

# Glutathione S-Transferase p Elicits Protection against H<sub>2</sub>O<sub>2</sub>-induced Cell Death via Coordinated Regulation of Stress Kinases<sup>1</sup>

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## Abstract

To elucidate mechanisms underlying glutathione S-transferase p (GSTp)-mediated cellular protection against oxidative stress-induced cell death, the effect of GSTp on stress signaling pathways was investigated before and after H<sub>2</sub>O<sub>2</sub> treatment. Under nonstressed conditions, increased expression of GSTp via a tet-off-inducible GSTp in NIH 3T3 cells increased the phosphorylation of mitogen-activated protein (MAP) kinase kinase 4, p38, extracellular receptor kinase (ERK), and inhibitor of  $\kappa$ -kinase (IKK), and reduced phosphorylation of MAP kinase kinase 7 and Jun NH<sub>2</sub>-terminal kinase (JNK). Whereas H<sub>2</sub>O<sub>2</sub> treatment of cells induced JNK, p38, and IKK activities, in the presence of H<sub>2</sub>O<sub>2</sub> and elevated GSTp expression there was an additional increase in ERK, p38, and IKK activities and a decrease in JNK activity. GSTp-mediated protection from H<sub>2</sub>O<sub>2</sub>-induced death was attenuated upon inhibition of p38, nuclear factor  $\kappa$ B, or MAP kinase by dominant negative or pharmacological inhibitors. Conversely, expression of a dominant negative JNK protected cells from H<sub>2</sub>O<sub>2</sub>-mediated death. These data suggest that the coordinated regulation of stress kinases by GSTp, as reflected by increased p38, ERK, and nuclear factor  $\kappa$ B activities together with suppression of JNK signaling, contributes to protection of cells against reactive oxygen species-mediated death.

## Introduction

Cell protection from external damage largely depends on the availability and activity of antioxidative enzymes, which maintain homeostatic control of ROS.<sup>3</sup> An altered balance of ROS directly affects cellular proliferation, apoptosis, and senescence (1, 2).

A key determinant of the cellular response to oxidative stress relates to the level and form of glutathione. Changes in glutathione levels have been associated with the activation of stress kinases (3), although the underlying mechanisms are not known. A major factor that affects glutathione homeostasis is its utilization by conjugation, primarily via GST (reviewed in Refs. 4, 5). The ability of GST to alter levels of cellular glutathione in response to production of ROS has been implicated in protection of cells from ROS-inducing agents (6, 7).

Accumulation of ROS in response to UV or H<sub>2</sub>O<sub>2</sub> treatment results

in the activation of multiple stress kinase cascades, including the ASK1, MEKK1, MAPK, (8, 9), ERK (10), and IKK-NF $\kappa$ B (11, 12) signaling pathways. Among the substrates for these signaling cascades are p53, NF $\kappa$ B, c-Jun, ATF2, and c-Fos, which dictate protection from, or promotion of, cell death (13, 14). Importantly, the balance between different stress signaling cascades appears to be among the key determinants in dictating the cell's fate (reviewed in Refs. 15, 16), although the mechanisms underlying the coordinated regulation of the kinases involved are not understood.

Recent studies revealed an association of GSTp with JNK through which it regulates the low basal level of JNK activity in nonstressed cells. Stress in the form of UV or H<sub>2</sub>O<sub>2</sub> causes multimerization of GSTp, which impedes its inhibition of JNK (17). This is remarkably similar to thioredoxin's association with and inhibition of ASK1 activities, which limit ASK1 activities under nonstressed growth conditions (18). Given the ability of ROS to activate stress kinases and the link between stress-activated kinases and altered redox potential, we explored mechanisms underlying the ability of GST to elicit protection against ROS-producing agents. The present study demonstrates that GSTp coordinates ERK/p38/IKK activation and JNK suppression as part of the mechanism underlying its ability to elicit protection against H<sub>2</sub>O<sub>2</sub>-induced cell death.

