx1 adducts and oxidized Trx1 (as disulfides), whereas acrolein-Trx1 adducts are predominant as acrolein is increased. While the vast majority of TrxR is inhibited in these cells, and TrxR activity does not show any signs of recovery 4-hr after acrolein is removed (above), the remaining TrxR activity could account for reduction of Trx1 disulfides. Alternatively, acrolein adducts to thiols are reversible, albeit at a slow rate [42], and it is possible that cells have some ability to enhance the reversal of such adducts and thereby regenerate modest amounts of reduced Trx1. In limited experiments, redox recovery of Trx2 in human endothelial cells was not observed [24]. In A549 cells, partial recovery of Trx activity was noted 4 hr after acrolein was removed, but in these cells TrxR activity had fully recovered [53].

Thiol antioxidants such as *N*-acetylcysteine (NAC) can protect cells from some oxidants. Pretreatment (2 hr) with NAC partially protects the Trx1 redox state in human endothelial cells, and significantly diminishes the cytotoxicity of 5 or 12.5 μM acrolein [24]. In limited experiments, NAC did not protect Trx2, however [24]. While NAC increased GSH levels by an average of 31%, GSH-monoethyl ester (cell-permeable) increased GSH by 64% but offered only a minor protection of the cells from 12.5 μM acrolein. The bulk of the protective effects of NAC cannot therefore be explained by elevated GSH. NAC also protects other cell types from acrolein [10, 79, 80], and it reduces the toxicity of cigarette smoke extract, a known source of acrolein [81–85]. While extracellular thiols, including NAC, would quickly scavenge acrolein and thereby protect cells, the protection afforded by NAC pre-treatment is noted even when extracellular NAC is washed away before acrolein treatment [24, 79]. It is possible that intracellular NAC, or

thiols resulting from NAC, act as a thiol buffer or trap for acrolein, reducing the likelihood of reaction with cellular components. There are other possibilities, however, and the mechanism(s) of protection afforded by NAC remain poorly understood.

Overall, studies with bovine and non-cancerous human cells demonstrate that acrolein inhibits/inactivates Trx1 and Trx2 at low micromolar concentrations that are coincident with acrolein toxicity [24, 29, 55]. These concentrations are also cytotoxic to porcine pulmonary cells [37] and murine lymphocytes [86]. Since both Trx1 and Trx2 are critical to cell survival and to the function of many proteins involved in cell growth and signaling [50, 71], the inactivation of Trx1 and/or Trx2 by acrolein could negatively impact cell survival. Disruption of the Trxs has been associated with diminished antioxidant defense, increased oxidant sensitivity, and the promotion of apoptosis [69, 74, 76].

5 Effects of acrolein on peroxiredoxins

Peroxiredoxins (Prxs) are ubiquitous peroxidases that degrade H_2O_2 and organic hydroperoxides. During this process, their active site Cys residues are oxidized to sulfenic acid (–SOH). In the 2-Cys Prxs (e.g. cytosolic Prx1 and Prx2, and mitochondrial Prx3), this sulfenic acid reacts with the resolving Cys on the other subunit to form a disulfide-linked dimer [87–89]. The Prxs are directly dependent on their respective Trxs to reduce these disulfides to thiols, thereby regenerating active Prx [90].

In addition to the formation of disulfides as just described, two other types of Prx thiol oxidation are possible: (1) direct reaction with electrophiles to form Prx-S-electrophile adducts, or (2) over-oxidation of sulfenic acid to sulfinic (Prx-SO_2) or sulfonic (Prx-SO_3) forms. Both of these possibilities would prevent disulfide linkages between the two subunits.

In acrolein-treated human bronchial cells, Prx1 and Prx3 were examined as representatives of cytosolic and mitochondrial 2-Cys Prxs, respectively. Acrolein treatment resulted in the near complete oxidation of Prx1 and Prx3 to dimers [55], consistent with the oxidation of their thiols to disulfides. Consistent with this, these Prx dimers were readily reduced to Prx monomers by disulfide reductants [55]. These observations are in contrast to what would be expected for Prx-S-acrolein adducts, or for oxidation to the sulfinic or sulfonic forms, both of which should block dimer formation. While it is possible that a small percentage of Prx thiols react directly with acrolein, the Prx active site thiols may well be protected from direct alkylation, e.g. the Prx2 thiols are poorly reactive with iodoacetamide and various chloroamines, even though these agents readily alkylate a number of cellular thiols [89]. The reversible effects of acrolein on Prx are in direct contrast to the irreversible effects on TrxR and Trx (above).

A significant increase in Prx1 and Prx3 oxidation does not occur in acrolein-treated cells until all, or nearly all, of their respective Trxs (Trx1 and Trx2) are inactivated, i.e. the reduced form is eliminated [55]. This implies that Prx oxidation results from the inability of the Trxs to keep the Prxs reduced. Because of their dependence on the Trxs, the Prxs are therefore indirectly dependent on the function of TrxR, and Prx oxidation occurs with acrolein treatments that cause extensive inhibition of TrxR [55]. It was previously noted that the low levels of TrxR in erythrocytes hinder the reduction of oxidized Prx [91]. Electrophilic isothiocyanates also react with thiols, and some of these can oxidize mitochondrial Prx3 in cells [92]. In contrast to acrolein, however, the isothiocyanates have little to no effect on cytosolic peroxiredoxins, and Prx3 is markedly oxidized by treatments that inhibit only ca. 20% of TrxR activity [92]. The oxidation of Prx3 is important for apoptosis, however, as other isothiocyanates that oxidize GSH, but not Prx3, do not promote apoptosis [92].

The Prxs have important roles as peroxidases and therefore as mediators of peroxide-dependent signaling [43, 59, 89, 93]. For example, Prx3 may be a critical regulator of mitochondrial peroxide signaling given that mitochondria lack catalase and they contain 30-fold more Prx3 than glutathione peroxidase [94]. The ability of acrolein to compromise the function of Prxs by irreversible effects on their source of electrons (i.e. the TrxR/Trx system) implies that peroxide levels would increase, and/or that cells would be less tolerant of peroxide exposure. The earlier observation that various aldehydes can increase peroxide production in cells [15] may in part result from the effects on the Trx/Prx system which were not known at the time. Acrolein-mediated Prx oxidation may also promote apoptosis or render cells more sensitive to apoptotic insults. For example, Prx3 is a primary defense against peroxides in mitochondria, and depletion/oxidation of Prx3 renders HeLa cells and Jurkat T lymphocytes more susceptible to apoptosis [92, 94]. Some cell types may be more susceptible to these effects, depending on their levels of TrxR/Trx/Prx relative to other antioxidant systems. For example, endothelial cells contain more TrxR than other human cells, and they have more TrxR than glutathione peroxidase [95]. It is therefore possible that the Trx/Prx system may be more critical for cell survival and for regulating redox- and peroxide-dependent signaling in human endothelium than in some other cell types. Such differences need to be considered when assessing the effects of acrolein and other electrophiles on different cells and tissues, as well as in different species.

