Expression of Multidrug Resistance Protein/GS-X Pump and γ-Glutamylcysteine Synthetase Genes Is Regulated by Oxidative Stress*

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Expression of the MRP1 gene encoding the GS-X pump and of the γ-GCSH gene encoding the heavy (catalytic) subunit of the γ-glutamylcysteine synthetase is frequently elevated in many drug-resistant cell lines and can be co-induced by many cytotoxic agents. However, mechanisms that regulate the expression of these genes remain to be elucidated. We report here that like γ-GCSH, the expression of MRP1 can be induced in cultured cells treated with pro-oxidants such as tert-butylhydroquinone, 2,3-dimethoxy-1,4-naphthoquinone, and menadione. Intracellular reactive oxygen intermediate (ROI) levels were increased in hepatoma cells treated with tert-butylhydroquinone for 2 h as measured by flow cytometry using an ROI-specific probe, dihydorhodamine 123. Elevated GSH levels in stably γ-GCSH-transfected cell lines down-regulated endogenous MRP1 and γ-GCSH expression. ROI levels in these transfected cells were lower than those in the untransfected control. In the cell lines in which depleting cellular GSH pools did not affect the expression of the MRP1 and γ-GCSH genes, only minor increased intracellular levels of ROIs were observed. These results suggest that intracellular ROI levels play an important role in the regulation of MRP1 and γ-GCSH expression. Our data also suggest that elevated intracellular GSH levels not only facilitate substrate transport by the MRP1/GS-X pump as previously demonstrated, but also suppress MRP1 and γ-GCSH expression.

Human MRP1 (multidrug resistance protein) encoded by MRP1 was first isolated by molecular cloning from doxorubicin-selected multidrug-resistant lung cancer cells (Ref. 1; reviewed in Ref. 2). Studies using plasma membrane vesicles prepared from MRP1-overproducing cell lines demonstrated increased ATP-dependent, high-affinity transport activities of cysteinyl leukotrienes (e.g. LTC₄) (3, 4). Deletion of homologous MRP1 alleles in mice results in impaired response to inflammatory stimulus in these animals because LTC₄ is a potent mediator of the inflammation reaction (5). These findings suggest that MRP1 encodes the previously described GS-X (ATP-dependent glutathione S-conjugate export) pump (6). In addition to transporting LTC₄ and its related glutathione S-conjugates, naturally occurring organic conjugates, including 17β-estradiol (17β-estradiol), and bile salt conjugates, including 6α-glucuronosylhydrodeoxychlorate and 3α-sulfatolithocholyltaurine, are also good substrates for the MRP1/GS-X pump (7–9). There are also reports suggesting that GSH may serve as a cofactor in MRP1/GS-X pump-mediated drug transport (8, 11). In addition, the MRP1/GS-X pump is responsible for the release of GSSG from cells. This active export of GSSG is considered to be an important mechanism to maintain the reduced status of intracellular thiols under oxidative stress (12, 13). These observations underscore the importance of GSH for the function of MRP1.

Biosynthesis of GSH is controlled by multiple enzyme systems (reviewed in Refs. 14 and 15). The first step of GSH biosynthesis, catalyzed by γ-glutamylcysteine synthetase (EC 6.3.2.2), is the rate-limiting step. The mammalian γ-glutamylcysteine synthetase holoenzyme is a heterodimer consisting of a 73-kDa heavy subunit (γ-GCSH) (16, 17) and a 28-kDa light subunit (18, 19). Although the heavy subunit contains the entire catalytic activity, its activity can be modulated by the association with the light subunit, the regulatory subunit. To facilitate the MRP1/GS-X pump-mediated transport, it is speculated that activities, particularly those of γ-GCSH, could be increased to furnish intracellular GSH. Indeed, we recently demonstrated the frequent coexpression patterns of MRP1 and γ-GCSH mRNAs in many drug-resistant cell lines (20–22) as well as in human colorectal cancers (23).