## Materials and Methods

**Cells and Protein Preparation.** The mouse fibroblast cell line NIH 3T3 and the NIH 3T3 cells that stably express the pSV40-Hyg plasmid were maintained in DMEM supplemented with 10% fetal bovine serum and antibiotics (Life Technologies). Cells were grown at 37°C with 5% CO<sub>2</sub>. The pTet-GSTp was constructed by subcloning the cDNA of wild-type GSTp (*Bam*HI-*Sal*I fragment) into the tet-regulated promoter of the pUHD-10-3 vector (Clontech). Cell clones that stably express both constructs were selected in 600  $\mu$ g/ml geneticin in the presence of hygromycin (100  $\mu$ g/ml). GSTp-tet-regulatable cells were maintained in DMEM containing 10% fetal bovine serum, 100  $\mu$ g/ml hygromycin, and 400  $\mu$ g/ml geneticin. To maintain suppression of GSTp expression, 1  $\mu$ g/ml tet was added to the medium every 3 days. Proteins were prepared from cells as described previously (19). In all cases, the buffer contained a cocktail of protease (1  $\mu$ g/ml pepstatin, leupeptin, and aprotinin) and the phosphatase inhibitors sodium vanadate (1 mM) and sodium fluoride (5 mM).

**Chemicals.** H<sub>2</sub>O<sub>2</sub>, GSTp, pepstatin, leupeptin, aprotinin, sodium vanadate, and sodium fluoride were purchased from Sigma. SB203580 and PD98059 were purchased from CalBiochem.

**Constructs.**  $\Delta$ MEKK1, a constitutively active form of MEKK1 that lacks amino acids 1–351, was kindly provided by Audrey Minden (Columbia University, New York, NY). MKK6<sup>DD</sup>, a constitutive activator of p38; JNK2<sup>APF</sup>, a dominant negative of JNK2; p38<sup>ASP</sup>, a dominant negative form of p38; flag-MKK7, flag-MKK4, and GST-ATF2 expression vectors were kindly provided by Roger Davis (University of Massachusetts, Worcester, MA). I $\kappa$ B $\Delta$ N, a superstable form of I $\kappa$ B, was provided by Dean Ballard (School of Medicine, Vanderbilt University, Nashville, TN). IKK $\beta$ <sup>SE</sup>, a constitutively active I $\kappa$ B kinase  $\beta$ S177; 181E; GST-I $\kappa$ B; 2xNF $\kappa$ B-Luciferase; and MEK<sup>EL</sup>, a constitutively active form of MEK, were kindly provided by Michael Karin (University

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<sup>3</sup> Abbreviations used are: ROS, reactive oxygen species; GST, glutathione S-transferase; ASK1, apoptosis signal-regulating kinase 1; MEKK1, MAPK/ERK kinase kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular receptor kinase; IKK, inhibitor of  $\kappa$ -kinase; NF $\kappa$ B, nuclear factor  $\kappa$ B; ATF2, activating transcription factor 2; GSTp, glutathione S-transferase p; JNK, Jun NH<sub>2</sub>-terminal kinase; MEK, MAPK/ERK kinase; MKK, MAPK kinase; I $\kappa$ B, inhibitor of nuclear factor  $\kappa$ B; tet, tetracycline; IP, immunoprecipitation; PKB, protein kinase B/AKT.

of California, San Diego, CA). GSTp cDNA was cloned into pcDNA3.1. 5xJun2tk-Luciferase was kindly provided by Hans Van Dam (Silvius Laboratory, Leiden, the Netherlands).

**Antibodies, Immunoprecipitations, and Immunoblots.** Antibodies to c-Jun were purchased (Santa Cruz Biotechnology). Polyclonal antibodies to JNK were generated using bacterially expressed JNK as an antigen. Polyclonal antibodies to GSTp were generated using purified GSTp (Sigma). Phospho-antibodies to MKK4 and MKK7 were gifts from Michael Comb of New England Biolabs., Beverly, MA. Phospho-antibodies to MKK6, ERK1/2, AKT, p38, IκB, and JNK were purchased (New England Biolabs). Immunoprecipitations were carried out using 1 mg of protein extract and 1 μg of the respective antibodies and protein G beads (Life Technologies) for 16 h at 4°C as described previously (17). Immunoblotting analysis was performed as described (17). Quantification of the results was performed by computerized densitometry.

**H<sub>2</sub>O<sub>2</sub> Treatment.** Medium taken from the culture dish was mixed with freshly diluted H<sub>2</sub>O<sub>2</sub> and immediately applied to the cultured fibroblasts.