6 Other downstream effects of the acrolein-mediated disruption of the TrxR/Trx system

In addition to loss of support for the Prxs, disruption of the TrxR/Trx system by acrolein would be expected to have multiple additional downstream effects [96], including: (i) interference with DNA replication during S phase,

because ribonucleotide reductase is largely dependent on Trx1 [97]; (ii) changes in protein folding through effects on protein disulfide isomerase; (iii) loss of support for methionine sulfoxide reductase which is involved in the repair of oxidative protein damage; (iv) effects on multiple redox-sensitive transcription factors including NF-κB, AP-1, p53 and others [50, 62, 71, 74]; and (v) promotion of apoptosis through activation of apoptosis signal regulating kinase (ASK1). While some of these downstream effects have not been specifically tested with acrolein, the effects on ASK1 and its downstream proteins have been examined and are consistent with Trx oxidation. In unstressed cells, reduced Trx binds to ASK1, keeping ASK1 inactive [50, 98]. Oxidized Trx dissociates from ASK1, facilitating ASK1 activation and promoting apoptosis [98–101] via activation of its MAP kinase kinase kinase activity. While the inactivation of Trx by acrolein is most likely due to acrolein-S-Trx adducts (above), acrolein treatments do result in the dissociation of Trx1 from ASK1 (Fig. 4), and this dissociation is consistent with ASK1 activation.

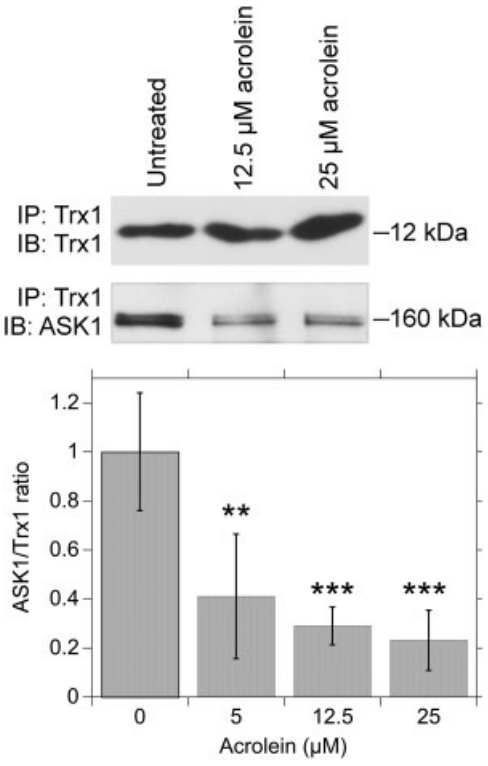

Figure 4. Immunoprecipitation (IP) shows that acrolein treatment (30 min) of human endothelial (HMEC-1) cells causes dissociation of ASK1 from Trx1. The relative ratio of ASK1 bound to Trx1 is shown in the plot. Total ASK1 did not change in these cells (not shown). $^{**}p<0.01$ and $^{***}p<0.001$ relative to the control cells treated without acrolein. In limited experiments, acrolein also caused the dissociation of 14-3-3 from ASK1 (not shown); this dissociation is also consistent with ASK1 activation [120]. Details of the IP protocol are described in the methods (see Supporting Information).

Activated ASK1 can mediate the activation (phosphorylation) of p38 MAPK via MKK3/MKK6, and of JNK (c-Jun N-terminal kinase) via MKK4/MKK7 [100]. These downstream effects are thought to be important contributors to the pro-apoptotic effects of ASK1, and to other ASK1-mediated stress responses. While studies typically focus on the dissociation of Trx1 from ASK1, ASK1 is also found in the mitochondria and its activation there has also been linked to MAP kinase activation [102]. Reactive aldehydes, including acrolein, have been implicated in MAP kinase activation [15], but the responsible mechanisms are not yet well defined. In CHO cells, acrolein increases ERK and p38 phosphorylation, and chemical inhibitors of MAP kinases decreased caspase activation [103]. However, Trx redox status or ASK1 activation were not determined in the CHO cells. In human endothelial cells, acrolein treatments that inactivate Trx1 cause significant activation (phosphorylation) of p38 MAPK and JNK, whereas total levels of p38 and JNK do not change (Figs. 5 and 6). The p38 inhibitor SB203580 decreases acrolein toxicity in human endothelial cells (Fig. 7), implying a role for p38 activation in acrolein-induced cell death. The JNK inhibitor SP600125 by itself increases cell death in human endothelial HMEC-1 cells, precluding its use to explore the role of JNK in acrolein-induced apoptosis. In airway smooth muscle cells, acrolein induces a prolonged phosphorylation of p38, and p38

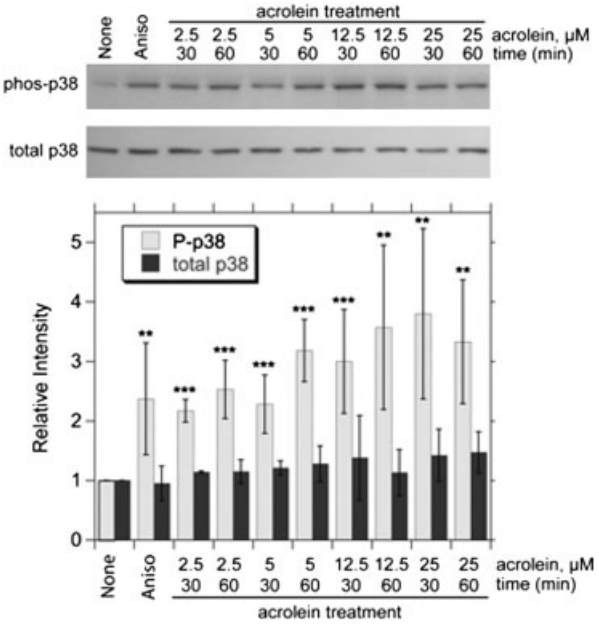

Figure 5. Acrolein causes phosphorylation of p38 in human endothelial cells (HMEC-1). Cells were treated with acrolein, or the known activator anisomycin (Aniso, 200 ng/mL), as indicated. Western blots were probed with anti-phospho-p38 (Thr180/Tyr182) or anti-p38α (total p38α). $^{**}p<0.01$ or $^{***}p<0.001$ relative to control cells (''None'') treated without acrolein or anisomycin ($n=3$–5 independent treatments for each). Supporting Information describes the details of the methods.

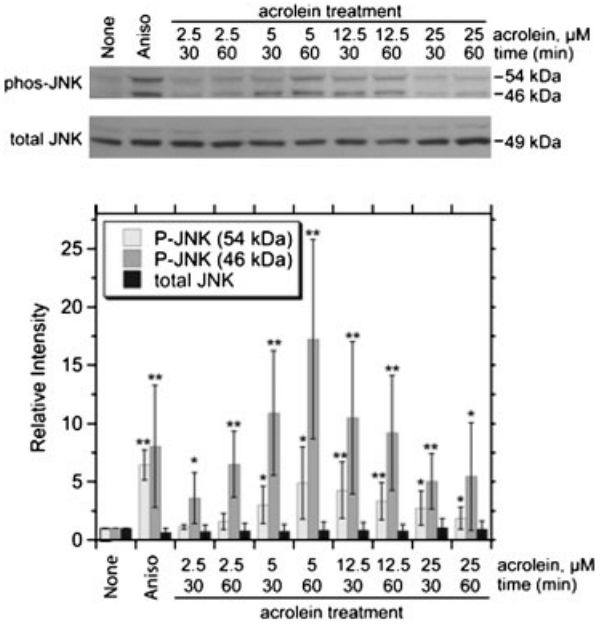

Figure 6. Acrolein causes phosphorylation of JNK in human endothelial cells (HMEC-1). Cells were treated with acrolein, or the known activator anisomycin (Aniso, 200 ng/mL), as indicated. Western blots were probed with anti-phospho-JNK (Thr183/Tyr185) or anti-pan-JNK. $^{*}p<0.05$ or $^{**}p<0.01$ relative to control cells (''None'') treated without acrolein or anisomycin ($n=3$–5 independent treatments for each). Supporting Information describes the details of the methods.