The frequent coexpression pattern between MRP1 and γ-GCSH mRNAs suggests that these genes may be coordinately regulated. However, the underlying mechanisms that regulate the expression of these genes are not known. In this study, we demonstrate that like γ-GCSH, the expression of MRP1 could be induced by many pro-oxidants. Moreover, elevated levels of the physiological antioxidant GSH, conferred by ectopic expression of γ-GCSH, down-regulated MRP1 and γ-GCSH expression.

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1 The abbreviation used are: LTC₄, leukotriene C₄; γ-GCSH, heavy subunit of γ-glutamylcysteine synthetase; ROI, reactive oxygen intermediate; BSO, l-buthionine(SR)-sulfoximine; t-BHQ, tert-butylhydroquinone; NQ, 2, 3-dimethoxy-1,4-naphthoquinone; DHR123, dihydorhodamine 123; R123, rhodamine 123; ORE, oxidative stress-responsive element.
We also measured intracellular levels of reactive oxygen intermediates (ROI) in those cells and demonstrated a potential link between ROI levels and MRP1 and \(\gamma\)-GCSH expression. Our results are consistent with the idea that expression of MRP1 and \(\gamma\)-GCSH is sensitive to the intracellular oxidation-reduction (redox) status.

**Experimental Procedures**

**Materials**—GSH, glutathione reductase, and the transcriptional kit for RNA probe synthesis were purchased from Boehringer Mannheim. 

\(\gamma\)-Buthionine-(SR) sulfoximine (BSO), doxorubicin, vinblastine, sodium arsenite, sodium chloride, L-carnitine, L-tert-butylhydroquinone (t-BHQ), 2,3-dimethoxy-1,4-naphthoquinone (NQ), and menadione were purchased from Sigma. \(\alpha\)-[\(\beta\)]UTP was from ICN Biomedicals (Irvine, CA). The fluorescent ROI probe dihydrorhodamine 123 (DHR123) was purchased from Molecular Probes, Inc. (Eugene, OR). All other chemicals were of analytical grade.

**Cell Cultures**—The rat hepatoma cell line H-4-II-E and the human hepatoma cell line HepG2 were purchased from the American Type Culture Collection Center (Rockville, MD). Human small cell lung cancer cell lines (SCLC and SR3A) were a generous gift from Dr. Niramol Savaraj (University of Miami, Miami, FL). SR3A was a doxorubicin-resistant cell population established from SCLC as described previously (20). SCLC cells contained 3- and 5.5-fold higher MRP1 and \(\gamma\)-GCSH mRNAs, respectively, compared with SCLC cells (22). The cells were maintained in Dulbecco’s modified Eagle’s medium (Sigma) supplemented with 10% fetal calf serum (Life Technologies, Inc.), 1 mM glutamine, and 50 \(\mu\)g/ml neomycin in a humidified incubator containing 5% CO2. SR3A and its transfectants were fed twice weekly with medium containing 0.17 mM doxorubicin.

**Transfection of \(\gamma\)-GCSH cDNA**—The calcium phosphate precipitation method was used to transfect cells with DNA, pCMV/GCSH, which contains a full-length human \(\gamma\)-GCSH cDNA under the transcriptional control of the cytomegalovirus promoter and the neomycin resistance marker for G418 selection, was used for transfection (24). G418-resistant cell lines, either from pooled colonies or from individual clones, were established. Each pool contained at least 20 independent colonies.

**RNA Isolation and RNA Protection Assay**—The procedures used for isolation of RNA, preparations of human MRP1 and \(\gamma\)-GCSH probes, and RNase protection assays have been described previously (20, 21). For the RNase protection assay of rat MRP1 mRNA, an antisense probe was synthesized from a recombinant plasmid DNA template containing the polymerase chain reaction product generated using the forward primer 5’-GGAGGAGGAGGGGGGGGCG-3’ and the backward primer 5’-GGATCCTGTGGAATGATG-3’ derived from the two conserved regions in Sequences I and II of rat MRP1 cDNA (25), respectively. This probe produced an 140-nucleotide protected fragment in the RNase protection assay. For the rat \(\gamma\)-GCSH probe, a 990-nucleotide fragment of cDNA was synthesized using the forward primer 5’-GGAGGAGGAGGGGGCGCGG-3’ and the backward primer 5’-TTCTCAGGGCTCCAGTCC-3’ (These primer sequences were selected because they are conserved in the human and rat homologues.) The polymerase chain reaction product was subcloned into pSP718 vector and sequenced. The plasmid was linearized by digestion at the internal Pst1 site, and the probe was synthesized with T7 polymerase to generate a 183-nucleotide fragment in the RNase protection assay.

