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Forum Review

Antioxidant Role of Glutathione *S*-Transferases: Protection Against Oxidant Toxicity and Regulation of Stress-Mediated Apoptosis

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ABSTRACT

It has been known that glutathione *S*-transferases (GSTs) can reduce lipid hydroperoxides through their Se-independent glutathione peroxidase activity and that these enzymes can also detoxify lipid peroxidation end products such as 4-hydroxynonenal (4-HNE). In this article, recent studies suggesting that the Alpha class GSTs provide a formidable defense against oxidative stress are critically evaluated and the role of these enzymes in the regulation of oxidative stress-mediated signaling is reviewed. Available evidence from earlier studies together with results of recent studies in our laboratories strongly suggests that lipid peroxidation products, particularly hydroperoxides and 4-HNE, are involved in the mechanisms of stress-mediated signaling and that it can be modulated by the Alpha class GSTs through the regulation of the intracellular concentrations of 4-HNE. *Antioxid. Redox Signal.* 6, 289–300.

INTRODUCTION

ANTIOXIDANTS are important means of negating the deleterious effects of oxidative stress, and are viewed as potential protective agents against age-related degenerative disorders such as atherosclerosis, cataractogenesis, carcinogenesis, Parkinson's disease, and Alzheimer's disease. Unless detoxified, the reactive oxygen species [ROS; *e.g.,* hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hydroxyl ($OH^\bullet$)] generated during processes such as mitochondrial electron transport, UV irradiation, inflammation, and metabolism of xenobiotics by the CYP450 system can attack the cellular macromolecules, including DNA, protein, and lipids. The interaction of ROS with lipids is particularly damaging to cells because a single ROS molecule can generate a number of toxicants such as the hydroperoxides, peroxyradicals, alkoxy radicals, and α,β-unsaturated aldehydes due to the autocatalytic propagation of lipid peroxidation reactions. Lipid peroxidation has been implicated in the etiology of age-related degenerative disorders (15, 34, 61, 66). Therefore, termination of ROS-induced lipid peroxidation and the detoxification of lipid peroxidation products are equally important as the disposition of ROS to protect cells from oxidative stress.

Aerobic organisms have a multitier defense system to combat oxidative stress that provides protection not only against the ROS, but also against the toxic electrophilic compounds generated by the interaction of ROS with cellular constituents, particularly the lipid peroxidation products. Enzymes such as catalase (CAT), superoxide dismutases (SOD), and glutathione peroxidases (GPxs) and nonenzymatic defense such as glutathione (GSH), urate, and tocopherols provide the first line of defense by inactivating ROS and scavenging the free radicals. However, even the small amounts of ROS escaping this first line of defense can initiate the autocatalytic chain of lipid peroxidation, resulting in the formation of a variety of toxic electrophilic species such as alkoxyradicals, peroxyradicals, epoxides, hydroperoxides, and relatively stable toxic and reactive end products such as 4-hydroxyalkenals [*e.g.,* 4-hydroxynonenal

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(4-HNE)], malondialdehyde, and acrolein. The defense mechanisms to provide protection against lipid peroxidation constitute the second line of defense against ROS. Recent studies suggest that glutathione *S*-transferases (GSTs) play a crucial role in defense mechanisms against lipid peroxidation. In this article, this antioxidant role of GSTs and its implications in stress-mediated signaling are reviewed.

DEFENSE MECHANISMS AGAINST LIPID PEROXIDATION

Se-dependent glutathione peroxidases (Se-GPxs) are known to provide protection against lipid peroxidation by terminating lipid peroxidation cascade through the reduction of fatty acid hydroperoxides (FA-OOH) and phospholipid hydroperoxides (PL-OOH). At least four Se-GPxs are known that can catalyze GSH-dependent reduction of lipid hydroperoxides. Of these selenoenzymes, GPx-1, GPx-2, and GPx-3, which are tetramers, can reduce H_2O_2 as well as FA-OOH, but not the intact PL-OOH, present in membranes (12, 20, 56). Only GPx-4, which is a membrane-associated monomeric enzyme, can reduce intact PL-OOH (58). Thus, Se-GPxs provide protection against H_2O_2 toxicity as well as the toxicity due to lipid peroxidation. In addition to Se-GPxs, GSTs can also reduce FA-OOH and PL-OOH, and their importance as antioxidant enzymes is beginning to be recognized only recently. Some of the GST isozymes can efficiently reduce FA-OOH as well as PL-OOH and can interrupt the autocatalytic chain of lipid peroxidation by reducing these hydroperoxides that propagate lipid peroxidation chain reactions (62, 63, 68). In addition, a subgroup of GST isozymes with substrate preference for α,β-unsaturated carbonyls (*e.g.,* 4-HNE and acrolein) can effectively detoxify these toxic end products of lipid peroxidation (1, 27, 48, 49, 52, 53, 69, 70). Thus, GSTs not only complement GPxs in attenuating lipid peroxidation by reducing hydroperoxides, but also protect cells from toxic end products of lipid peroxidation. Furthermore, compelling evidence suggesting the role of GSTs in the regulation of ROS-mediated cell cycle signaling has emerged in recent years. In this article, we have evaluated these physiological roles of GSTs.

ROLE OF GSTS IN DEFENSE AGAINST LIPID PEROXIDATION

Mammalian GSTs belong to a multifunctional family of phase II detoxification enzymes whose primary function is to catalyze the conjugation of electrophilic xenobiotics (or their metabolites) to GSH (13, 24, 28, 32). Currently, mammalian cytosolic GSTs are divided into four major gene families: Alpha, Mu, Pi, and Theta (24). In addition, at least four minor families (Zeta, Sigma, Kappa, and Omega) have also been identified (54). With the exception of the microsomal GSTs that are trimers (36), all mammalian GSTs are dimers of subunits within the class. In general, GST isozymes within a class have similar substrate specificities, but significant variations in substrate preferences and kinetic properties are often observed among the isozymes within a class. GST isozymes are expressed in a gender- (47) and tissue- (13, 24, 60) specific manner. Except for the microsomal GSTs, all other isozymes are presumed to be cytosolic, but recent studies from our laboratories suggest a strong association of some of the Alpha class GSTs with plasma membrane (45). Crystal structures of most mammalian GSTs are now available, and excellent reviews containing details of GST gene family, their nomenclature, and their role in detoxification of xenobiotics are available (24, 33). As the protection against lipid peroxidation is mainly provided by the Alpha class GSTs via their Se-independent GPx activity, a brief description of these isozymes given below is pertinent to this article.