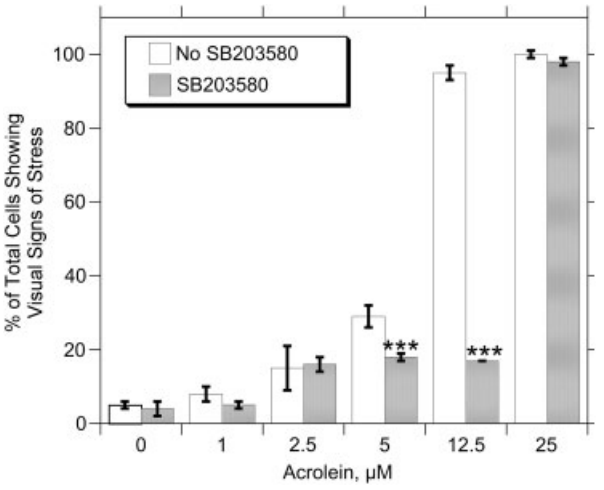

Figure 7. Pretreatment with the p38 inhibitor SB203580 (10 μM, 2 h) protects human endothelial HMEC-1 cells from 5 and 12.5 μM acrolein. Following acrolein (30 min), cells were washed and incubated for 14 h in fresh medium. The percentage of cells that displayed visual stress (e.g. rounded, condensed) were determined (mean ± SD, $n=3$). $^{***}p<0.001$ relative to the corresponding samples without SB203580 pretreatment.

activation facilitates increases in vascular endothelial growth factor and interleukin-8 [104, 105]. The activation of p38 is also important for airway inflammation and mucus hypersecretion in mice exposed to acrolein aerosols [106]. While the effects on the Trx system or ASK1 activation were not assessed in these airway studies, the exposure levels were consistent with those that would be expected to affect Trx and Trx-dependent events. Similar to acrolein, electrophilic isothiocyanates can induce p38 and JNK activation in T24 bladder carcinoma cells [107]. In contrast to acrolein, however, the inhibition of p38 activity does not protect these cells from isothiocyanate-induced apoptosis [107]. Such differences from acrolein could be attributed to differential effects of the electrophiles themselves, and/or to the different cell types.

While ASK1 activation is a plausible mechanism for p38 and JNK activation in acrolein-treated cells, there are other possible contributors. For example, 4-HNE can inhibit protein tyrosine phosphatases that dephosphorylate (inactivate) MAP kinases such as JNK [108], and trans-2-nonenal can inhibit membrane-associated phosphotyrosine phosphatases [109]. Reaction of acrolein with the active site Cys or other key residues in these phosphatases represent plausible alternative mechanisms that may contribute to the initiation or prolongation of MAP kinase activation.

Some studies have shown that acrolein can affect redox-sensitive transcription factors such as AP-1 and NF-κB [29, 110–112]. AP-1 is localized in the nucleus where it exists as c-Jun dimers or c-Jun-c-Fos heterodimers [113]. Active AP-1 binds to tetradecanoyl phorbal acetate response elements in DNA, with the resulting promotion of cell proliferation and the suppression of pro-apoptotic proteins including p53 and p21 [113, 114]. The phosphorylation of c-Jun by JNK enhances AP-1 activity and can promote its binding to DNA [69, 113]. Treatment of bovine endothelial cells and rat vascular smooth muscle cells with moderate doses of acrolein can increase c-Jun phosphorylation [29, 110], although c-Jun phosphorylation does not occur with higher acrolein levels [110]. Both c-Jun and c-Fos have a conserved Cys motif (KCR) in their DNA binding domains, and the oxidation or elimination of this Cys prevents DNA binding [113, 115]. In A549 cells, acrolein decreases AP-1 binding to DNA and AP-1 activity (as measured by a reporter construct) [111]. Two likely explanations for this decreased AP-1 activity, in spite of enhanced c-Jun phosphorylation, would be the formation of acrolein adducts on AP-1 [111], or the loss of reduced Trx1 which, together with redox factor-1 (Ref-1), reduces the critical Cys residues in AP-1 [113, 116]. The oxidation of nuclear Trx1 can inhibit the binding of AP-1 to DNA [69], and this would be predicted to decrease proliferation and enhance cell death through increases in p53 and other tumor suppressors.

Acrolein can also affect NF-κB-dependent signaling [29, 117]. The cytosolic NF-κB dimer (p50/p65) is bound to IκB, keeping it inactive [113]. Cytosolic oxidant stress and other insults can promote IκB phosphorylation, the release of NF-κB and its translocation to the nucleus where it can activate a number of genes including several that promote survival and suppress apoptosis [113]. While cytosolic

