Glutathione *S*-Transferase p Elicits Protection against H_2O_2-induced Cell Death via Coordinated Regulation of Stress Kinases[1]

Zhimin Yin, Vladimir N. Ivanov, Hasem Habelhah, Kenneth Tew, and Ze'ev Ronai[2]

Ruttenberg Cancer Center, Mount Sinai School of Medicine, New York, New York 10029 [Z. Y., V. N. I., H. H., Z. R.]; Department of Pharmacology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111 [K. T.]

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_2O_2 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 κ-kinase (IKK), and reduced phosphorylation of MAP kinase kinase 7 and Jun NH_2-terminal kinase (JNK). Whereas H_2O_2 treatment of cells induced JNK, p38, and IKK activities, in the presence of H_2O_2 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_2O_2-induced death was attenuated upon inhibition of p38, nuclear factor κB, or MAP kinase by dominant negative or pharmacological inhibitors. Conversely, expression of a dominant negative JNK protected cells from H_2O_2-mediated death. These data suggest that the coordinated regulation of stress kinases by GSTp, as reflected by increased p38, ERK, and nuclear factor κB 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.[3] 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_2O_2 treatment results in the activation of multiple stress kinase cascades, including the ASK1, MEKK1, MAPK, (8, 9), ERK (10), and IKK-NFκB (11, 12) signaling pathways. Among the substrates for these signaling cascades are p53, NFκB, 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_2O_2 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_2O_2-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_2. 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 μg/ml geneticin in the presence of hygromycin (100 μg/ml). GSTp-tet-regulatable cells were maintained in DMEM containing 10% fetal bovine serum, 100 μg/ml hygromycin, and 400 μg/ml geneticin. To maintain suppression of GSTp expression, 1 μg/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 μg/ml pepstatin, leupeptin, and aprotinin) and the phosphatase inhibitors sodium vanadate (1 mM) and sodium fluoride (5 mM).

Chemicals. H_2O_2, GSTp, pepstatin, leupeptin, aprotinin, sodium vanadate, and sodium fluoride were purchased from Sigma. SB203580 and PD98059 were purchased from CalBiochem.

Constructs. ΔMEKK1, a constitutively active form of MEKK1 that lacks amino acids 1–351, was kindly provided by Audrey Minden (Columbia University, New York, NY). $MKK6^{D/D}$, a constitutive activator of p38; $JNK2^{APF}$, a dominant negative of JNK2; $p38^{ASP}$, 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). IκBΔN, a superstable form of IκB, was provided by Dean Ballard (School of Medicine, Vanderbilt University, Nashville, TN). $IKK\beta^{SE}$, a constitutively active IκB kinase βS177; 181E; GST-IκB; 2xNFκB-Luciferase; and MEK^{EL}, a constitutively active form of MEK, were kindly provided by Michael Karin (University

Received 3/7/00; accepted 6/8/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Support from the National Cancer Institute (Grant CA77389) to Z. R. is gratefully acknowledged.

[2] To whom requests for reprints should be addressed, at Ruttenberg Cancer Center, Mount Sinai School of Medicine, One Gustave Levy Place, Box 1130, New York, NY 10029. Fax: (212) 849-2446; E-mail: ronaiz01@doc.mssm.edu.

3 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 κ-kinase; NFκB, nuclear factor κB; ATF2, activating transcription factor 2; GSTp, glutathione *S*-transferase π; JNK, Jun NH_2-terminal kinase; MEK, MAPK/ERK kinase; MKK, MAPK kinase; IκB, inhibitor of nuclear factor κB; 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_2O_2 Treatment. Medium taken from the culture dish was mixed with freshly diluted H_2O_2 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^{1-87}, 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. 1*a*). 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. 1*a*). 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. 1*a*). 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. 1*a*). A modest increase was seen in phosphorylation of MKK7 after 24 h of elevated GSTp expression (Fig. 1*a*). 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