Alpha class GSTs as antioxidant enzymes

In humans and rodents, at least four major Alpha class GST subunits designated as GSTA1, GSTA2, GSTA3, and GSTA4 have been characterized (24, 27). Corresponding dimeric isozymes are designated as GSTA1-1, GSTA2-2, GSTA3-3, and GSTA4-4. Recently, an additional subunit GSTA5 (35) has been cloned, and an Alpha class GST designated as GST5.8 has been partially characterized in human tissues (48, 49). Recent studies (62, 64) suggest that the Alpha class GSTs perhaps play a more important role than the Se-GPxs (GPx-1, GPx-2, GPx-3, or GPx-4) in defense mechanisms against lipid peroxidation. Thus, the Alpha class GSTs can provide protection against the electrophilic xenobiotics or the drugs not only via their conjugation to GSH, but also by alleviating oxidative stress and subsequent lipid peroxidation that is often associated with exposure to xenobiotics. GST isozymes, GSTA1-1 and GSTA2-2, can reduce PL-OOH as well as FA-OOH with high catalytic efficiency (62, 68). Kinetic properties of the Alpha class GSTs toward lipid peroxidation products presented in Table 1 suggest that these enzymes can interrupt lipid peroxidation chain reactions by reducing hydroperoxides. The Alpha class GST isozymes mGSTA4-4 (mice), rGSTA4-4 (rats), hGST5.8, and hGSTA4-4 (humans) have high activity toward 4-HNE and other α,β-unsaturated aldehyes (Table 2). These isozymes can also detoxify the toxic end products of lipid peroxidation, easing the burden of electrophilic stress on the cellular environment. More importantly, recent studies suggest that these enzymes can also affect cell cycle signaling by regulating the intracellular concentrations of 4-HNE. These roles of the Alpha class GSTs in defense against oxidative stress are outlined in Fig. 1.

The Alpha class GSTs hGSTA1-1 and hGSTA2-2 constitute the bulk of GSTs in human and rodent liver (13, 28, 62, 64). Among the known mammalian GSTs, the Alpha class GSTs are the most efficient in catalyzing the GSH-dependent reduction of lipid hydroperoxides (46, 62, 68). Considering the high abundance of these isozymes in tissues such as liver (~3% of total soluble protein), these enzymes can contribute a major portion of the total GPx activity toward lipid hydroperoxides. In fact, immunotitration studies using highly specific antibodies against the Alpha class GSTs have shown that more than half of the GPx activity of human and rodent liver toward lipid hydroperoxides can be immunoprecipitated by these antibodies (62, 64). Kinetic properties of hGSTA1-1 and hGSTA2-2 toward physiologically relevant products of lipid peroxidation (Table 1) indicate that both hGSTA1-1 and

TABLE 1. KINETIC CONSTANTS OF THE GPx ACTIVITY OF hGSTA1-1 AND hGSTA2-2 AGAINST LIPID HYDROPEROXIDES

	hGSTA1-1				*hGSTA2-2*			
Substrates	*Specific activity (μmol/min/mg)*	K_m *(mM)*	k_{cat} (s^{-1})	K_{cat}/K_m $(s^{-1}mM^{-1})$	*Specific activity (μmol/min/mg)*	K_m *(mM)*	k_{cat} (s^{-1})	K_{cat}/K_m $(s^{-1}mM^{-1})$
Dilinoleoyl phosphatidylcholine hydroperoxide	12.50	0.08	14.5	181.3	14.58	0.05	16.6	353
Dilinoleoyl phosphatidyl ethanolamine hydroperoxide	11.6	0.057	11.4	200	15.23	0.04	12.7	318
5-Hydroperoxyeicosatetraenoic acid	6.2	0.005	5.92	1183	7.52	0.007	9.1	1379

Data are from our published studies (68).

hGSTA2-2 have relatively high catalytic efficiency for the reduction of FA-OOH and PL-OOH. In general, the activity of hGSTA2-2 towards these substrates is higher than that of hGSTA1–1 (68). However, the relative abundance of hGSTA1-1 in human liver is ~10-fold higher than that of hGSTA2-2, indicating a major role of GSTA1-1 in the reduction of lipid hydroperoxides. It is possible that the substrate specificities of hGSTA1-1 and hGSTA2-2 toward individual FA-OOH or PL-OOH may vary, and further studies into the kinetic properties of these enzymes toward individual FA-OOH and PL-OOH may reveal specific functions of these isozymes. The role of the minor Alpha class enzyme, hGSTA3-3, in reduction of hydroperoxides may also be minimal because of its very low constitutive levels.

Overexpression of GSTA1-1 and GSTA2-2 protects cells against oxidant toxicity

Both hGSTA1-1 and hGSTA2-2 can use membrane PL-OOH as substrates *in situ* (62, 63). Therefore, the protection provided by these isozymes against lipid peroxidation is not dependent on release of the oxidized fatty acids from membrane phospholipids as suggested previously (57), and these enzymes can protect cell membranes at the site of damage (62, 63). The protective role of hGSTA1-1 and hGSTA2-2 against oxidant toxicity has been demonstrated in studies (62) showing that transfection of K562 cells with hGSTA1-1 or hGSTA2-2 protects these cells from H_2O_2 cytotoxicity (Fig. 2). These studies have shown that as compared with the wild-type or vector-transfected cells, lower levels of basal lipid peroxidation are observed in the cells transfected with hGSTA1-1 or hGSTA2-2. During the oxidative stress, the attenuation of lipid peroxidation in the transfected cells is even more remarkable (Fig. 3), and transfected cells are relatively more resistant to the cytotoxic effects of H_2O_2 and other oxidants such as naphthalene (63). As H_2O_2 is not a substrate for GSTA1-1 or GSTA2-2, the protection provided by these enzymes against H_2O_2 or oxidant toxicity must come from their ability to attenuate lipid peroxidation by reducing the hydroperoxides. *In vivo* studies also suggest a protective role of the Alpha class GSTs against the deleterious effects of chronic oxidative stress. Oxidative stress-induced cataractogenesis in rodents can be attenuated by administration of curcumin, which selectively induces the Alpha class GSTs in lens epithelial cells (3). Oxidative stress is involved in the mechanisms of cataractogenesis induced by administration of naphthalene or high galactose diet, and the inhibition of naphthalene- and galactose-induced cataractogenesis in mice by curcumin correlates with the induction of the Alpha class GSTs in lens epithelial cells (38, 39).