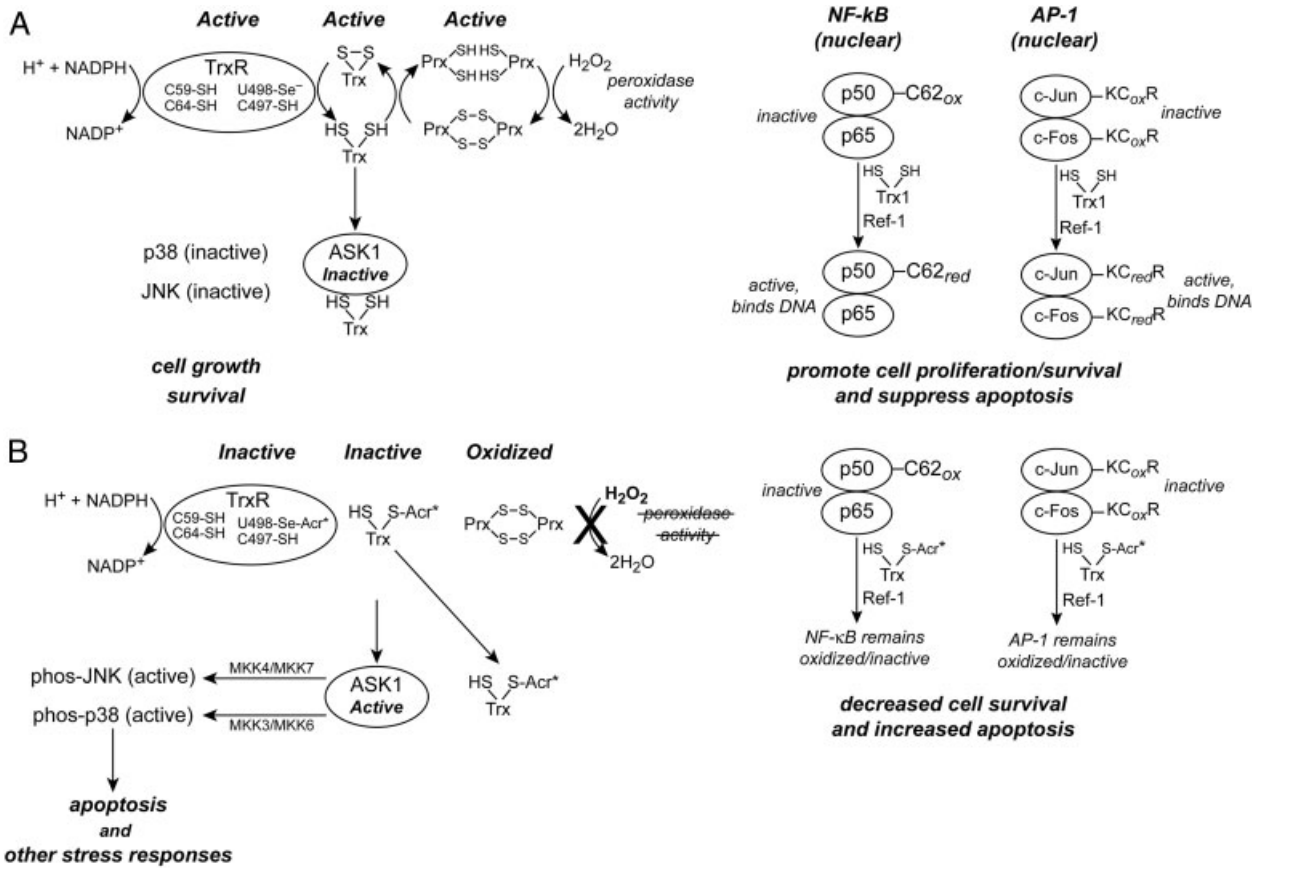

Figure 8. Examples of functions of the TrxR/Trx system in untreated cells (A), and some proposed effects of acrolein on this system (B). TrxR is a major regulator of intracellular thiol redox balance through its support of the thioredoxins and a multitude of Trx-dependent proteins and signaling events, of which only a few examples are shown here. Acrolein causes irreversible inhibition of TrxR and Trx, which would be most consistent with the propensity of acrolein (shown as Acr* in (B)) to form adducts with soft nucleophiles. The data are consistent with acrolein adducts on the SeCys of TrxR, and with adducts on one or more thiols of Trx. This results in an inability to reduce Trx-dependent proteins such as the Prxs which then accummulate in the oxidized form. Oxidized Prxs are unable to function as peroxidases that could increase peroxide levels and alter peroxide signaling. Acrolein-inactivated Trx also dissociates from ASK1 and this can promote many downstream effects through activation of the MAP kinases p38 and JNK. Acrolein-induced p38 activation plays an important role in the induction of apoptosis and other stress responses. Acrolein adducts on Trx1 in the nucleus (shown at right) could interfere with the activity of redox-sensitive transcription factors such as NF-κB and AP-1 by blocking the reduction of critical Cys residues in their DNA-binding domains. It is also possible that acrolein may form adducts with the critical Cys residues in these transcription factors (not shown in the diagram), which could also inhibit their DNA binding. While not shown here, it is predicted that a number of other TrxR/Trx-dependent systems would be affected by acrolein's disruption of TrxR and Trx (see text). Since both Trx1 and Trx2 are affected, this could include a number of cytosolic, nuclear, and mitochondrial Trx-regulated events.

oxidants can promote NF-κB translocation, nuclear oxidation can inhibit the binding of NF-κB to DNA [69, 113, 118]. Cys62 in the p50 subunit of NF-κB must be reduced in order to bind DNA [69, 117], and nuclear Trx1 in conjunction with Ref-1 reduce this Cys [113, 118]. Consistent with this, acrolein treatment of A549 cells blocks both the binding and activity of NF-κB but does not change the phosphorylation status of IκB [117]. Similarly, treatment of bovine endothelial cells with acrolein blocks NF-κB DNA binding [29]. Aqueous cigarette smoke extract, which is a significant source of acrolein, initially disrupts the binding of NF-κB to DNA in Swiss 3T3 cells with coincident disruption of Trx-NF-κB complexes [112]. Cinnamaldehyde, another α,β-unsaturated aldehyde that can form thiol adducts, also blocks NF-κB activity [119]. The inhibition of NF-κB activity/binding could result from possible acrolein adducts to NF-κB [117] and/or to acrolein-inactivated Trx1 in the nucleus. The resulting decrease in NF-kB activity would be predicted to decrease cell proliferation and enhance cell death.

7 Concluding remarks

A schematic overview of the proposed effects of acrolein on TrxR, Trx, and Prx is shown in Fig. 8. In untreated cells (Fig. 8A), TrxR maintains the Trxs in the reduced state,

which supports a number of Trx-dependent proteins including the peroxidase activity of the peroxiredoxins. Reduced Trx binds to ASK1, thereby inhibiting the activation of ASK1 and downstream signals that may otherwise be transmitted through ASK1, e.g. p38 and JNK activation. In acrolein-treated cells (Fig. 8B), it is likely that acrolein forms adducts with the active site-SeCys of TrxR, and with one or more thiols of the Trxs, thus causing extended inhibition of TrxR and Trx, and thus Trx-dependent functions. One example is the accumulation of Prxs in their oxidized (inactive) state (Fig. 8B), blocking their peroxidase activity. Acrolein-inactivated Trx also dissociates from ASK1, which facilitates ASK1 activation and subsequent downstream events such as the phosphorylation (activation) of p38 and JNK. In acrolein-treated endothelial cells, p38 activation contributes to cell death. In airways, p38 activation can promote inflammation, mucus secretion, and other stress responses that are not shown in the diagram. Acrolein-Trx1 adducts in the nucleus could compromise the DNA binding of redox-sensitive transcription factors such as NF-kB and AP-1 (Fig. 8). This would be expected to markedly decrease cell proliferation and promote apoptosis.

TrxR and Trx1 exhibit approximately similar sensitivity to the effects of acrolein, at least in the non-cancerous human cells examined to date. TrxR and Trx1 are, however, considerably more sensitive to acrolein than a number of other oxidant-sensitive and redox-active proteins such as glutathione reductase, glyceraldehyde-3-phosphate dehydrogenase, glutathione peroxidase, and superoxide dismutase. The irreversible effects on TrxR and Trx imply that the inhibition could extend well beyond exposure to free acrolein which can be transient or intermittent. Since the TrxR/Trx system controls many redox-sensitive proteins in cells, the downstream effects could be widespread, well beyond the few examples shown in Fig. 8. Since control of thiol redox balance is critical for normal function and cell viability, thiol redox disruptions resulting from inhibition of the TrxR/Trx system could be a significant contributor to acrolein-induced redox stress and cell death. The effects of acrolein on the TrxR/Trx system could therefore contribute to a number of conditions associated with elevated acrolein including acute and chronic respiratory damage, atherosclerosis and other aspects of vascular disease, and neurodegenerative disorders such as Alzheimer's disease. The effects of acrolein on the TrxR/Trx system and the implications for redox signaling as it contributes to these disorders warrants further study.