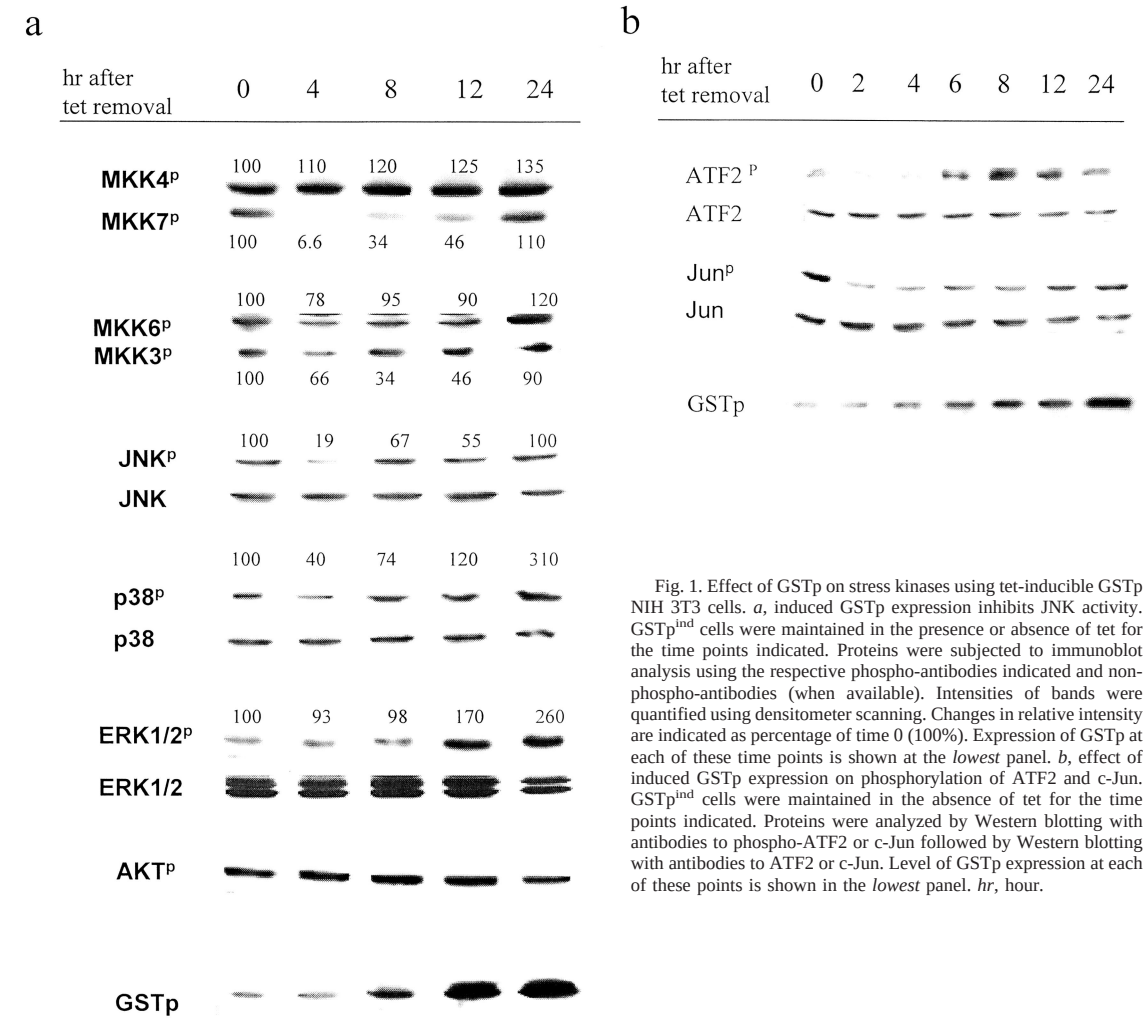

Fig. 1. Effect of GSTp on stress kinases using tet-inducible GSTp NIH 3T3 cells. *a*, induced GSTp expression inhibits JNK activity. $GSTp^{ind}$ cells were maintained in the presence or absence of tet for the time points indicated. Proteins were subjected to immunoblot analysis using the respective 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. $GSTp^{ind}$ 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.