TABLE 2. SPECIFIC ACTIVITY AND KINETIC CONSTANTS OF MAMMALIAN GSTs TOWARD 4-HNE

Isozymes	*Specific activity (μmol/min/mg protein)*	K_m *(μM)*	k_{cat} (s^{-1})	k_{cat}/K_m $(s^{-1}mM^{-1})$
hGSTA4-4 (27)	189 ± 9	37 ± 4	113 ± 4	3,100
hGST5.8 (48)	176.0 ± 17.6	97 ± 2	227 ± 16	2,340
mGSTA4-4 (48)	65.2 ± 3.1	108 ± 3.0	89 ± 6	820
rGSTA4-4 (24, 26)	170	7.4 ± 0.2	144 ± 4	19,459 ± 782
hGSTA1-1 (68)	2.52 ± 0.22	50	2.94	58.8
hGSTA2-2 (68)	1.76 ± 0.18	80	2.1	26.3
hGSTM1-1 (48)	3.23 ± 0.32	121 ± 3.0	6.0 ± 0.20	49
hGSTP1-1 (48)	0.56 ± 0.03	154 ± 12.0	1.07 ± 0.05	7

Data were compiled from studies cited in parentheses. The nomenclature of GSTs is based on reference 33. In brief, a lowercase letter identifies species and an uppercase letter identifies the class (Alpha). A1-1 or A2-2 means that the enzyme is a homodimer of these subunits. The primary structure of hGST5.8 is unknown yet, and the enzyme is provisionally named according to its pI value. hGST5.8, rGSTA4-4, and mGSTA4-4 are immunologically similar, but distinct from hGSTA1-1, hGSTA2-2, hGSTA3-3, and hGSTA4-4.

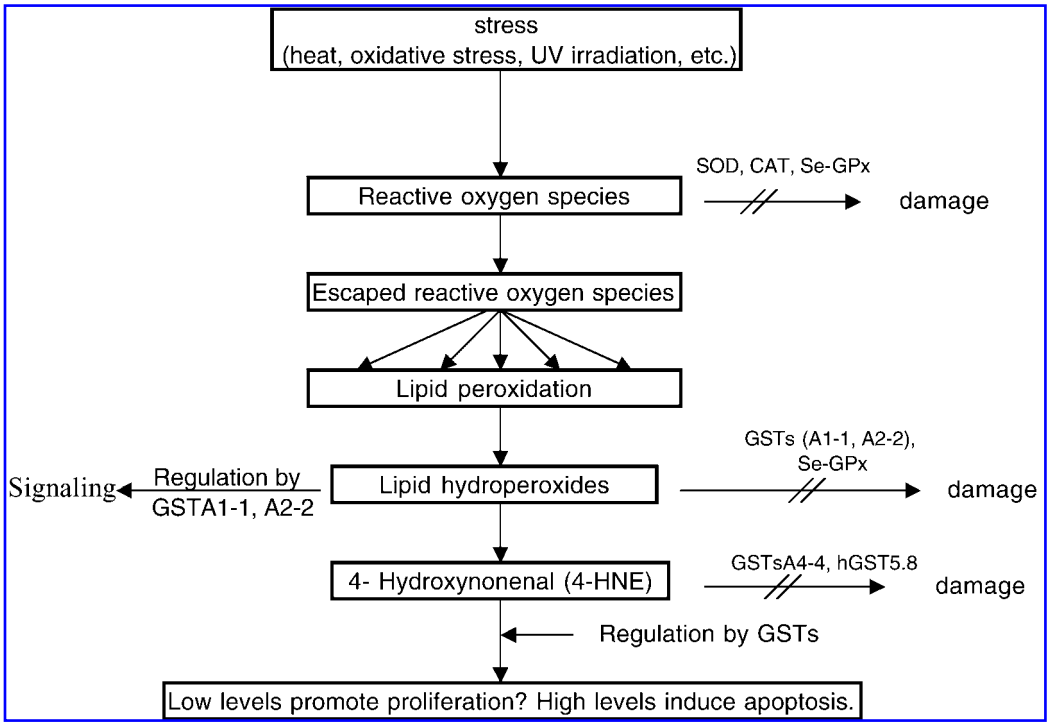

FIG. 1. Role of GSTs in protection against oxidant toxicity and regulation of signaling. The concentration of ROS generated upon exposure of cells to stress is regulated by primary antioxidant enzymes such as CAT, SOD, and Se-GPxs. Lipid peroxidation initiated by ROS escaping these defense mechanisms leads to amplification of oxidative stress. Lipid peroxidation products are involved in stress-mediated signaling mechanisms, and the Alpha class GSTs by regulating the intracellular concentrations of lipid hydroperoxides and 4-HNE can modulate these mechanisms.