The authors' research on this topic is supported by grants to C. R. M. from Advancing a Healthier Wisconsin, the National Institute of Environmental Health Sciences (NIH ES012707), and by the Department of Pharmacology and Toxicology at the Medical College of Wisconsin. They are grateful to Elias Arnér and his colleagues at the Karolinska Institute for their generous collaborations and expertise on TrxR. The HMEC-1 cells were provided by the CDC, and were developed by Dr. Edwin Ades and Mr. Fransisco J. Candal of the CDC, and Dr. Thomas Lawley of Emory University.

The authors have declared no conflict of interest.

8 References

[1] Esterbauer, H., Schaur, R. J., Zollner, H., Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic. Biol. Med.* 1991, *11*, 81–128.

[2] Carnevali, S., Nakamura, Y., Mio, T., Liu, X. et al., Cigarette smoke extract inhibits fibroblast-mediated collagen gel contraction. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 1998, *274*, L591–L598.

[3] Ghilarducci, D. P., Tjeerdema, R. S., Fate and effects of acrolein. *Rev. Environ. Contam. Toxicol.* 1995, *144*, 95–146.

[4] Beauchamp, R. O., Andjelkovich, D. A., Kligerman, A. D., Morgan, K. T., Heck, H. A., A critical review of the literature on acrolein toxicity. *CRC Crit. Rev. Toxicol.* 1985, *14*, 309–380.

[5] Barnoya, J., Glantz, S. A., Cardiovascular effects of secondhand smoke: nearly as large as smoking. *Circulation* 2005, *111*, 2684–2698.

[6] Castelli, W. P., Garrison, R. J., Dawber, T. R., McNamara, P. M. et al., The filter cigarette and coronary heart disease: the Framingham study. *Lancet* 1981, *2*, 109–113.

[7] Daimon, M., Sugiyama, K., Kameda, W., Saitoh, T. et al., Increased urinary levels of pentosidine, pyrraline and acrolein adduct in type 2 diabetes. *Endocrine J.* 2003, *50*, 61–67.

[8] ATSDR, Toxicological profile for acrolein. *U.S. Dept of Health and Human Services* 2007.

[9] Borchers, M. T., Carty, M. P., Leikauf, G. D., Regulation of human airway mucins by acrolein and inflammatory mediators. *Am. J. Physiol.* 1999, *276*, L549–L555.

[10] Kachel, D. L., Martin, W. J., II, Cyclophosphamide-induced lung toxicity: mechanism of endothelial cell injury. *J. Pharmacol. Exp. Ther.* 1994, *268*, 42–46.

[11] Zhang, J., Tian, Q., Zhou, S.-F., Clinical pharmacology of cyclophosphamide and ifosfamide. *Curr. Drug Ther.* 2006, *1*, 55–84.

[12] Batista, C. K. L. P., Brito, G. A. C., Souza, M. L. P., Leitao, B. T. A. et al., A model of hemorrhagic cystitis induced with acrolein in mice. *Brazil. J. Med. Biol. Res.* 2006, *39*, 1475–1481.

[13] Batista, C. K. L. P., Mota, J. M. S. C., Souza, M. L. P., Leitao, B. T. A. et al., Amifostine and glutathione prevent ifosfamide- and acrolein-induced hemorrhagic cystitis. *Cancer Chemother. Pharmacol.* 2007, *59*, 71–77.

[14] Conklin, D. J., Langford, S. D., Boor, P. J., Contribution of serum and cellular semicarbazide-sensitive amine oxidase to amine metabolism and cardiovascular toxicity. *Toxicol. Sci.* 1998, *46*, 386–392.

[15] Uchida, K., Role of reactive aldehyde in cardiovascular diseases. *Free Radic. Biol. Med.* 2000, *28*, 1685–1696.

[16] Uchida, K., Kanematsu, M., Sakai, K., Matsuda, T. et al., Protein-bound acrolein: potential markers for oxidative stress. *Proc. Natl. Acad. Sci. USA* 1998, *95*, 4882–4887.

[17] Markesbery, W. R., Oxidative stress hypothesis in Alzheimer's disease. *Free Radic. Biol. Med.* 1997, *23*, 134–147.

[18] Uchida, K., Kanematsu, M., Morimitsu, Y., Osawa, T. et al., Acrolein is a product of lipid peroxidation reaction. Formation of free acrolein and its conjugate with lysine residues in oxidized low density lipoproteins. *J. Biol. Chem.* 1998, *273*, 16058–16066.

[19] Nelson, T. J., Boor, P. J., Allylamine cardiotoxicity-IV. Metabolism to acrolein by cardiovascular tissues. *Biochem. Pharmacol.* 1982, *31*, 509–514.

[20] Boor, P. J., Hysmith, R. M., Allylamine cardiovascular toxicity. *Toxicology* 1987, *44*, 129–145.

[21] Misra, P., Srivastava, S. K., Singhal, S. S., Awasthi, S. et al., Glutathione *S*-transferase 8-8 is localized in smooth muscle cells of rat aorta and is induced in an experimental model of atherosclerosis. *Toxicol. Appl. Pharmacol.* 1995, *133*, 27–33.

[22] Cox, L. R., Ramos, K., Allylamine-induced phenotypic modulation of aortic smooth muscle cells. *J. Exp. Pathol. (Oxford)* 1990, *71*, 11–18.

[23] Awasthi, S., Boor, P. J., Semicarbazide protection from in vivo oxidant injury of vascular tissue by allylamine. *Toxicol. Lett.* 1993, *66*, 157–163.

[24] Szadkowski, A., Myers, C. R., Acrolein oxidizes the cytosolic and mitochondrial thioredoxins in human endothelial cells. *Toxicology* 2008, *243*, 164–176.

[25] Satoh, K., Yamada, S., Koike, Y., Igarashi, Y. et al., A 1-hour enzyme-linked immunosorbent assay for quantitation of acrolein- and hydroxynonenal-modified proteins by epitope-bound casein matrix method. *Anal. Biochem.* 1999, *270*, 323–328.

[26] Anderson, M. M., Hazen, S. L., Hsu, F. F., Heinecke, J. W., Human neutrophils employ the myeloperoxidase-hydrogen peroxide-chloride system to convert hydroxy-amino acids into glycolaldehyde, 2-hydroxypropanal, and acrolein. A mechanism for the generation of highly reactive alpha-hydroxy and alpha,beta-unsaturated aldehydes by phagocytes at sites of inflammation. *J. Clin. Invest.* 1997, *99*, 424–432.

[27] Offenbacher, S., Odle, B. M., Van Dyke, T. E., The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. *J. Periodontal Res.* 1986, *21*, 101–112.

[28] Kondo, M., Oya-Ito, T., Kumagai, T., Osawa, T., Uchida, K., Cyclopentenone prostaglandins as potential inducers of intracellular oxidative stress. *J. Biol. Chem.* 2001, *276*, 12076–12083.

[29] Go, Y. M., Halvey, P. J., Hansen, J. M., Reed, M. et al., Reactive aldehyde modification of thioredoxin-1 activates early steps of inflammation and cell adhesion. *Am. J. Pathol.* 2007, *171*, 1670–1681.

[30] Hazen, S. L., Hsu, F. F., d'Avignon, A., Heinecke, J. W., Human neutrophils employ myeloperoxidase to convert alpha-amino acids to a battery of reactive aldehydes: a pathway for aldehyde generation at sites of inflammation. *Biochemistry* 1998, *37*, 6864–6873.