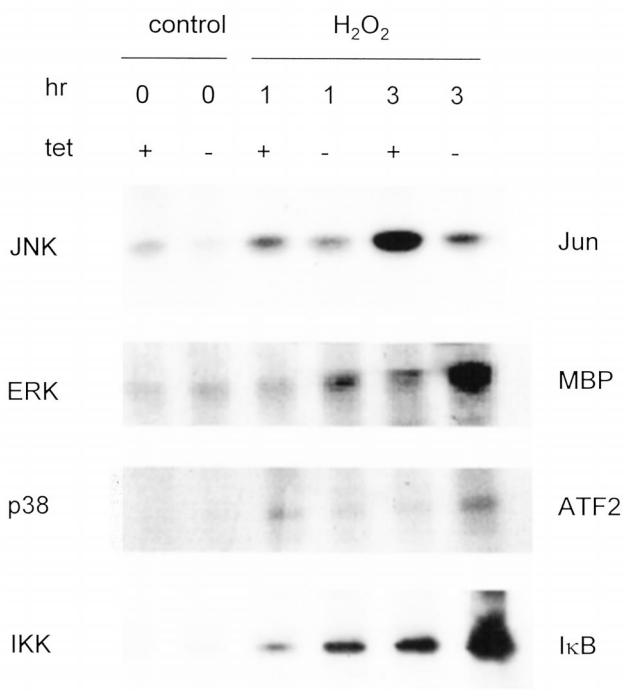

Fig. 2. Effect of GSTp on H_2O_2 induction of stress kinases using tet-inducible GSTp NIH 3T3 cells. $GSTp^{ind}$ cells were maintained in the presence or absence of tet with or without H_2O_2. Proteins were prepared for analysis 1 or 3 h after H_2O_2 treatment. Analysis was carried out via immunokinase 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. 1*a*). 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. 1*a*, *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. 1*b*) but not c-Jun, probably because of an increase in p38 activity (Fig. 1*a*). 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_2O_2 Treatment. We next examined the effect of GSTp expression on the activities of stress kinases after exposure to H_2O_2 treatment, which generates high levels of ROS (1, 24). Analysis of H_2O_2-elicited changes revealed increased activity of ERK, p38, JNK, and IKK, measured via immunokinase reactions of the respective substrates (Fig. 2). H_2O_2 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_2O_2-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_2O_2 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_2O_2 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_2O_2-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_2O_2 in the presence and absence of GSTp expression. Treatment with 50 μM H_2O_2 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_2O_2 used (not shown). Exposure to higher doses of H_2O_2 (100 μM) also revealed GSTp-mediated protection against cell death (39% *versus* 25%). GSTp maintained its protection even at higher doses of H_2O_2 (40% *versus* 30% at 200 μM; Fig. 3). These data suggest that the H_2O_2-induced cell death of 3T3 cells was efficiently reduced by GSTp expression. Analysis of the type of H_2O_2-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_2O_2 (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_2O_2-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^{APF}, a dominant negative form of JNK,