**Other Methods**—Measurements of total cellular glutathione (GSH + 2\(\times\)GSSG) and transport of LTC4 using membrane vesicles followed the methods described previously (20).

**Results**

**Induction of MRP1 and \(\gamma\)-GCSH Gene Expression by Prooxidants**—Shi et al. (27) demonstrated that expression of \(\gamma\)-GCSH in cultured rat cells could be induced by NQ and menadione. Mulcahy et al. (28) reported that exposing human hepatoma HepG2 cells to \(\beta\)-naphthoflavone resulted in an increase in \(\gamma\)-GCSH mRNA levels. These results demonstrated that expression of \(\gamma\)-GCSH could be up-regulated by pro-oxidants. Because we previously demonstrated coordinated expression of MRP1 and \(\gamma\)-GCSH mRNAs in many experimental systems (20–23), we examined whether the expression of MRP1 could also be induced by the pro-oxidants. Fig. 1A shows an RNase protection assay of MRP1 mRNA levels in rat hepatoma H-4-II-E cells treated with t-BHQ, NQ, and menadione for time periods from 6 to 48 h. In all cases, increased MRP1 mRNA levels could be seen 6 h after the treatment and declined 48 h after the treatment, except for those treated with NQ. The time course rise and fall of MRP1 mRNA varied among the different pro-oxidants used. These results demonstrate that expression of MRP1, like that of \(\gamma\)-GCSH (27, 28), is regulated by pro-oxidants. Co-induction of MRP1 and \(\gamma\)-GCSH by t-BHQ was also observed in human HepG2 cells (Fig. 1B). Maximal levels of induction by these pro-oxidants ranged from 2.5- to 4.2-fold. These pro-oxidants exert oxidative stress on the cells by forming highly reactive, short half-life ROIs such as superoxide and hydroperoxide (29–31).

**Increased LTC4 Transport Activities in Membrane Vesicles Prepared from t-BHQ-treated Cells**—Previous studies have demonstrated that membrane vesicles prepared from MRP1-overexpressing cells exhibit increased ATP-dependent transport of LTC4 (3, 4). To investigate whether the pro-oxidant-treated cells exhibit enhancement of such activities, we prepared membrane vesicles from H-4-II-E cells treated with 100 \(\mu\)M t-BHQ for 24 h and from those that were untreated. LTC4 uptake activities were measured in an incubation mixture with or without ATP. Elevated ATP-dependent LTC4 transport activity was observed (Fig. 2). Similar results were observed in H-4-II-E cells treated with 50 \(\mu\)M NQ for 24 h (data not shown). These results demonstrate that the functional MRP1/GS-X pump was induced by these pro-oxidants.

**Measurement of t-BHQ-induced ROI Formation by Flow Cytometry**—A previous study demonstrated, using EPR spectroscopy, that the formation of ROIs in t-BHQ-treated cells is concentration- and time-dependent (26). Production of ROIs apparently leveled off within 1 h. In this study, we used a fluorescent probe, DHR123, to monitor the formation of ROIs. DHR123, an uncharged and fluorescent dye, passively diffuses across most cell membranes and reacts with ROIs, resulting in the formation of cationic fluorescent R123 that can be measured by flow cytometry. DHR123 has been used as a molecular probe for the measurement of ROI formation in cultured endothelial cells (32) and in murine fibrosarcoma cells treated with tumor necrosis factor (26).

**Measurement of LTC4 transport activity**—DHR123 was prepared as a 5 mM stock in dimethyl sulfoxide (Me2SO) and used at a final concentration of 1 \(\mu\)M. Cells (6 \(\times\) 10^5) were plated on a 65-mm plate for 24 h. Cells were treated with t-BHQ or BSO according to the specifications described, followed by DHR123. Cells were trypsinized into a single-cell suspension. R123 fluorescence intensity resulting from DHR123 oxidation was measured by a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) with excitation at 488 nm and was detected between 515 and 550 nm according to the procedure described by Goossens et al. (26). Cell debris and dead cells (<5%) were calculated and eliminated with forward light scatter. Data analysis was performed using LYSYSII software (Becton Dickinson), which provided linearized values for the logarithmic fluorescent histograms.