[31] Penn, A., Chen, L. C., Snyder, C. A., Inhalation of steady-state sidestream smoke from one cigarette promotes arteriosclerotic plaque development. *Circulation* 1994, *90*, 1363–1367.

[32] Penn, A., Snyder, C. A., Inhalation of sidestream cigarette smoke accelerates development of arteriosclerotic plaques. *Circulation* 1993, *88*, 1820–1825.

[33] McGill, H. C., Jr., Smoking and the pathogenesis of atherosclerosis. *Adv. Exp. Med. Biol.* 1990, *273*, 9–16.

[34] Wei, W., Kim, Y., Boudreau, N., Association of smoking with serum and dietary levels of antioxidants in adults: NHANES III, 1988–1994. *Am. J. Public Health* 2001, *91*, 258–264.

[35] Pignatelli, B., Li, C. Q., Boffetta, P., Chen, Q. et al., Nitrated and oxidized plasma proteins in smokers and lung cancer patients. *Cancer Res.* 2001, *61*, 778–784.

[36] Valkonen, M., Kuusi, T., Passive smoking induces atherogenic changes in low-density lipoprotein. *Circulation* 1998, *97*, 2012–2016.

[37] Patel, J. M., Block, E. R., Acrolein-induced injury to cultured pulmonary artery endothelial cells. *Toxicol. Appl. Pharmacol.* 1993, *122*, 46–53.

[38] Lovell, M. A., Xie, C., Markesbery, W. R., Acrolein is increased in Alzheimer's disease brain and is toxic to primary hippocampal cultures. *Neurobiol. Aging* 2001, *22*, 187–194.

[39] Williams, T. I., Lynn, B. C., Markesbery, W. R., Lovell, M. A., Increased levels of 4-hydroxynonenal and acrolein, neurotoxic markers of lipid peroxidation, in the brain in Mild Cognitive Impairment and early Alzheimer's disease. *Neurobiol. Aging* 2006, *27*, 1094–1099.

[40] Calingasan, N. Y., Uchida, K., Gibson, G. E., Protein-bound acrolein: a novel marker of oxidative stress in Alzheimer's disease. *J. Neurochem.* 1999, *72*, 751–756.

[41] LoPachin, R. M., Gavin, T., Petersen, D. R., Barber, D. S., Molecular mechanisms of 4-hydroxy-2-nonenal and acrolein toxicity: nucleophilic targets and adduct formation. *Chem. Res. Toxicol.* 2009, *22*, 1499–1508.

[42] Cai, J., Bhatnagar, A., Pierce, W. M., Jr., Protein modification by acrolein: formation and stability of cysteine adducts. *Chem. Res. Toxicol.* 2009, *22*, 708–716.

[43] Winterbourn, C. C., Hampton, M. B., Thiol chemistry and specificity in redox signaling. *Free Radic. Biol. Med.* 2008, *45*, 549–561.

[44] Carvalho, A. T., Fernandes, P. A., Ramos, M. J., Determination of the ΔpKa between the active site cysteines of thioredoxin and DsbA. *J. Comput. Chem.* 2006, *27*, 966–975.

[45] Jacob, C., Giles, G. I., Giles, N. M., Sies, H., Sulfur and selenium: the role of oxidation state in protein structure and function. *Angew. Chem. Int. Ed. Engl.* 2003, *42*, 4742–4758.

[46] Johansson, L., Gafvelin, G., Arnér, E. S., Selenocysteine in proteins-properties and biotechnological use. *Biochim. Biophys. Acta* 2005, *1726*, 1–13.

[47] Arnér, E. S., Selenoproteins – what unique properties can arise with selenocysteine in place of cysteine? *Exp. Cell Res.* 2010, *316*, 1296–1303.

[48] Sandalova, T., Zhong, L., Lindqvist, Y., Holmgren, A., Schneider, G., Three-dimensional structure of a mammalian thioredoxin reductase: implications for mechanism and evolution of a selenocysteine-dependent enzyme. *Proc. Natl. Acad. Sci. USA* 2001, *98*, 9533–9538.

[49] Arnér, E. S., Focus on mammalian thioredoxin reductases – important selenoproteins with versatile functions. *Biochim. Biophys. Acta* 2009, *1790*, 495–526.

[50] Nordberg, J., Arnér, E. S., Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic. Biol. Med.* 2001, *31*, 1287–1312.

[51] Cheng, Q., Sandalova, T., Lindqvist, Y., Arnér, E. S., Crystal structure and catalysis of the selenoprotein thioredoxin reductase 1. *J. Biol. Chem.* 2009, *284*, 3998–4008.

[52] Cheng, Q., Antholine, W. E., Myers, J. M., Kalyanaraman, B. et al., The selenium-independent inherent pro-oxidant NADPH oxidase activity of mammalian thioredoxin reductase and its selenium-dependent direct peroxidase activities. *J. Biol. Chem.* 2010, *285*, 21708–21723.

[53] Yang, X., Wu, X., Choi, Y. E., Kern, J. C., Kehrer, J. P., Effect of acrolein and glutathione depleting agents on thioredoxin. *Toxicology* 2004, *204*, 209–218.

[54] Park, Y. S., Misonou, Y., Fujiwara, N., Takahashi, M. et al., Induction of thioredoxin reductase as an adaptive response to acrolein in human umbilical vein endothelial cells. *Biochem. Biophys. Res. Commun.* 2005, *327*, 1058–1065.

[55] Myers, C. R., Myers, J. M., The effects of acrolein on peroxiredoxins, thioredoxins, and thioredoxin reductase in human bronchial epithelial cells. *Toxicology* 2009, *257*, 95–104.

[56] Watjen, W., Beyersmann, D., Cadmium-induced apoptosis in C6 glioma cells: influence of oxidative stress. *Biometals* 2004, *17*, 65–78.

[57] Eriksson, S. E., Prast-Nielsen, S., Flaberg, E., Szekely, L., Arnér, E. S., High levels of thioredoxin reductase 1 modulate drug-specific cytotoxic efficacy. *Free Radic. Biol. Med.* 2009, *47*, 1661–1671.

[58] Fang, J., Holmgren, A., Inhibition of thioredoxin and thioredoxin reductase by 4-hydroxy-2-nonenal in vitro and in vivo. *J. Am. Chem. Soc.* 2006, *128*, 1879–1885.

[59] Baty, J. W., Hampton, M. B., Winterbourn, C. C., Proteomic detection of hydrogen peroxide-sensitive thiol proteins in Jurkat cells. *Biochem. J.* 2005, *389*, 785–795.

[60] Schuppe-Koistinen, I., Moldeus, P., Bergman, T., Cotgreave, I. A., *S*-thiolation of human endothelial cell glyceraldehyde-3-phosphate dehydrogenase after hydrogen peroxide treatment. *Eur. J. Biochem.* 1994, *221*, 1033–1037.

[61] Arnér, E. S., Bjornstedt, M., Holmgren, A., 1-Chloro-2, 4-dinitrobenzene is an irreversible inhibitor of human thioredoxin reductase. Loss of thioredoxin disulfide reductase activity is accompanied by a large increase in NADPH oxidase activity. *J. Biol. Chem.* 1995, *270*, 3479–3482.