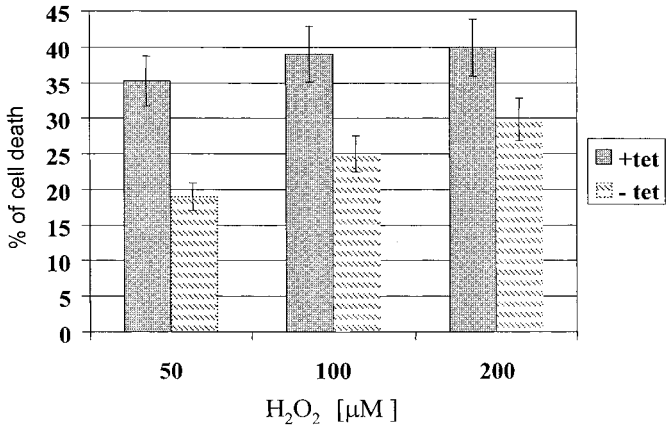

Fig. 3. Effect of GSTp on H_2O_2-induced cell death. $GSTp^{ind}$ cells were maintained in the presence (*gray columns*) or absence (*hatched columns*) of tet for the time points indicated with or without H_2O_2 treatment at the doses indicated. In all cases, cells were prepared for cell death analysis 30 h after H_2O_2 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.

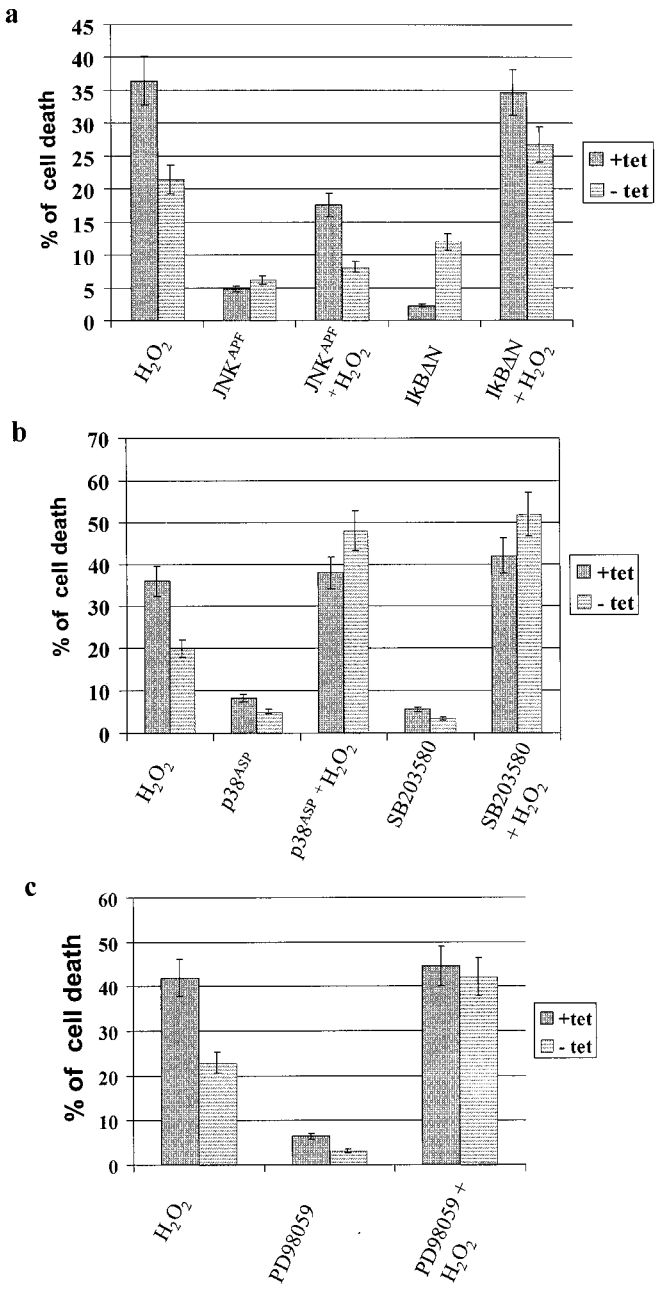

Fig. 4. Inhibition of p38, ERK, or NFκB attenuates GSTp ability to elicit protection from H_2O_2-induced cell death. *a*, $GSTp^{ind}$ cells were transfected with JNK^{APF} or IκBΔN and 24 h later exposed to H_2O_2 (100 μM) in the presence (*gray column*) or absence (*hatched column*) of tet. Degree of cell death was determined 24 h after exposure to H_2O_2, 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 ($p38^{ASP}$) 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_2O_2-induced cell death was reduced upon expression of GSTp (from 36% to 22%) and upon coexpression of JNK^{APF} and GSTp (from 17% to 8%; Fig. 4*a*). This observation suggests that JNK promotes cell death after exposure to H_2O_2. The ability of GSTp expression to further reduce the degree of H_2O_2-mediated cell death in the presence of JNK^{APF} expression could be attributed to the limited effectiveness of this dominant negative vector, and suggests that GSTp complements the limited effect of JNK^{APF} through inhibition of additional JNK molecules.