**Immunokinase Assays.** Immunokinase assays were carried out using immunoprecipitated material (of 1 mg of protein) that had been incubated with the respective substrates (17). IP with JNK was followed by phosphorylation of GST-Jun<sup>1-87</sup>, IP of p38 was followed by phosphorylation of GST-ATF2, IP of ERK was followed by phosphorylation of myelin basic protein, and IP of IKK was followed by phosphorylation of GST-IκB.

**Apoptosis Studies.** Analysis of cell death was carried out as described by Kumar *et al.* (20), using triplicates of >5000 cells per measurement, at the time points indicated in "Results."

**Results**

**GSTp Expression Alters Activity of Stress Kinases.** To elucidate the nature of GSTp-elicited changes in stress kinases, we established a tet-off-based GSTp-inducible 3T3 cell line. This cell line was selected because of its low basal levels of GSTp (Fig. 1a). Using cells maintained in the presence or absence of tet, we monitored changes in stress kinases. Removal of tet caused a time-dependent gradual increase in GSTp expression (Fig. 1a). Within the initial 8 h, the levels of GSTp were increased 2–4-fold, whereas during the 12–24 h period, GSTp expression was increased 10–20-fold (Fig. 1a). MKK4 and MKK6 were among the kinases that were not affected within the first 8 h after increased GSTp expression in nonstressed 3T3 cells. Kinases that were inhibited by low levels of GSTp expression included JNK, MKK3, p38, and MKK7. Conversely, upon higher expression of GSTp, p38 exhibited a substantial increase in its phosphorylation. ERK phosphorylation revealed a marked increase at later time points (Fig. 1a). A modest increase was seen in phosphorylation of MKK7 after 24 h of elevated GSTp expression (Fig. 1a). Higher levels of GSTp expression (absence of tet for 12–24 h) attenuated inhibition of JNK phosphorylation. This observation is in line with our former findings, which revealed a concentration-dependent inhibition of JNK activity by GSTp (17). Increased GSTp expression also caused a noticeable increase in the degree of IκB phosphorylation (not shown). Of the various stress-activated kinases tested, PKB/AKT was not