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tions of the mean fluorescence intensity. These results suggest that ROIs were produced in the initial phase of t-BHQ treatments. The reduced accumulation of R123 in the prolonged t-BHQ-treated cells may reflect the short half-life of the pro-oxidant or the elimination/sequestration of the produced R123 by a yet to be elucidated transporter. Although indirect evidence suggests that R123 may be a substrate for the MRP1/GS-X pump, the reduction of the R123 fluorescence in the 4-h treated cells was not likely due to the enhanced expression of MRP1 because an increase in MRP1 mRNA levels was not observed 6 h after the treatment (Fig. 1A).

**Suppression of MRP1 and γ-GCSH Expression by Elevated Intracellular GSH Levels**—To further substantiate the effects of oxidative stress on MRP1 and γ-GCSH expression, we analyzed the expression of these genes in cells expressing elevated levels of the important physiological antioxidant GSH. GSH levels can be elevated by overexpressing γ-GCSH (24, 33). A recombinant plasmid encoding human γ-GCSH was used to transfect cell line SCLC and its doxorubicin-resistant line, SR3A. This plasmid DNA also contains a neomycin-resistant marker for selection. Positive transfectants were established from the pooled colonies or from single-clone isolation. The use of pooled colonies in the analyses could minimize clonal variations among individual clones.

Eight γ-GCSH-positive SR3A cell lines have been obtained: five from pools of NeoR colonies (pools 1–5) and three from individual colonies (clones 13–15). One γ-GCSH-transfected

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*Y. Yamane, M. Furuichi, R. Song, N. T. Van, R. T. Mulcahy, T. Ishikawa, and M. T. Kuo, unpublished observations.*
SCLC cell line was established from a pooled population (Table I). RNase protection assays were performed to determine the steady-state levels of MRP1 and \( \gamma\)-GCSm mRNAs (Fig. 4), and results from three independently prepared RNA samples were densitometrically analyzed. The transfected cell lines exhibited elevated \( \gamma\)-GCSm mRNA levels, ranging from 1.8- to 10-fold (Table I), but only the values for cell lines 1, 4, 13, and 14 were statistically significant, despite the fact that all the cell lines displayed statistically significant increases in GSH levels (increases ranging from 1.93- to 4.97-fold). These results may reflect the differences in sensitivities of detecting methods: for the biochemical determination of GSH levels, only 1.9-fold increases were required to be statistically significant, whereas 3-fold increases in mRNA levels were required for the RNase protection assay. A similar explanation may also apply to the transfected SCLC cell line, which displayed \( \gamma\)-GCSm mRNA levels of 2-fold increases in both the transfected cell lines (data not shown). These results suggest that the RNase protection assay employed here was more sensitive than the Northern hybridization in the analyses of these mRNAs. Our results are consistent with previous findings showing that transfection of \( \gamma\)-GCSm cDNA results in elevated intracellular GSH levels (24, 33).

RNase protection assays also revealed that, although many transfected cell lines had reduced endogenous MRP1 mRNA levels (Table I), cell lines 1 and 14 showed statistically significant reduction of MRP1 mRNA compared with the untransfected SR3A cell line. These two cell lines contained the highest levels of \( \gamma\)-GCSm mRNA, i.e. 10.09- and 9.62-fold, respectively (Table I). The inability to detect a significant reduction of MRP1 mRNA in other transfected cell lines may reflect the technical limitation of RNase protection. Alternatively, these results may suggest that \( \gamma\)-GCSm mRNA levels must reach a sufficient threshold to exert suppressive effects on MRP1 expression.