Exposure of IκBΔN-expressing cells to H_2O_2 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_2O_2. Importantly, upon expression of GSTp, there was an increase in H_2O_2-induced cell death (from 21% to 28%), indicating that the lack of NFκB attenuates GSTp-elicited protection against H_2O_2-induced death (Fig. 4*a*). Forced expression of the IκB superrepressor IκBΔN in the presence of elevated GSTp caused a 5-fold increase in the basal level of cell death before exposure to H_2O_2 (Fig. 4*a*), suggesting that without exogenous damage, NFκB cooperates with GSTp to promote cell survival. These findings are in line with the effect of GSTp on IκB phosphorylation (Fig. 3) and suggest that NFκB is a part of the GSTp-coordinated response that elicits protection against H_2O_2-induced cell death.

Forced expression of $p38^{ASP}$, a dominant negative p38 construct, did not alter the level of H_2O_2-induced cell death; however, it attenuated the decrease mediated by GSTp. Whereas GSTp led to a 40% inhibition of H_2O_2-mediated cell death (from 37% to 20%), in the presence of $p38^{ASP}$, there was no protection from H_2O_2-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_2O_2-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_2O_2-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_2O_2-mediated cell death (Fig. 4*c*). This finding points to the role of ERK in the ability of GSTp to protect against H_2O_2-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_2O_2-mediated cell death. At the same time, the increased survival of H_2O_2-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, NFκB, 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_2O_2. 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_2O_2 is efficiently inhibited by forced expression of the dominant negative form of JNK. Conversely, inhibition of p38/ERK/NFκB 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_2O_2-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, IκB 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.

Acknowledgments

We thank Stuart Aaronson for providing NIH 3T3 cells that stably express the pSV40-Hyg plasmid, and Roger Davis, Michael Karin, H. Van Dam, and Audrey Minden for stress kinase constructs. We also thank Thomas Buschmann for kind assistance and Arthur Cederbaum and Serge Fuchs for comments.