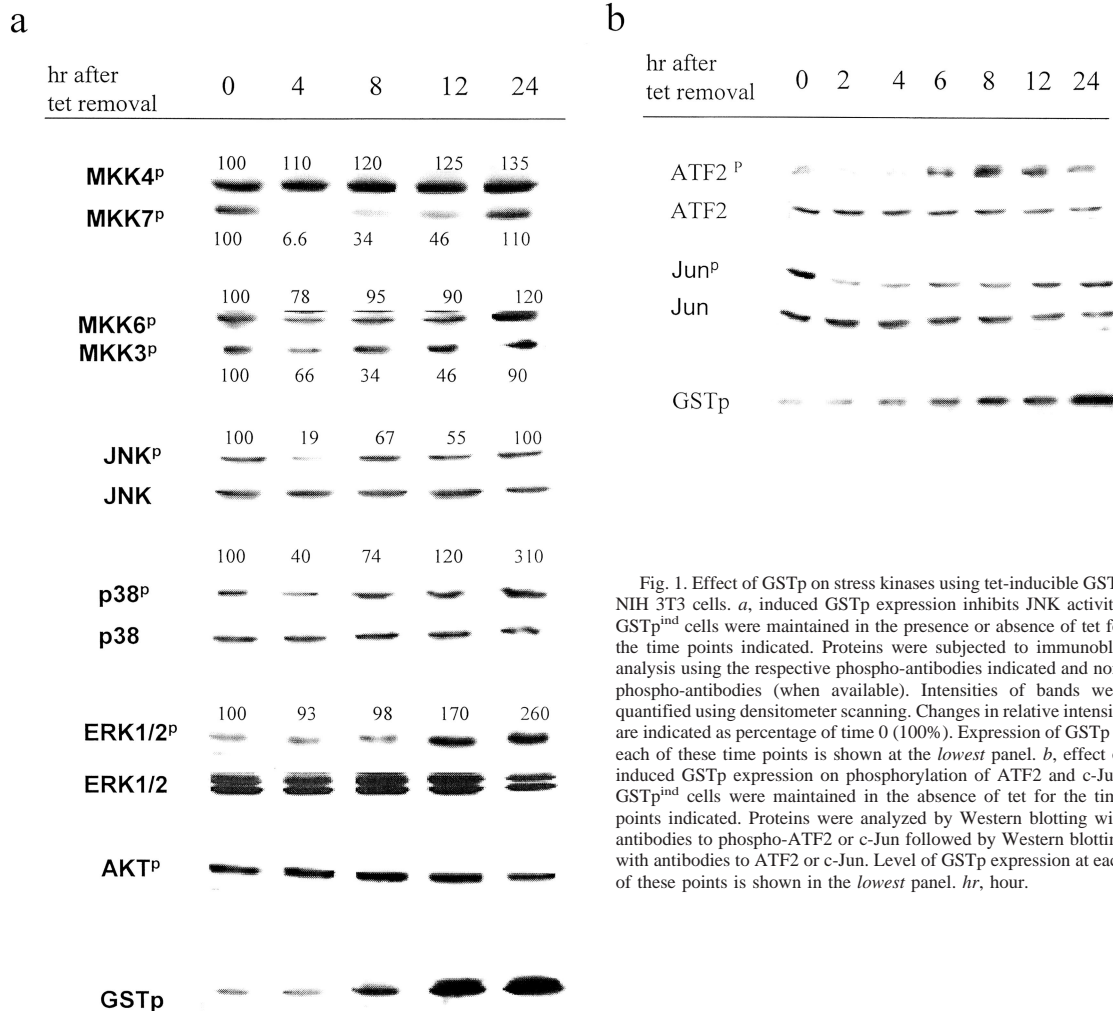


Fig. 1. Effect of GSTp on stress kinases using tet-inducible GSTp NIH 3T3 cells. *a*, induced GSTp expression inhibits JNK activity. GSTp<sup>ind</sup> cells were maintained in the presence or absence of tet for the time points indicated. Proteins were subjected to immunoblot analysis using the respective phospho-antibodies indicated and non-phospho-antibodies (when available). Intensities of bands were quantified using densitometer scanning. Changes in relative intensity are indicated as percentage of time 0 (100%). Expression of GSTp at each of these time points is shown at the lowest panel. *b*, effect of induced GSTp expression on phosphorylation of ATF2 and c-Jun. GSTp<sup>ind</sup> cells were maintained in the absence of tet for the time points indicated. Proteins were analyzed by Western blotting with antibodies to phospho-ATF2 or c-Jun followed by Western blotting with antibodies to ATF2 or c-Jun. Level of GSTp expression at each of these points is shown in the lowest panel. *hr*, hour.

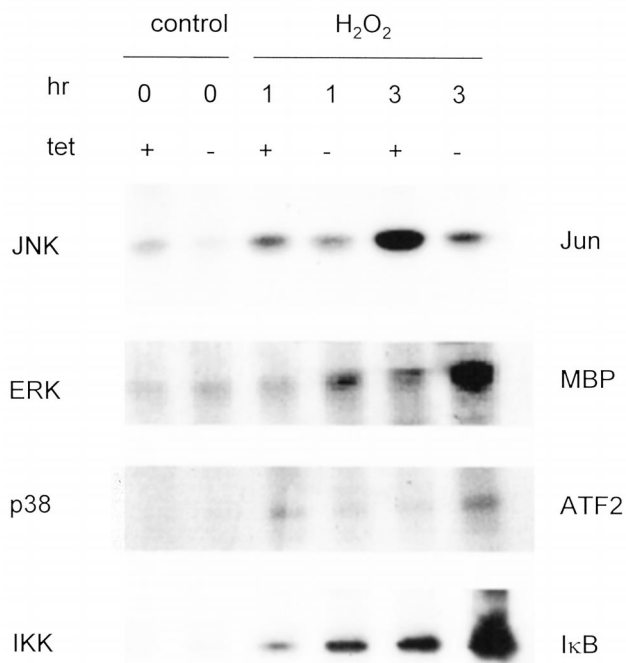


Fig. 2. Effect of GSTp on H<sub>2</sub>O<sub>2</sub> induction of stress kinases using tet-inducible GSTp NIH 3T3 cells. GSTp<sup>ind</sup> cells were maintained in the presence or absence of tet with or without H<sub>2</sub>O<sub>2</sub>. Proteins were prepared for analysis 1 or 3 h after H<sub>2</sub>O<sub>2</sub> treatment. Analysis was carried out via immunokinase reaction using antibodies to the kinases indicated on the left and respective substrates as indicated on the right. hr, hour; MBP, myelin basic protein.