Whether elevated expression of \( \gamma\)-GCSm down-regulates endogenous \( \gamma\)-GCSm expression could not be concluded from these experiments because human \( \gamma\)-GCSm cDNA was used to transfect the human cells. To address this issue, we performed similar experiments by transfecting human \( \gamma\)-GCSm cDNA into rat hepatoma H-4-II-E cells. Two cell lines, designated H9 and H17, were obtained from individual colonies. Expression of the transfected human \( \gamma\)-GCSm cDNA in the rat cells was measured by RNase protection assay using human antisense \( \gamma\)-GCSm as a probe, and the results were compared with those obtained in human HepG2 cells. Levels of human \( \gamma\)-GCSm mRNA in H9 cells were comparable to those in HepG2 cells, whereas H17 cells contained about one-third of the amount (Fig. 5B). Levels of endogenous MRP1 and \( \gamma\)-GCSm mRNAs in the transfected cells were then determined using the rat probes that gave rise to protection fragments of 140 and 183 nucleo-

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**FIG. 2. MRP1/GS-X pump activity in plasma membrane preparations from H-4-II-E cells and \( \tau\)-BHQ-treated cells.** Membrane vesicles prepared from H-4-II-E cells (○, ●) and \( \tau\)-BHQ-treated (100 \( \mu\)M, 24 h) (■, □) cells were used for LTC\(_4\) uptake assay in the presence (○, ■) or absence (●, □) of ATP. Data are expressed as the means of three independent experiments (n = 3). Bars indicate S.D. Note that the ● line superimposes the ■ line.
Redox Regulation of MRP1 and γ-GCSH Expression

These results demonstrate that overexpression of MRP1 in the transfected rat H-4-II-E cells compared with the transfected SR3A cells (Table I).

**Reduction of ROI Formation in GSH-overexpressing Cell Lines**—The SR3A cell line and its γ-GCSH-overexpressing cell lines (13–15) exhibited reduced intracellular ROI levels. Cells were harvested, and the fluorescence intensities in the treated cells and in the vehicle-treated cells (measuring autofluorescence) were analyzed by flow cytometry. Results from duplicate experiments showed that clones 13–15 exhibited 19, 25, and 22% reduction, respectively, of the mean fluorescence intensities compared with untransfected cells (Fig. 6A). Similar analyses revealed that the γ-GCSH-overexpressed 4-II-E cell lines H9 and H17 exhibited 22 and 23% reduction, respectively, of the mean fluorescence intensities in comparison with the untransfected control (Fig. 6B). These results suggest that overexpression of γ-GCSH is associated with reduced intracellular ROI levels.

**Fig. 3. Measurement of ROI formation in t-BHQ-treated cells.** H-4-II-E cells (5 × 10⁵) were treated with (open bars) or without (hatched bars) 100 μM t-BHQ for the time intervals as indicated. Two h prior to harvest, cells were treated with DHR123 and then analyzed by flow cytometry. Values are the linearized mean fluorescence intensity for the t-BHQ-treated samples in arbitrary units. The results shown are averages of duplicate experiments.

**TABLE I**

| Cell lines  | GSH levels (nmol/mg protein) | Relative level of mRNA*<sup>a</sup> |
|-------------|-----------------------------|-----------------------------------|
| H-4-II-E    | 0.79 ± 0.05<sup>b</sup> (1.00) | 1.00                              |
| Transfectant| 0.49 ± 0.05 (1.47)           | 0.66 ± 0.06 (1.00)                 |
| 1<sup>c</sup> | 7.97 ± 0.06 (1.17)           | 0.09 ± 0.01 (1.00)                 |

*Relative γ-GCSH and MRP1 mRNA levels were derived from densitometric analyses of the RNase protection data shown in Figs. 4 and 5.

**Values are means ± S.D. (n = 3).**

**Cells were derived from pooled populations.**

**Significant difference (p < 0.05), analyzed by unpaired Student's t test.**

**Cells derived from individual clones.**

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**TABLE I**

| GSH levels and relative mRNA contents in γ-GCSH-transfected cell lines |
|--------------------------|--------------------------|--------------------------|
| Cell lines  | Cellular GSH | Relative level of mRNA<sup>a</sup> |
|-------------|--------------|-----------------------------------|
| H-4-II-E    | 0.79 ± 0.05<sup>b</sup> (1.00) | 1.00                              |
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tides, respectively (Fig. 5A, lanes 1–3). These two protected fragments were not detected in the same analysis when total RNA from human hepatoma HepG2 cells was used (Fig. 5A, lane 4), although a strong protection signal corresponding to 165 nucleotides, possibly due to the cross-hybridization to the human γ-GCSH mRNA, was present. These results suggest that these probes could adequately measure endogenous MRP1 and γ-GCSH mRNA levels in the H-4-II-E cell line and its transfected cells.