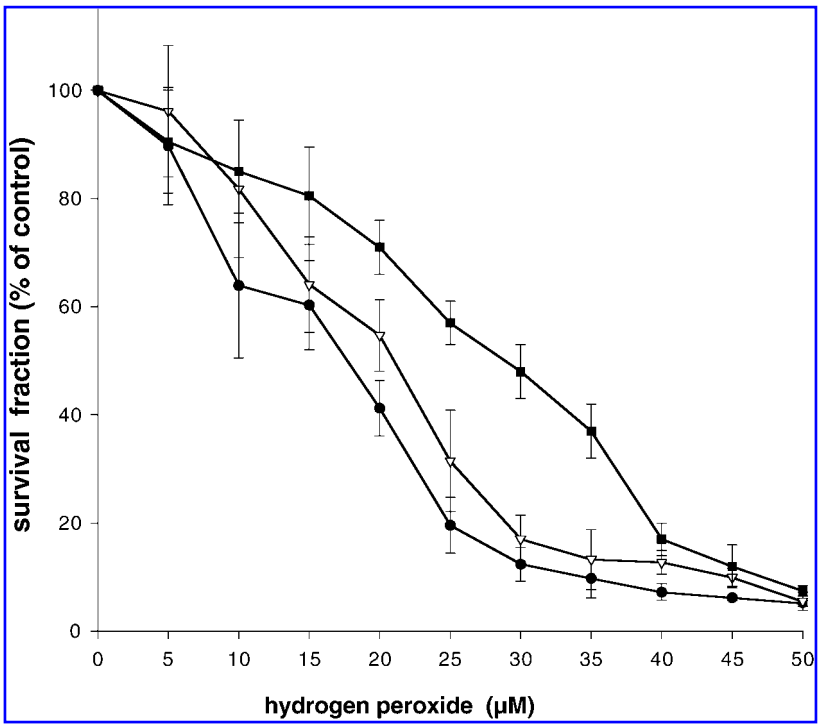

FIG. 2. K562 cells transfected with hGSTA2–2 acquire relative resistance to the cytotoxicity of H_2O_2. Cells in log-phase growth from wild-type (●), vector-transfected (▽), and hGSTA2-2-transfected (■) K562 cells were washed twice, resuspended in phosphate-buffered saline, and inoculated at a density of 2×10^5 cells/ml (50 μl/well) into eight replicate wells with various H_2O_2 concentrations (0–50 μ*M*) in a 96-well plate. The MTT assays were performed according to a previously described method (25). Blank (no cells) subtracted OD_{590} values were normalized to control (cells without H_2O_2 treatment). The figure represents results from one of several independent experiments on H_2O_2 cytotoxicity. Data were compiled from our previously published work (62).

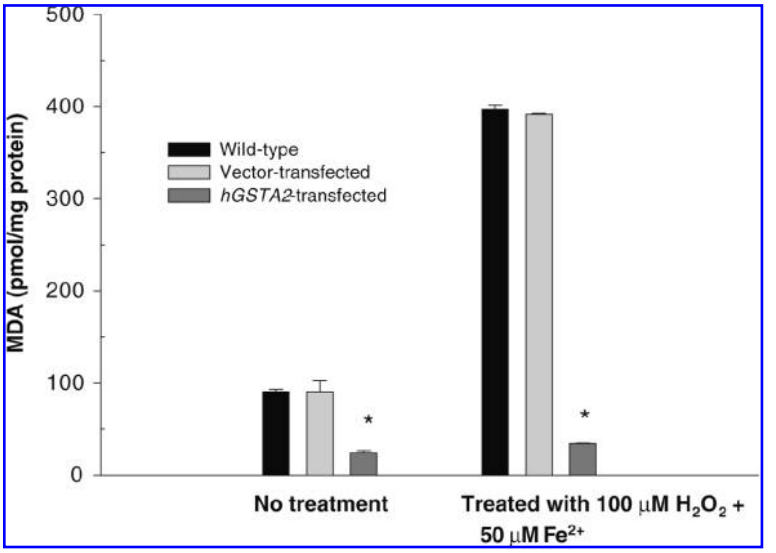

FIG. 3. hGSTA2-2 overexpression suppresses oxidative stress-induced lipid peroxidation. K562 cells (1×10^7) were incubated with RPMI complete medium alone or RPMI complete medium containing 100 μ*M* H_2O_2 and 50 μ*M* $FeSO_4$ for 30 min. The cells were pelleted by centrifugation, washed with phosphate-buffered saline, and homogenized in 10 m*M* potassium phosphate buffer, pH 7.0, containing 0.4 m*M* butylated hydroxytoluene. The whole homogenate was immediately assayed for malonaldehyde (MDA) by determining thiobarbituric acid reactive substances. The values are presented as means ± SD, ($n = 3$). *Significantly different from the controls ($p < 0.01$). Data were compiled from our previously published studies (62).

LIPID HYDROPEROXIDES AND SIGNALING: REGULATION BY ALPHA CLASS GSTS

There is substantial evidence suggesting involvement of lipid hydroperoxides in signaling cascades. PL-OOH can affect the hydrolytic activity of cytosolic phospholipase A_2 without marked changes in the intracellular concentration of free Ca^{2+} (41, 55). Lipid hydroperoxides have been shown to stimulate interleukin-1-induced nuclear factor-κB activation in a human endothelial cell line, and platelet-activating factor-like activity has been attributed to hydroperoxides isolated from oxidized low-density lipoprotein (25). Recent studies have shown that FA-OOH can activate NADPH oxidase and enhance production of O_2^- in vascular smooth muscle cells (31). PL-OOH can also induce apoptosis in human cell lines through a sustained activation of stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) and caspase 3 (62). As the Alpha class GSTs can regulate the intracellular levels of lipid hydroperoxides, we have studied the possible role of GSTs in the oxidative stress-mediated signaling for apoptosis.

Overexpression of hGSTA1-1 or hGSTA2-2 protects against oxidative stress-induced apoptosis

Studies in our laboratory (62) have shown that transfection of human erythroleukemia K562 cells with the Alpha class GSTs, hGSTA1-1 or hGSTA2-2, results in ~10-fold higher GPx activity toward PL-OOH and FA-OOH in the transfected cells without any compensatory response on the expression of antioxidant enzymes such as CAT, SOD, and the GPx activity toward H_2O_2. Upon treatment with H_2O_2, the transfected cells show minimal lipid peroxidation (Fig. 3) and only a transient activation of JNK, which quickly returns to basal levels. H_2O_2 does not cause caspase 3 activation in the transfected cells, and only a minimal number of these cells undergo apoptosis (62). In contrast, upon treatment with H_2O_2 under identical conditions, the wild-type and empty vector-transfected cells show a remarkable increase in lipid peroxidation and a sustained activation of JNK and caspase 3, and a significant fraction of cells undergo apoptosis (Fig. 4). Resistance of hGSTA1-1 or hGSTA2-2 transfected cells to H_2O_2-induced apoptosis should be attributed to their enhanced capability to reduce PL-OOH and FA-OOH because hGSTA1-1 and hGSTA2-2 display no detectable activity toward H_2O_2. This would suggest that lipid hydroperoxides formed as a consequence of oxidative stress mediate stress-induced apoptosis. This idea is supported by studies that show that wild-type K562 cells undergo apoptosis when treated with PL-OOH and transfection with *hGSTA2* cDNA prevents PL-OOH-induced apoptosis (62).