affected by either low or high levels of GSTp expression (Fig. 1a). These results document the ability of GSTp to elicit coordinated regulation of various stress kinases in a dose-dependent manner. These observations also imply that the subset of activated stress kinases affected by GSTp depends on the expression level of GSTp. Whereas at low levels GSTp inhibited phosphorylation of JNK, MKK3, MKK7, and p38, higher expression of GSTp led to marked ERK1/2, MKK4, and p38 phosphorylation. Changes in the level of GSTp expression, as generated in these GSTp-inducible cells (Fig. 1a, lowest panel), mimic the increase in GSTp expression seen after exposure to various DNA-damaging agents (21, 22). Similarly, elevated expression of GSTp has been reported to exist in a wide range of human tumors and often is associated with increased drug resistance (7, 23).

The effects of GSTp on stress kinases were also reflected at the level of the respective transcription factor substrates. Increased expression of GSTp coincided with increased phosphorylation of ATF2 (Fig. 1b) but not c-Jun, probably because of an increase in p38 activity (Fig. 1a). Together, these data establish the effect of GSTp expression on the activity of key stress kinases and their respective substrates. The biological significance of GSTp-mediated changes in various stress kinases was elucidated using cell death as a relevant biological end point.

**Effect of GSTp Expression on Stress Kinases following Exposure to H<sub>2</sub>O<sub>2</sub> Treatment.** We next examined the effect of GSTp expression on the activities of stress kinases after exposure to H<sub>2</sub>O<sub>2</sub> treatment, which generates high levels of ROS (1, 24). Analysis of H<sub>2</sub>O<sub>2</sub>-elicited changes revealed increased activity of ERK, p38, JNK, and IKK, measured via immunokinase reactions of the respective substrates (Fig. 2). H<sub>2</sub>O<sub>2</sub> treatment in the presence of GSTp expression further increased ERK, p38, and IKK activities as clearly seen at the 3-h time point (Fig. 2). Conversely, GSTp expression reduced the

degree of H<sub>2</sub>O<sub>2</sub>-induced JNK phosphorylation, although the level of JNK phosphorylation was still higher than in control or in GSTp-expressing cells (Fig. 2). The ability of GSTp to limit the degree of JNK activation after H<sub>2</sub>O<sub>2</sub> treatment points to a mechanism by which GSTp may also serve to limit the magnitude of this stress kinase output. Together, changes seen in stress kinases upon H<sub>2</sub>O<sub>2</sub> treatment of GSTp-expressing cells revealed the nature of coordinated regulation of stress kinases as reflected in the increased activities of IKK, p38, and ERK while limiting the JNK output.

**Elevated GSTp Expression Elicits Protection against H<sub>2</sub>O<sub>2</sub>-induced Cell Death.** Given the well-documented protection elicited by GSTp in cells subjected to treatments that generate high levels of ROS (reviewed in Refs. 5, 7), we determined whether GSTp-mediated changes in stress kinases play a causative role in GSTp-elicited protection against ROS-generating treatments. To this end, we first compared the levels of cell death in response to various doses of H<sub>2</sub>O<sub>2</sub> in the presence and absence of GSTp expression. Treatment with 50 μM H<sub>2</sub>O<sub>2</sub> in the absence of GSTp expression led to the death of 35% of the cells after 24 h; this was reduced to 20% upon GSTp expression (Fig. 3). Analysis at a later time point (48 h) revealed higher degrees of cell death, which were dependent on the dose of H<sub>2</sub>O<sub>2</sub> used (not shown). Exposure to higher doses of H<sub>2</sub>O<sub>2</sub> (100 μM) also revealed GSTp-mediated protection against cell death (39% versus 25%). GSTp maintained its protection even at higher doses of H<sub>2</sub>O<sub>2</sub> (40% versus 30% at 200 μM; Fig. 3). These data suggest that the H<sub>2</sub>O<sub>2</sub>-induced cell death of 3T3 cells was efficiently reduced by GSTp expression. Analysis of the type of H<sub>2</sub>O<sub>2</sub>-induced cell death revealed that it is primarily late apoptosis/necrosis (not shown), which is in line with previous studies indicating that necrosis is the major form of death induced by H<sub>2</sub>O<sub>2</sub> (11).