[62] Cassidy, P. B., Edes, K., Nelson, C. C., Parsawar, K. et al., Thioredoxin reductase is required for the inactivation of tumor suppressor p53 and for apoptosis induced by endogenous electrophiles. *Carcinogenesis* 2006, *27*, 2538–2549.

[63] Fang, J., Lu, J., Holmgren, A., Thioredoxin reductase is irreversibly modified by curcumin: a novel molecular mechanism for its anticancer activity. *J. Biol. Chem.* 2005, *280*, 25284–25290.

[64] Myers, C. R., Cheng, Q., Myers, J. M., Antholine, W. E., Arnér, E. S. J., The interaction of thioredoxin reductase with quinones and acrolein: covalent adducts and stimulation of NADPH oxidase activity. *Free Radic. Biol. Med.* 2010, *49*, S98–S99.

[65] Bondareva, A. A., Capecchi, M. R., Iverson, S. V., Li, Y. et al., Effects of thioredoxin reductase-1 deletion on embryogenesis and transcriptome. *Free Radic. Biol. Med.* 2007, *43*, 911–923.

[66] Anestål, K., Arnér, E. S., Rapid induction of cell death by selenium-compromised thioredoxin reductase 1 but not by the fully active enzyme containing selenocysteine. *J. Biol. Chem.* 2003, *278*, 15966–15972.

[67] Anestål, K., Prast-Nielsen, S., Cenas, N., Arnér, E. S., Cell death by SecTRAPs: thioredoxin reductase as a prooxidant killer of cells. *PLoS ONE* 2008, *3*, e1846.

[68] Nkabyo, Y. S., Ziegler, T. R., Gu, L. H., Watson, W. H., Jones, D. P., Glutathione and thioredoxin redox during differentiation in human colon epithelial (Caco-2) cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2002, *283*, G1352–G1359.

[69] Hansen, J. M., Go, Y. M., Jones, D. P., Nuclear and mitochondrial compartmentation of oxidative stress and redox signaling. *Annu. Rev. Pharmacol. Toxicol.* 2006, *46*, 215–234.

[70] Hansen, J. M., Zhang, H., Jones, D. P., Differential oxidation of thioredoxin-1, thioredoxin-2, and glutathione by metal ions. *Free Radic. Biol. Med.* 2006, *40*, 138–145.

[71] Powis, G., Montfort, W. R., Properties and biological activities of thioredoxins. *Annu. Rev. Biophys. Biomol. Struct.* 2001, *30*, 421–455.

[72] Watson, W. H., Pohl, J., Montfort, W. R., Stuchlik, O. et al., Redox potential of human thioredoxin 1 and identification of a second dithiol/disulfide motif. *J. Biol. Chem.* 2003, *278*, 33408–33415.

[73] Myers, J. M., Antholine, W. E., Myers, C. R., Hexavalent chromium causes the oxidation of thioredoxin in human bronchial epithelial cells. *Toxicology* 2008, *246*, 222–233.

[74] Arnér, E. S. J., Holmgren, A., Physiological functions of thioredoxin and thioredoxin reductase. *Eur. J. Biochem.* 2000, *267*, 6102–6109.

[75] Halvey, P. J., Watson, W. H., Hansen, J. M., Go, Y. M. et al., Compartmental oxidation of thiol-disulphide redox couples during epidermal growth factor signalling. *Biochem. J.* 2005, *386*, 215–219.

[76] Chen, Y., Cai, J., Jones, D. P., Mitochondrial thioredoxin in regulation of oxidant-induced cell death. *FEBS Lett.* 2006, *580*, 6596–6602.

[77] Myers, J. M., Antholine, W. E., Myers, C. R., The iron-chelating drug triapine causes pronounced mitochondrial thiol redox stress. *Toxicol. Lett.* 2011, *201*, 130–136.

[78] Haendeler, J., Hoffmann, J., Tischler, V., Berk, B. C. et al., Redox regulatory and anti-apoptotic functions of thioredoxin depend on S-nitrosylation at cysteine 69. *Nat. Cell Biol.* 2002, *4*, 743–749.

[79] Tanel, A., Averill-Bates, D. A., Inhibition of acrolein-induced apoptosis by the antioxidant *N*-acetylcysteine. *J. Pharmacol. Exp. Ther.* 2007, *321*, 73–83.

[80] Wood, P. L., Khan, M. A., Moskal, J. R., Cellular thiol pools are responsible for sequestration of cytotoxic reactive aldehydes: Central role of free cysteine and cysteamine. *Brain Res.* 2007, *1158*, 158–163.

[81] Nagy, J., Demaster, E. G., Wittmann, I., Shultz, P., Raij, L., Induction of endothelial cell injury by cigarette smoke. *Endothelium* 1997, *5*, 251–263.

[82] Baglole, C. J., Bushinsky, S. M., Garcia, T. M., Kode, A. et al., Differential induction of apoptosis by cigarette smoke extract in primary human lung fibroblast strains: implications for emphysema. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2006, *291*, L19–L29.

[83] Vayssier-Taussat, M., Camilli, T., Aron, Y., Meplan, C. et al., Effects of tobacco smoke and benzo[a]pyrene on human endothelial cell and monocyte stress responses. *Am. J. Physiol. Heart Circ. Physiol.* 2001, *280*, H1293–H1300.

[84] Yang, Q., Hergenhahn, M., Weninger, A., Bartsch, H., Cigarette smoke induces direct DNA damage in the human B-lymphoid cell line Raji. *Carcinogenesis* 1999, *20*, 1769–1775.

[85] Hoshino, Y., Mio, T., Nagai, S., Miki, H. et al., Cytotoxic effects of cigarette smoke extract on an alveolar type II cell-derived cell line. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2001, *281*, L509–L516.

[86] Kern, J. C., Kehrer, J. P., Acrolein-induced cell death: a caspase-influenced decision between apoptosis and oncosis/necrosis. *Chem. Biol. Interact.* 2002, *139*, 79–95.

[87] Wood, Z. A., Poole, L. B., Karplus, P. A., Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. *Science* 2003, *300*, 650–653.

[88] Wood, Z. A., Schroder, E., Robin Harris, J., Poole, L. B., Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem. Sci.* 2003, *28*, 32–40.

[89] Peskin, A. V., Low, F. M., Paton, L. N., Maghzal, G. J. et al., The high reactivity of peroxiredoxin 2 with H_2O_2 is not reflected in its reaction with other oxidants and thiol reagents. *J. Biol. Chem.* 2007, *282*, 11885–11892.

[90] Kim, J. A., Park, S., Kim, K., Rhee, S. G., Kang, S. W., Activity assay of mammalian 2-cys peroxiredoxins using yeast thioredoxin reductase system. *Anal. Biochem.* 2005, *338*, 216–223.

[91] Low, F. M., Hamptom, M. B., Peskin, A. V., Winterbourn, C. C., Peroxiredoxin 2 functions as a noncatalytic scavenger of low-level hydrogen peroxide in the erythrocyte. *Blood* 2007, *109*, 2611–2617.

[92] Brown, K. K., Eriksson, S. E., Arner, E. S., Hampton, M. B., Mitochondrial peroxiredoxin 3 is rapidly oxidized in cells treated with isothiocyanates. *Free Radic. Biol. Med.* 2008, *45*, 494–502.