4-HNE has been shown to cause apoptosis in a variety of human cell lines (16–18, 51). Transfection of cells with hGSTA1-1 or hGSTA2-2 is not expected to provide protection against 4-HNE-induced apoptosis because 4-HNE is downstream to PL-OOH in the cascade of lipid peroxidation reactions and it is not a preferred substrate for hGSTA1-1 or hGSTA2-2. Consistent with this idea, 4-HNE-induced apoptosis in K562 cells is not inhibited by transfection with hGSTA1-1 or hGSTA2-2 (62), but is inhibited by transfection with a 4-HNE-metabolizing enzyme mGSTA4-4 (16, 18). Together, these studies suggest that oxidative stress-induced signaling for apoptosis is transduced through lipid hydroperoxides or their downstream product, 4-HNE. This contention is further supported by our unpublished studies that show that overexpression of hGSTA1-1 or hGSTA2-2 protects various cell types from UVA-induced lipid peroxidation and apoptosis. Cells overexpressing hGSTA1-1 or hGSTA2-2 are also resistant to apoptosis induced by oxidative stress-causing agents such as xanthine/xanthine oxidase, adriamycin, and naphthalene (63). It has been demonstrated that human lens epithelial cells (HLE B-3) show a persistent activation of JNK and caspases and undergo apoptosis when naphthalene is introduced in the culture medium. On the other hand, hGSTA1-1-overexpressing HLE B-3 cells neither show activation of JNK and caspases nor undergo apoptosis under similar conditions of naphthalene exposure (63). These findings strongly suggest that lipid peroxidation products may be a common link among the

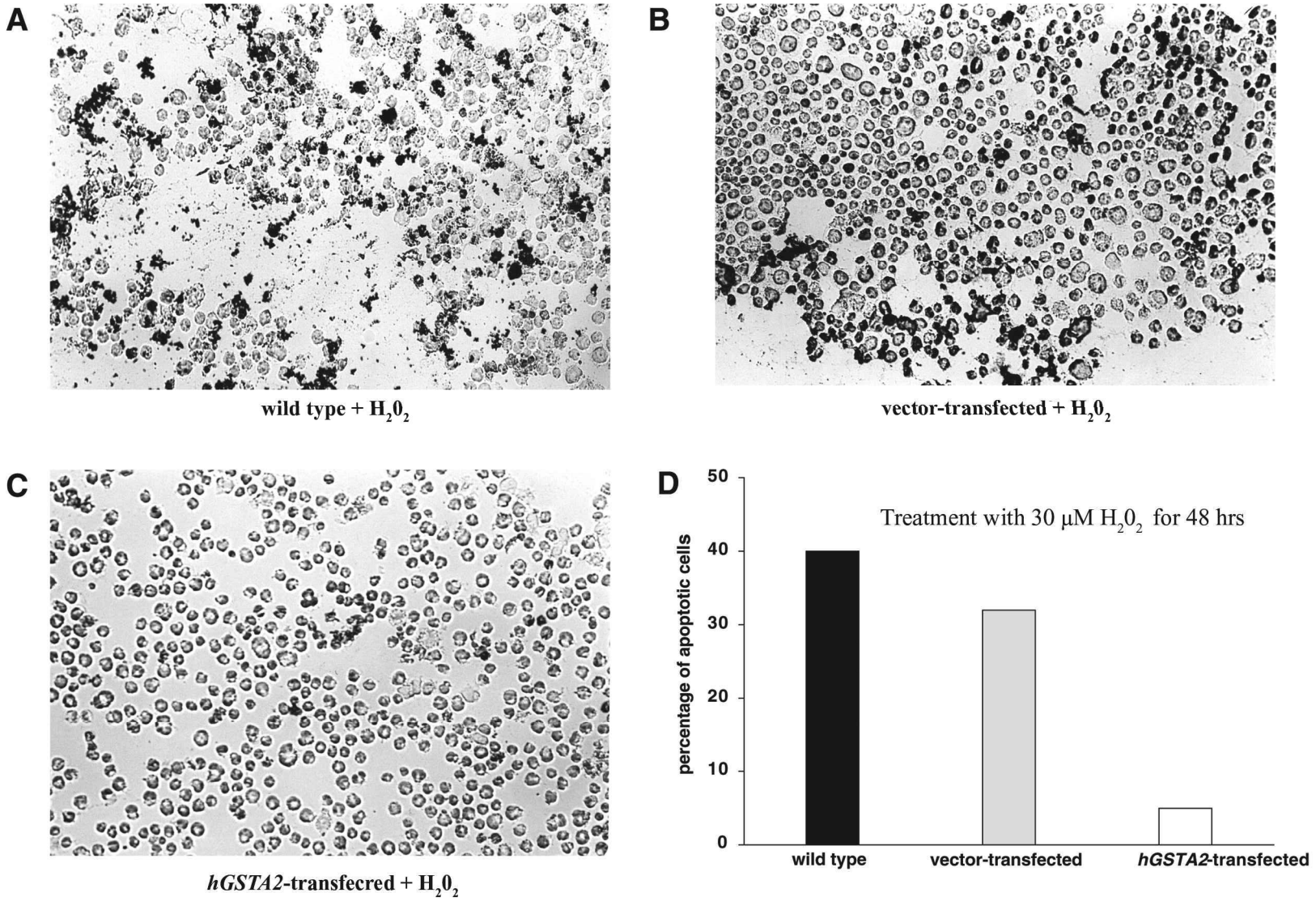

FIG. 4. hGSTA2-2 overexpression inhibits H_2O_2–induced apoptosis in K562 cells. Wild-type (**A**), vector-transfected (**B**), and *hGSTA2*-transfected (**C**) K562 cells were treated with 30 μ*M* H_2O_2 for 48 h. After this, cells were cytospun and fixed in 4% paraformaldehyde, and DNA fragmentation was detected by colorimetric TUNEL assay. The nuclei of apoptotic cells stained dark brown. (**D**) In each slide, 200 cells were counted to determine the percentage of apoptotic cells.

mechanisms of the signaling for apoptosis by oxidative stress, chemical agents, and UV irradiation. More importantly, these studies strongly indicate that GSTs can influence stress-mediated signaling by regulating the intracellular levels of lipid peroxidation products.