**p38, ERK, and NFκB Activation and JNK Suppression Are Required for GSTp's Ability to Mediate Protection against ROS-induced Cell Death.** To directly assess the possible contribution of GSTp-modified kinases to GSTp-mediated protection from H<sub>2</sub>O<sub>2</sub>-induced cell death, 3T3 cells were transfected with a dominant negative form of p38, with JNK, or with the superrepressor of IκB, or were subjected to treatment with pharmacological inhibitors of MAPK (PD98059) or p38 (SB203580). An ability to reduce the degree of GSTp-elicited protection would point to the role of the respective kinase in this response.

Forced expression of JNK<sup>APF</sup>, a dominant negative form of JNK,

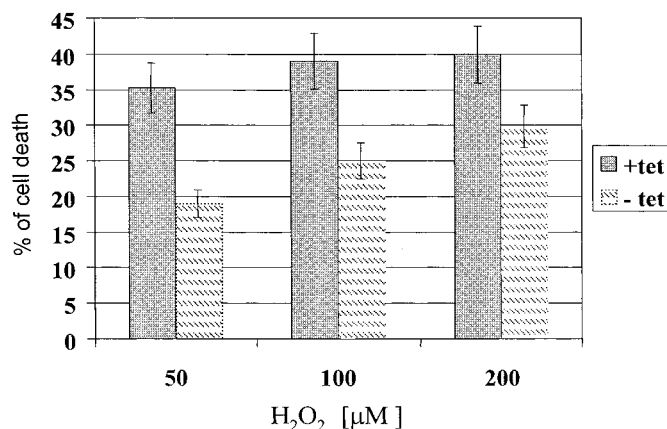


Fig. 3. Effect of GSTp on H<sub>2</sub>O<sub>2</sub>-induced cell death. GSTp<sup>ind</sup> cells were maintained in the presence (gray columns) or absence (hatched columns) of tet for the time points indicated with or without H<sub>2</sub>O<sub>2</sub> treatment at the doses indicated. In all cases, cells were prepared for cell death analysis 30 h after H<sub>2</sub>O<sub>2</sub> treatment. Each analysis was performed in triplicate (counting 50,000 cells per point). Data shown represent values over control, which varied between 2.5 and 5.0% of cell death. Data shown represent three independent experiments.