[93] Cox, A. G., Brown, K. K., Arner, E. S., Hampton, M. B., The thioredoxin reductase inhibitor auranofin triggers apoptosis through a Bax/Bak-dependent process that involves peroxiredoxin 3 oxidation. *Biochem. Pharmacol.* 2008, *76*, 1097–1109.

[94] Chang, T. S., Cho, C. S., Park, S., Yu, S. et al., Peroxiredoxin III, a mitochondrion-specific peroxidase, regulates apoptotic signaling by mitochondria. *J. Biol. Chem.* 2004, *279*, 41975–41984.

[95] Anema, S. M., Walker, S. W., Howie, A. F., Arthur, J. R. et al., Thioredoxin reductase is the major selenoprotein expressed in human umbilical-vein endothelial cells and is regulated by protein kinase C. *Biochem. J.* 1999, *342*, 111–117.

[96] Holmgren, A., Lu, J., Thioredoxin and thioredoxin reductase: current research with special reference to human disease. *Biochem. Biophys. Res. Commun.* 2010, *396*, 120–124.

[97] Avval, F. Z., Holmgren, A., Molecular mechanisms of thioredoxin and glutaredoxin as hydrogen donors for mammalian S phase ribonucleotide reductase. *J. Biol. Chem.* 2009, *284*, 8233–8240.

[98] Saitoh, M., Nishitoh, H., Fujii, M., Takeda, K. et al., Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J.* 1998, *17*, 2596–2606.

[99] Tobiume, K., Saitoh, M., Ichijo, H., Activation of apoptosis signal-regulating kinase 1 by the stress-induced activating phosphorylation of pre-formed oligomer. *J. Cell. Physiol.* 2002, *191*, 95–104.

[100] Ichijo, H., Nishida, E., Irie, K., ten Dijke, P. et al., Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* 1997, *275*, 90–94.

[101] Hatai, T., Matsuzawa, A., Inoshita, S., Mochida, Y. et al., Execution of apoptosis signal-regulating kinase 1 (ASK1)-induced apoptosis by the mitochondria-dependent caspase activation. *J. Biol. Chem.* 2000, *275*, 26576–26581.

[102] Zhang, R., Al-Lamki, R., Bai, L., Streb, J. W. et al., Thioredoxin-2 inhibits mitochondria-located ASK1-mediated apoptosis in a JNK-independent manner. *Circ. Res.* 2004, *94*, 1483–1491.

[103] Tanel, A., Averill-Bates, D. A., P38 and ERK mitogen-activated protein kinases mediate acrolein-induced apoptosis in Chinese hamster ovary cells. *Cell. Signal.* 2007, *19*, 968–977.

[104] Moretto, N., Facchinetti, F., Southworth, T., Civelli, M. et al., alpha,beta-Unsaturated aldehydes contained in cigarette smoke elicit IL-8 release in pulmonary cells through mitogen-activated protein kinases. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2009, *296*, L839–L848.

[105] Volpi, G., Facchinetti, F., Moretto, N., Civelli, M., Patacchini, R., Cigarette smoke and alpha,beta-unsaturated aldehydes elicit VEGF release through the p38 MAPK pathway in human airway smooth muscle cells and lung fibroblasts. *Br. J. Pharmacol.* 2011, *163*, 649–661.

[106] Liu, D. S., Wang, T., Han, S. X., Dong, J. J. et al., p38 MAPK and MMP-9 cooperatively regulate mucus overproduction in mice exposed to acrolein fog. *Int. Immunopharmacol.* 2009, *9*, 1228–1235.

[107] Pullar, J. M., Thomson, S. J., King, M. J., Turnbull, C. I. et al., The chemopreventive agent phenethyl isothiocyanate sensitizes cells to Fas-mediated apoptosis. *Carcinogenesis* 2004, *25*, 765–772.

[108] Rinna, A., Forman, H. J., SHP-1 inhibition by 4-hydroxynonenal activates Jun N-terminal kinase and glutamate cysteine ligase. *Am. J. Respir. Cell. Mol. Biol.* 2008, *39*, 97–104.

[109] Hernandez-Hernandez, A., Garabatos, M. N., Rodriguez, M. C., Vidal, M. L. et al., Structural characteristics of a lipid peroxidation product, trans-2-nonenal, that favour inhibition of membrane-associated phosphotyrosine phosphatase activity. *Biochim. Biophys. Acta* 2005, *1726*, 317–325.

[110] Ranganna, K., Yousefipour, Z., Nasif, R., Yatsu, F. M. et al., Acrolein activates mitogen-activated protein kinase signal transduction pathways in rat vascular smooth muscle cells. *Mol. Cell. Biochem.* 2002, *240*, 83–98.

[111] Biswal, S., Acquaah-Mensah, G., Datta, K., Wu, X., Kehrer, J. P., Inhibition of cell proliferation and AP-1 activity by acrolein in human A549 lung adenocarcinoma cells due to thiol imbalance and covalent modifications. *Chem. Res. Toxicol.* 2002, *15*, 180–186.

[112] Gebel, S., Muller, T., The activity of NF-kappaB in Swiss 3T3 cells exposed to aqueous extracts of cigarette smoke is dependent on thioredoxin. *Toxicol. Sci.* 2001, *59*, 75–81.

[113] Gius, D., Botero, A., Shah, S., Curry, H. A., Intracellular oxidation/reduction status in the regulation of transcription factors NF-kappaB and AP-1. *Toxicol. Lett.* 1999, *106*, 93–106.

[114] Shaulian, E., Karin, M., AP-1 in cell proliferation and survival. *Oncogene* 2001, *20*, 2390–2400.

[115] Abate, C., Patel, L., Rauscher, F. J., 3rd, Curran, T., Redox regulation of fos and jun DNA-binding activity in vitro. *Science* 1990, *249*, 1157–1161.

[116] Hirota, K., Matsui, M., Iwata, S., Nishiyama, A. et al., AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1. *Proc. Natl. Acad. Sci. USA* 1997, *94*, 3633–3638.

[117] Horton, N. D., Biswal, S. S., Corrigan, L. L., Bratta, J., Kehrer, J. P., Acrolein causes inhibitor kappaB-independent decreases in nuclear factor kappaB activation in human lung adenocarcinoma (A549) cells. *J. Biol. Chem.* 1999, *274*, 9200–9206.

[118] Hirota, K., Murata, M., Sachi, Y., Nakamura, H. et al., Distinct roles of thioredoxin in the cytoplasm and in the nucleus. A two-step mechanism of redox regulation of transcription factor NF-kappaB. *J. Biol. Chem.* 1999, *274*, 27891–27897.

[119] Cabello, C. M., Bair 3rd, W. B., Lamore, S. D., Ley, S. et al., The cinnamon-derived Michael acceptor cinnamic aldehyde impairs melanoma cell proliferation, invasiveness, and tumor growth. *Free Radic. Biol. Med.* 2009, *46*, 220–231.

[120] Goldman, E. H., Chen, L., Fu, H., Activation of apoptosis signal-regulating kinase 1 by reactive oxygen species through dephosphorylation at serine 967 and 14-3-3 dissociation. *J. Biol. Chem.* 2004, *279*, 10442–10449.