ROLE OF ALPHA CLASS GSTs IN REGULATION OF 4-HNE-MEDIATED SIGNALING

4-HNE and signaling

Being electrophilic in nature, 4-HNE is a potent alkylating agent; which can react with a variety of nucleophilic sites in DNA and proteins, generating various types of adducts (22). Its role in signaling mechanisms has been suggested for quite some time (14, 21, 42). Submicromolar concentrations of 4-HNE have been shown to activate protein kinase C-βII in rat hapotocytes, whereas micromolar concentrations of 4-HNE inhibit its activity (19). 4-HNE can affect nitric oxide homeostasis by inhibiting nuclear factor-κB dependent activation of inducible nitric oxide synthase (23). Recent studies indicate that intracellular 4-HNE levels are correlated with transforming growth factor-β1 levels in colon cancer (67). It has also been proposed that 4-HNE induces cyclooxygenase-2 via the activation of p38 mitogen-activated protein kinase (MAPK) (29, 30). Studies with a variety of cell lines suggest that 4-HNE activates SAPK/JNK (17, 18, 40, 59). In hepatic stellate cells, 4-HNE activates JNK through direct binding and not by phosphorylation (40), whereas in other cell types, 4-HNE may activate JNK through the redox-sensitive MAPK kinase cascade (59). Activation of JNK by 4-HNE is accompanied by the activation of caspase 3 and eventual apoptosis (17, 18, 51). Although the majority of studies show that 4-HNE is pro-apoptotic, it can also stimulate cell proliferation at relatively lower intracellular concentrations (16, 43), and it has been postulated that the intracellular concentration of 4-HNE may differentially affect the signals for proliferation, differentiation, and apoptosis (16–18, 21).

GSTs as determinants of the intracellular levels of 4-HNE

4-HNE being an α,β-unsaturated aldehyde has an electrophilic center, and it can be nonenzymatically conjugated to cellular nucleophiles such as GSH. The conjugation of 4-HNE to GSH in cells is, however, facilitated by GSTs that catalyze this reaction (1). A rat enzyme initially designated as GST8-8 (now rGSTA4-4) was shown to have high catalytic efficiency for 4-HNE (53). GST isozymes with substrate preference for 4-HNE and a high degree of homology with rGSTA4-4 have since been identified in mice (mGSTA4-4; 70), bovine (bGST5.8; 52), and human (GST5.8, 48; hGSTA4-4, 27). These enzymes belonging to a subgroup of the Alpha class GSTs have substrate preference for 4-HNE (Table 2) and are immunologically distinct from GSTA1-1, GSTA2-2, and GSTA3-3. Interestingly, in humans two distinct 4-HNE-metabolizing enzymes (hGSTA4-4 and hGST5.8) with K_{cat}/K_m values in the range of >2,000 s^{-1} mM^{-1} are present. Whereas hGSTA4-4 has been cloned (27), the primary structure of hGST5.8 is still not known and its cDNA has not been cloned perhaps due to its very low constitutive levels (17). Kinetic properties of tissue purified hGST5.8 have been studied, and its immunological similarity to mouse enzyme mGSTA4-4 suggests structural similarities between these two enzymes.

The relative abundance of 4-HNE-metabolizing GST isozymes is much lower than that of GSTA1-1, GSTA2-2, or the Mu and Pi class GSTs, which constitute the bulk of GST protein in mammalian tissues. In extrahepatic tissues, Pi and Mu class GSTs are predominant and the contribution of these enzymes in the metabolism of 4-HNE could also be substantial despite their low catalytic efficiency toward 4-HNE (Table 2). This is consistent with the results of our as yet unpublished studies showing that ~40% of residual GST activity toward 4-HNE is retained in the tissues of mGSTA4-4 null (−/−) mice. Redundancy in enzymes responsible for the metabolism of 4-HNE is similar to that observed with GPxs, which are responsible for the detoxification of H_2O_2 and lipid hydroperoxides and provide formidable defense against oxidative stress. This perhaps underscores the physiological significance of the mechanisms for maintaining the intracellular levels of 4-HNE. Recent studies reviewed below strongly suggest that GSTs can modulate stress-mediated signaling by regulating intracellular levels of 4-HNE.

Overexpression of GSTA4-4 promotes proliferation in some cell lines

Overexpression of 4-HNE-metabolizing GST isozyme in cells results in lower intracellular levels of 4-HNE (16–18, 65). K562 cells transfected with mGSTA4-4 having about fivefold higher GST activity toward 4-HNE as compared with the controls show only ~10% of 4-HNE levels as compared with the empty vector-transfected or wild-type cells (16). Interestingly, mGSTA4-4-transfected cells grow at a 50% higher rate as compared with their wild-type or vector-transfected counterparts, suggesting that lowering the levels of 4-HNE promotes proliferation (Fig. 5). Promotion of proliferation in cells having low intracellular levels of 4-HNE has also been observed in other cell lines. Unpublished studies in our laboratory also show that HLE B-3 cells transfected with hGSTA4-4 have lower basal levels of intracellular 4-HNE and grow at a rate about threefold faster as compared with the wild-type or vector-transfected cells. Promotion of the proliferation of aortic smooth muscle cells by low levels of 4-HNE has also been observed by Ruef *et al.* (43).

Overexpression of GSTA4–4 protects against oxidative stress-induced apoptosis

We have shown that increasing the concentrations of 4-HNE in the medium differentially affects mGSTA4-4-transfected and empty vector-transfected K562 cells. Exposure of 20 μ*M* 4-HNE to the wild-type or empty vector-transfected K562 cells results in a marked erythroid differentiation, whereas the cells transfected with mGSTA4-4 do not undergo such differentiation (16), suggesting a role of 4-HNE in signaling for differentiation and its modulation by GSTs. Prolonged exposure of the wild-type or vector-transfected K562 cells to rela-