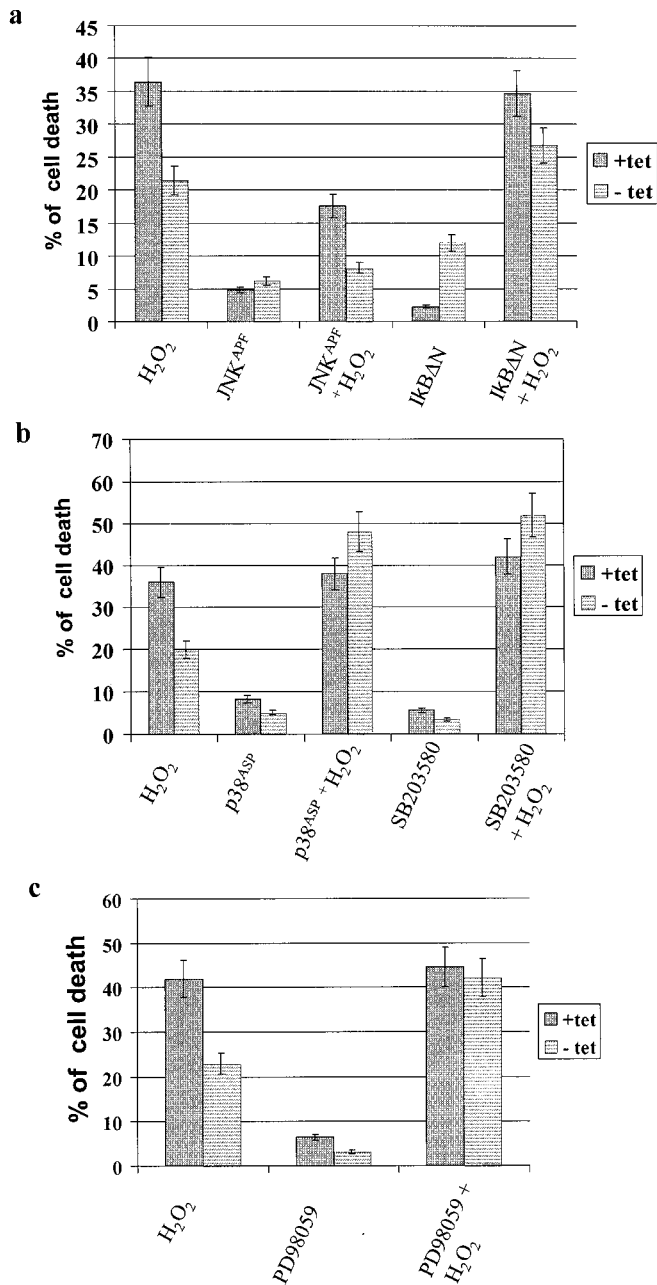


Fig. 4. Inhibition of p38, ERK, or NF $\kappa$ B attenuates GSTp ability to elicit protection from H<sub>2</sub>O<sub>2</sub>-induced cell death. *a*, GSTp<sup>ind</sup> cells were transfected with JNK<sup>APF</sup> or I $\kappa$ BAN and 24 h later exposed to H<sub>2</sub>O<sub>2</sub> (100  $\mu$ M) in the presence (gray column) or absence (hatched column) of tet. Degree of cell death was determined 24 h after exposure to H<sub>2</sub>O<sub>2</sub>, using vital staining analysis (20). Data shown represent three experiments performed in triplicate. Data shown reflect numbers over control nontreated cells (which varied between 3 and 5%). *b*, experiment performed similar to the one described in *a*, with the exception that the pharmacological inhibitor SB203580 was used to inhibit p38 catalytic activity. The dominant negative construct of p38 (p38<sup>ASP</sup>) was also used in this setting, as indicated. *c*, experiment performed similar to the one described in *a*, with the exception that the pharmacological inhibitor PD98059 was used to inhibit the MEK signaling pathway. Inhibitor was added 6 h before tet was removed (or not, as indicated).

caused a minimal increase (5%) in the level of cell death in untreated cells, in a manner that was not affected by GSTp expression (Fig. 4*a*). The level of H<sub>2</sub>O<sub>2</sub>-induced cell death was reduced upon expression of GSTp (from 36% to 22%) and upon coexpression of JNK<sup>APF</sup> and GSTp (from 17% to 8%; Fig. 4*a*). This observation suggests that JNK promotes cell death after exposure to H<sub>2</sub>O<sub>2</sub>. The ability of GSTp expression to further reduce the degree of H<sub>2</sub>O<sub>2</sub>-mediated cell death in

the presence of JNK<sup>APF</sup> expression could be attributed to the limited effectiveness of this dominant negative vector, and suggests that GSTp complements the limited effect of JNK<sup>APF</sup> through inhibition of additional JNK molecules.

Exposure of I $\kappa$ BAN-expressing cells to H<sub>2</sub>O<sub>2</sub> did not cause additional changes in the degree of cell death (from 36% to 34%), probably because of experimental conditions that were set to a high dose effect of H<sub>2</sub>O<sub>2</sub>. Importantly, upon expression of GSTp, there was an increase in H<sub>2</sub>O<sub>2</sub>-induced cell death (from 21% to 28%), indicating that the lack of NF $\kappa$ B attenuates GSTp-elicited protection against H<sub>2</sub>O<sub>2</sub>-induced death (Fig. 4*a*). Forced expression of the I $\kappa$ B super-repressor I $\kappa$ BAN in the presence of elevated GSTp caused a 5-fold increase in the basal level of cell death before exposure to H<sub>2</sub>O<sub>2</sub> (Fig. 4*a*), suggesting that without exogenous damage, NF $\kappa$ B cooperates with GSTp to promote cell survival. These findings are in line with the effect of GSTp on I $\kappa$ B phosphorylation (Fig. 3) and suggest that NF $\kappa$ B is a part of the GSTp-coordinated response that elicits protection against H<sub>2</sub>O<sub>2</sub>-induced cell death.