References

1. Gamaley, I. A., and Klyubin, I. V., Roles of reactive oxygen species: signaling and regulation of cellular functions. Int. Rev. Cytol., *188:* 203–255, 1999.
2. Powis, G., Briehl, M., and Oblong, J. Redox signalling and the control of cell growth and death. Pharmacol. Ther., *68:* 149–173, 1995.
3. Wilhelm, D., Bender, K., Knebel, A., Angel, P. The level of intracellular glutathione is a key regulator for the induction of stress-activated signal transduction pathways including jun N-terminal protein kinases and p38 kinase by alkylating agents. Mol. Cell. Biol., *17:* 4792–4800, 1997.
4. Tew, K. D. Glutathione-associated enzymes in anticancer drug resistance. Cancer Res., *54:* 4313–4320, 1994.
5. Hayes, J. D., and McLellan, L. I. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free Radical Res., *31:* 273–300, 1999.
6. Baez, S., Segura-Aguilar, J., Widersten, M., Johnson, A. S., and Mannervik, B. Glutathione transferases catalyse the detoxification of oxidized metabolites (*o*-quinones) of catecholamines and may serve as an antioxidant system preventing degenerative cellular processes. Biochem. J., *324:* 25–28, 1997.
7. Tew, K. D., and Ronai, Z. GST function in drug and stress response. Drug Resist. Updates, *2:* 143–147, 1999.
8. Cobb, M. H. MAP kinase pathways. Prog. Biophys. Mol. Biol., *71:* 479–500, 1999.
9. Adler, V., Yin, Z., Tew, K. D., and Ronai, Z. Role of redox potential and reactive oxygen species in stress signaling. Oncogene, *18:* 6104–6111, 1999.
10. Guyton, K. Z., Liu Y., Gorospe M., Xu, Q., and Holbrook, N. J. Activation of mitogen-activated protein kinase by H_2O_2. Role in cell survival following oxidant injury. J. Biol. Chem., *271:* 4138–4142, 1996.
11. Lee, Y., and Shacter, E. Oxidative stress inhibits apoptosis in human lymphoma cells. J. Biol. Chem., *274:* 19792–19798, 1999.
12. Kamata, H., and Hirata, H. Redox regulation of cellular signalling. Cell. Signal., *11:* 1–14, 1999.
13. Fuchs, S. Y., Adler, V., Pincus, M. R., and Ronai, Z. MEKK1/JNK signaling stabilizes and activates p53. Proc. Natl. Acad. Sci. USA, *95:* 10541–10546, 1998.
14. Xia, M., Dickens, J., Raingeaud, R., Davis, J., and Greenberg, M. E. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science (Washington DC), *270:* 1326–1331, 1995.
15. Karin, M. Mitogen-activated protein kinase cascades as regulators of stress responses. Ann. NY Acad. Sci., *851:* 139–146, 1998.
16. Davis, R. J. Signal transduction by the c-Jun N-terminal kinase. Biochem. Soc. Symp., *64:* 1–12, 1999.
17. Adler V., Yin, Z., Fuchs, S. Y., Benezra, M., Rosario, L., Tew, K. D., Pincus, M. R., Sardana, M., Henderson, C. J., Wolf, J. R., Davis, R., and Ronai, Z. Regulation of JNK signaling by GSTp. EMBO J., *18:* 1321–1334, 1999.
18. Saitoh, M., Nishitoh, H., Fujii, M., Takeda, K., Tobiume, K., Sawada, Y., Kawabata, M., Miyazono, K., and Ichijo, H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J., *17:* 2596–2606, 1998.
19. Adler, V., Shaffer, A., Kim, J., Dolan, L., and Ronai, Z. UV-irradiation and heat shock mediate JNK activation *via* alternate pathways. J. Biol. Chem., *270:* 26071–26077, 1995.
20. Kumar, S., Kinoshita, M., Noda, M., Copeland, N. G., and Jenkins, N. A. Gene Dev., *8:* 1613–1626, 1994.
21. Ainbinder, E., Bergelson, S., Pinkus, R., and Daniel, V. Regulatory mechanisms involved in activator-protein-1 (AP-1)-mediated activation of glutathione-S-transferase gene expression by chemical agents. Eur. J. Biochem., *243:* 49–57, 1997.
22. Sukuki, T., Morimura, S., Diccianni, M. B., Yamada, R., Hochi, S., Hirabayashi, M., Yuki, A., Nomura, K., Kitagawa, T., Imagawa, M., and Muramatsu, M. Activation of glutathione transferase P gene by lead requires glutathione transferase P enhancer I. J. Biol. Chem., *271:* 1626–1632, 1996.
23. Salinas, A. E., and Wong, M. G. Glutathione *S*-transferases—a review. Curr. Med. Chem., *6:* 279–309, 1999.
24. Scharffetter-Kochanek, K., Wlaschek, M., Brenneisen, P., Schauen, M., Blaudschun, R., and Wenk, J. UV-induced reactive oxygen species in photocarcinogenesis and photoaging. Biol. Chem., *378:* 1247–1257, 1997.
25. Xia, C., Hu, J., Ketterer, B., and Taylor, J. B. The organization of the human GSTP1-1 gene promoter and its response to retinoic acid and cellular redox status. Biochem. J., *313:* 155–161, 1996.