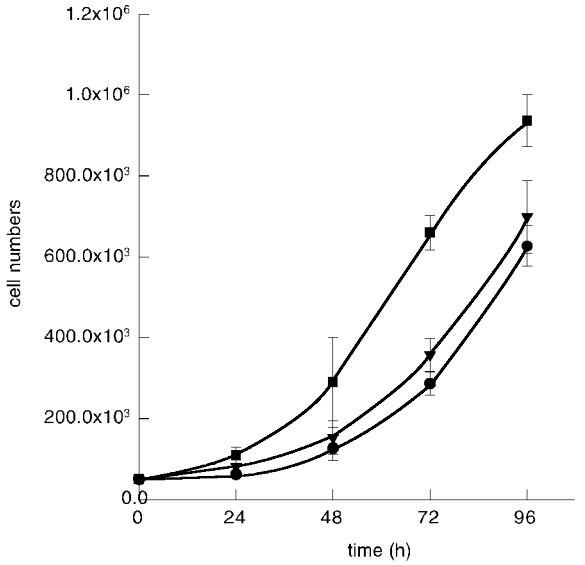

FIG. 5. mGSTA4-4 overexpression resulting in lower 4-HNE levels leads to increased growth rate of K562 cells. Wild-type (●), vector-transfected (▼), and mGSTA4-4-transfected (■) K562 cells were inoculated at a density of 1×10^5 cells/ml in 10 ml of RPMI 1640 medium containing 10% (vol/vol) fetal bovine serum and 1% (vol/vol) penicillin/streptomycin solution. Aliquots (100 µl) were removed at 24-h intervals, and trypan blue-excluding cells were counted using a hemocytometer. Average cell density and standard deviations from three separate experiments are presented. Data were compiled from our previous studies (16).

tively higher concentrations of 4-HNE in the medium leads to apoptosis. In contrast, the cells transfected with mGSTA4-4 are resistant to 4-HNE-induced apoptosis under these conditions (16). More importantly, the cells transfected with mGSTA4-4 also show resistance to H_2O_2-induced apoptosis, which implies that the signaling for H_2O_2-induced apoptosis may be conveyed through 4-HNE. Transfection with mGSTA4–4 does not affect the antioxidant enzymes such as CAT, GPx, and SOD. Therefore, the apoptotic effect of H_2O_2 in the transfected cells can be blocked only if 4-HNE is directly involved in H_2O_2-mediated signaling for apoptosis. Similar effects of mGSTA4-4 transfection on H_2O_2-induced apoptosis have also been observed in HL-60 cells (18). These studies show that in mGSTA4-4-transfected HL-60 cells, H_2O_2-mediated activation of JNK and caspase 3 is inhibited and the transfected cells are resistant to H_2O_2-induced apoptosis.

Induction of hGST5.8 and RLIP76 protects against oxidative stress and UVA-induced apoptosis

As suggested by the results of studies discussed above, the intracellular concentrations of 4-HNE play an important role in the transduction of signals for apoptosis in stressed cells. Under stress conditions, a rise in 4-HNE levels is expected, and in response to stress, cells may be expected to up-regulate the mechanisms that determine the intracellular concentrations of 4-HNE. In humans, a coordinated action of GSTs and the transporters that catalyze the ATP-dependent transport of the GSH conjugate of 4-HNE (GS-HNE) regulates the intracellular concentrations of 4-HNE (17). GST isozyme, hGST5.8, catalyzes the conjugation of 4-HNE to GSH to form GS-HNE, which must be transported out of the cells to sustain GST-mediated conjugation of 4-HNE. We have shown that the majority of GS-HNE transport is mediated by RLIP76 (6, 8, 44), a novel transporter capable of transporting a variety of xeno- and endobiotics with diverse structures (2, 4–11, 44, 50). Immunoprecipitation studies with highly specific antibodies against RLIP76 and MRP1 have shown that, in a variety of cell lines of human origin, ~70% of the ATP-dependent transport of GS-HNE is mediated by RLIP76 (44) and that it can be blocked by coating the cells with anti-RLIP76 IgG.

Our recent studies on the effect of stress on the intracellular concentrations of 4-HNE show that a rapid increase in 4-HNE levels is observed when cells are transiently exposed to low levels of H_2O_2, heat (42°C), or mild UVA irradiation (17, 65). The increase in 4-HNE levels is accompanied by a rapid induction of hGST5.8 and RLIP76, which regulate the intracellular levels of 4-HNE. The cells exposed to these transient and mild stressors acquire the capability to transport GS-HNE at a severalfold faster rate as compared with the control cells and acquire resistance to 4-HNE-induced apoptosis by blocking the activation of JNK and caspases. Interestingly, the stress-preconditioned cells also acquire resistance to H_2O_2-, UVA-, and O_2^--induced apoptosis because of their capability to exclude 4-HNE from the intracellular environment at a faster pace (17, 65). The resistance of stress-preconditioned cells to oxidative stress-mediated apoptosis can be abrogated by coating cells with anti-RLIP76 IgG, which blocks the efflux of GS-HNE resulting in increased intracellular levels of 4-HNE (Fig. 6). This phenomenon of mild stress preconditioning resulting in the induction of hGST5.8 and RLIP76 and the acquisition of resistance against oxidative stress-mediated apoptosis is observed in a variety of cell lines of human origin (17). Therefore, the involvement of 4-HNE in stress-mediated signaling does not appear to be limited only to specific cell types and that GSTs play an important physiological role in its regulation.

Overexpression of hGSTA4-4 affects expression of genes involved in cell cycle signaling

Further evidence for a pivotal role of GSTs in the modulation of cell cycle signaling is suggested by unpublished studies in our laboratory showing that the transfection of HLE B-3 cells with human 4-HNE-metabolizing GST isozyme hGSTA4-4 results in transformation and rapid growth of these cells. HLE B-3 are human lens epithelium cells immortalized with SV-40 transformation and are adherent cells. When these cells are transfected with hGSTA4-4, as expected the intracellular level of 4-HNE goes down. Surprisingly, hGSTA4-4-overexpressing cells with reduced levels of 4-HNE show rounding and detachment from the surface that is accompanied by a faster growth rate. These results strongly suggest a role of GSTs and perhaps other 4-HNE-metabolizing enzymes including aldose reductase and aldehyde dehydrogenase in cell cycle signaling. The mechanisms through which HLE B-3 cells undergo transformation subsequent to hGSTA4-4 transfection are being currently elucidated in our laboratory. Preliminary studies indi-