Forced expression of p38<sup>ASP</sup>, a dominant negative p38 construct, did not alter the level of H<sub>2</sub>O<sub>2</sub>-induced cell death; however, it attenuated the decrease mediated by GSTp. Whereas GSTp led to a 40% inhibition of H<sub>2</sub>O<sub>2</sub>-mediated cell death (from 37% to 20%), in the presence of p38<sup>ASP</sup>, there was no protection from H<sub>2</sub>O<sub>2</sub>-induced cell death (Fig. 4*b*), but rather an increase from 38% to 48%. Observations made with the dominant negative form of p38 were confirmed using the pharmacological inhibitor SB203580, which efficiently increased the degree of H<sub>2</sub>O<sub>2</sub>-mediated cell death in the presence of GSTp expression from 20% to 52% (Fig. 4*b*). These results indicate that p38 is among the stress kinases used by GSTp to elicit its protective effect against H<sub>2</sub>O<sub>2</sub>-induced cell death. Treatment of cells with the pharmacological inhibitor of MAPK kinase, PD98059, attenuated GSTp-elicited protection as reflected in the substantial increase (from 22% to 42%) in the level of H<sub>2</sub>O<sub>2</sub>-mediated cell death (Fig. 4*c*). This finding points to the role of ERK in the ability of GSTp to protect against H<sub>2</sub>O<sub>2</sub>-induced cell death.

The ability to attenuate protection elicited by elevated expression of GSTp via the genetic or pharmacological inhibitors of the respective kinases establishes the contribution of ERK, p38, and IKK to GSTp-elicited protection from H<sub>2</sub>O<sub>2</sub>-mediated cell death. At the same time, the increased survival of H<sub>2</sub>O<sub>2</sub>-treated cells by the dominant negative form of JNK points to its proapoptotic signal, which is down-regulated by GSTp, as part of the GST-elicited integrated stress response.

## Discussion

The present study points to the role of the glutathione-conjugating enzyme GSTp in the coordinated regulation of stress kinases in response to ROS-generating treatments. Integrated regulation of stress kinases by GSTp entails the activation of p38, NF $\kappa$ B, and ERK cascades, while limiting the degree of JNK signaling. Although each of the stress kinases alone, when uncoordinated (*i.e.*, overexpression), is capable of eliciting either promotion of or protection against cell death, GSTp contributes to a coordinated regulation that is likely to play an important role in its ability to elicit protective effects. The coordinated regulation of stress kinases by GSTp is better appreciated in light of the differences seen in the activities of the kinases following exposure to ROS-generating treatment, as demonstrated in the present study for H<sub>2</sub>O<sub>2</sub>. GSTp efficiently amplified the degree of IKK, p38, and ERK activities while suppressing the level of the JNK-elicited signal. Cell death elicited by H<sub>2</sub>O<sub>2</sub> is efficiently inhibited by forced expression of the dominant negative form of JNK. Conversely, inhibition of p38/ERK/NF $\kappa$ B attenuated GSTp-elicited protection.

The system used in the present studies reflects two physiological

scenarios where GSTp expression is elevated: (a) an increase in the expression of GSTp has been reported in response to ROS-generating agents and is mediated by c-Jun (21, 25); and (b) elevated levels of GSTp expression are found as often in human tumors as in their cell line derivatives. The latter observation has been correlated with multidrug resistance and poor apoptotic response to chemotherapeutic drugs (4, 7, 23).

Because ROS-generating agents also activate transcription of GSTp via the JNK/Jun cascade, our finding points to the possible existence of a feedback regulatory loop for regulation of stress kinases. According to this model, exposure to ROS-generating agents generates multimers of GSTp that no longer elicit JNK inhibition and thus enables the activation of JNK. Through transcriptionally active JNK substrates, including c-Jun, a new synthesis of GSTp takes place, which is expected to resume JNK inhibition. Such a feedback loop points to redox-dependent regulation of the duration and magnitude of stress kinase activity.

Among possible explanations for coordinated regulation of various stress kinases by GSTp are (a) the possible association of GSTp with other upstream signal transduction components; (b) the effect of GSTp on scaffold proteins; and (c) a possible link between GSTp and caspases, which are required for H<sub>2</sub>O<sub>2</sub>-mediated cell death.

Altogether, the current study provides insight into the mechanism underlying the regulation of key signal transduction components that play pivotal roles in the response to stress and damage by altered ROS and redox potential. The delicate balance between inhibition of JNK and activation of ERK, I $\kappa$ B kinase, and p38 points to the mechanism by which GSTp is capable of eliciting protection against cell death induced by ROS-generating agents. The widely documented deregulation of GSTp expression in human tumors represents one important setting where changes in the coordinated regulation of stress kinases are expected to take place and impact on cell protection from cell death in response to ROS-generating treatments.

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