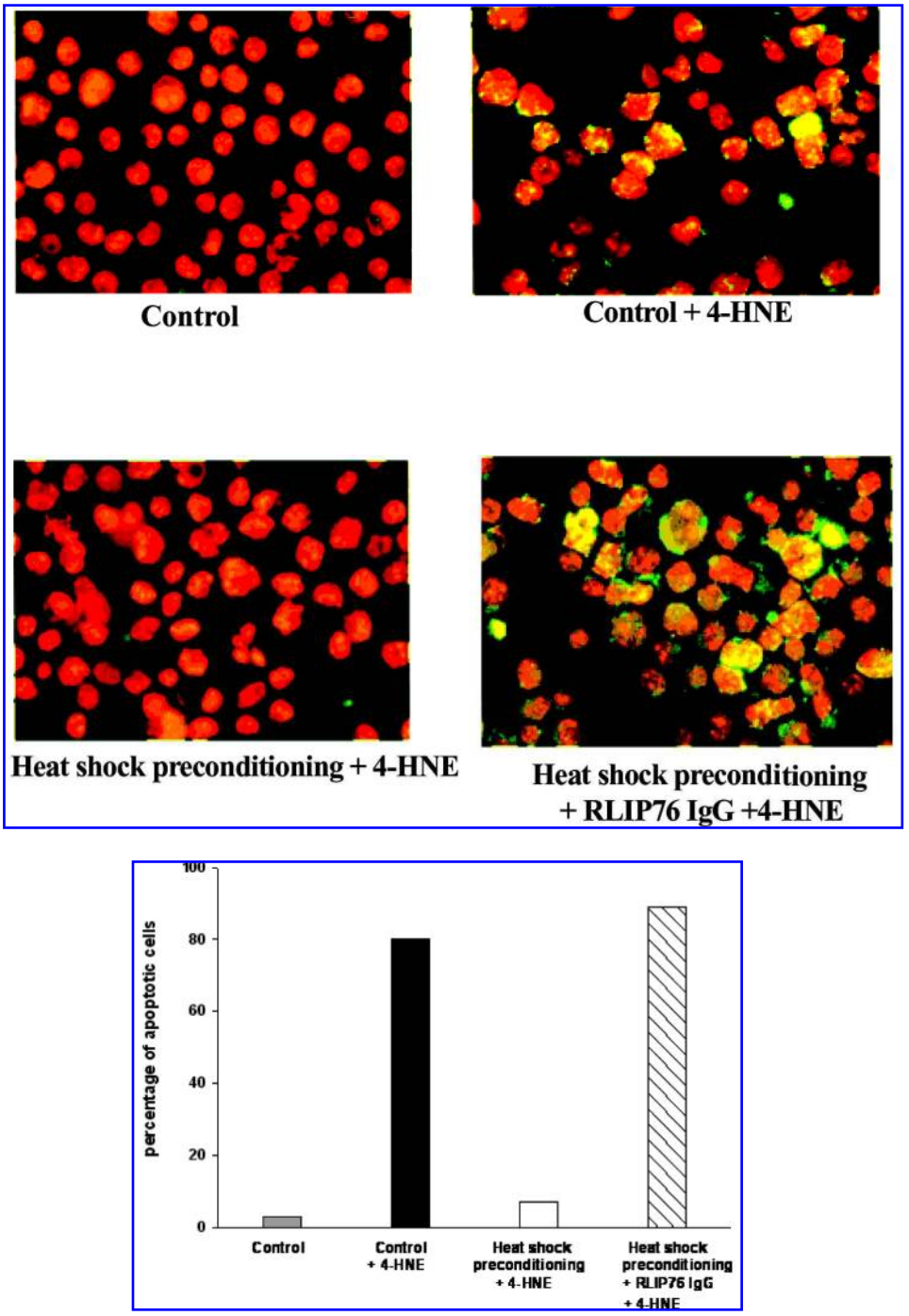

FIG. 6. Cells preconditioned with mild transient stress acquire resistance to 4-HNE-, H_2O_2, O_2^-, and UVA-induced apoptosis, and this resistance can be compromised by blocking the efflux of GS-HNE by anti-RLIP76 IgG. K562 cells (1×10^6) were fixed onto poly-L-lysine-coated slides by cytospin at 500 *g* for 5 min, and the TUNEL apoptosis assay was performed to detect apoptosis. The slides were analyzed by fluorescence microscope (Nikon Eclipse 600, Japan). Apoptotic cells showed characteristic green fluorescence. (**Left upper panel**) Control K562 cells pretreated with heat shock (42°C, 30 min) and allowed to recover for 2 h at 37°C. (**Right upper panel**) Control cells without heat shock pretreatment, incubated with 20 μ*M* 4-HNE for 2 h. (**Left lower panel**) Cells pretreated with heat shock, allowed to recover for 2 h at 37°C, followed by incubation in medium containing 20 μ*M* 4-HNE for 2 h at 37°C. (**Right lower panel**) Heat shock-pretreated cells, allowed to recover for 1 h at 37°C, after which anti-RLIP76 IgG was added to medium (20 μg/ml final concentration) and incubated for an additional 1 h to coat the cells with anti-RLIP76 IgG for blocking the efflux of GS-HNE. Anti-RLIP76 IgG-coated cells were then incubated for 2 h at 37°C in medium containing 20 μ*M* 4-HNE. (**Middle panel**) The percentage of the apoptotic cells counted from each slide. Similar results were obtained when cells were preconditioned with mild UVA or H_2O_2 exposure and instead of 4-HNE, prolonged exposure to H_2O_2, UVA, or O_2^- was used to induce apoptosis (17, 65).

cate that hGSTA4-4-transfected cells show substantial down-regulation of p53 and up-regulation of transforming growth factor-β and extracellular signal-regulated kinase, suggesting that the expression of these proteins involved in cell cycle signaling is modulated by GSTs. The role of GSTs in regulating cell cycle signaling and the mechanism through which 4-HNE modulates these processes should be vigorously pursued.

ABBREVIATIONS

CAT, catalase; FA-OOH, fatty acid hydroperoxides; GPx, glutathione peroxidase; GSH, glutathione (reduced form); GS-HNE, glutathione conjugate of 4-hydroxynonenal; GST, glutathione *S*-transferase; HLE B-3 cells, human lens epithelial cells; 4-HNE, 4-hydroxynonenal; H_2O_2, hydrogen peroxide; K562 cells, human erythroleukemia cells; MAPK, mitogen-activated protein kinases; O_2^-, superoxide anion; PL-OOH, phospholipid hydroperoxides; RLIP76, 76-kDa Ral-binding GTPase activating protein (RalBP1); ROS, reactive oxygen species; SAPK/JNK, stress-activated protein kinase/c-Jun N-terminal kinase; Se-GPx, Se-dependent glutathione peroxidase; SOD, superoxide dismutase.

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