H9 and H17 cells exhibited 1.48- and 1.2-fold increases, respectively, in GSH levels (Table I). RNase protection assays revealed that endogenous MRP1 and γ-GCSH mRNA levels were reduced in these transfectants, but only the results for the H9 line were statistically significant (Fig. 5A and Table I). These results demonstrate that overexpression of γ-GCSH down-regulated both MRP1 and γ-GCSH expression. Furthermore, it appears that smaller GSH increases were sufficient to
To investigate whether overexpression of γ-GCSH resulted in reduced MRP1/GS-X pump activities, we prepared membrane vesicles from SR3A and H-4-II-E cells and their corresponding γ-GCSH-transfected cell lines, clones 14 and H9. Reduced ATP-dependent LTC₄ transport activities were observed in the samples obtained from the transfected cells compared with those from the corresponding untreated cells (Fig. 7). These results demonstrate that overproduction of γ-GCSH resulted in reduction of MRP1/GS-X pump function.

No Alteration of MRP1 and γ-GCSH Expression in GSH-depleted Cells—Having established that increased GSH levels down-regulated MRP1 and γ-GCSH expression, we then investigated whether depleting intracellular GSH levels could affect the expression of MRP1 and γ-GCSH. We used BSO, which is a highly selective inhibitor of γ-GCSH that binds to the active site of the enzyme. Administration of BSO to cultured cells and animals effectively turns off cellular GSH synthesis (34–37). Moreover, these agents are known to introduce intracellular oxidation-reduction labile conditions to induce intracellular oxidation-reduction labile conditions (Fig. 9). Such low levels of ROI increase may not exert sufficient stress to alter MRP1 and γ-GCSH expression in these cells.

**DISCUSSION**

The roles of MRP1 in conferring multidrug resistance in cultured cells have been conclusively demonstrated by transfection experiments (39, 40). The identification of the function of MRP1 as a transporter of GSH-containing substrates underscores the important roles of GSH in MRP1-mediated drug transport. In this study, we present evidence showing that expression of MRP1, like that of γ-GCSH, can be induced in cultured cells treated with the pro-oxidants t-BHQ, NQ, and menadione (Fig. 1A) (27, 28). These results further substantiate the nature of the coordinate expression pattern between MRP1 and γ-GCSH (20–23). Moreover, these agents are known to induce intracellular oxidation-reduction labile conditions by virtue of their capacities to undergo 1- and/or 2-electron valency changes, leading to an alteration of intracellular redox reactions and generation of oxidative stress to the cells (29–31). Our present results suggest that expression of MRP1 and γ-GCSH is redox-sensitive.

Further support for redox regulation of MRP1 and γ-GCSH expression is demonstrated by the transfection experiment showing that overexpression of antioxidant GSH, conferred by ectopic expression of γ-GCSH, down-regulated MRP1 and...
Fig. 6. Measurement of relative ROI levels in γ-GCSh-transfected SR3A and H-4-II-E lines. A, the SR3A cell line and its transfectants; B, the H-4-II-E cell line and its transfectants. In each case, 5 × 10⁵ cells each were incubated with DHR123 (open profiles) or with Me₂SO (vehicles; filled profiles) for 6 h and analyzed by flow cytometry. Linearized mean fluorescence intensity values for the DHR123-treated (a) and untreated (b) (background fluorescence) cells and the differences between a and b as indicated (Δ) are shown. Panel e in A and panel d in B show relative mean fluorescence intensities of the transfected cells in reference to those in the untransfected controls. Results are from duplicate experiments.
FIG. 7. Measurement of MRP1/GS-X pump activities in plasma membranes prepared from γ-GCS-h-transfected cells. A, membrane vesicles prepared from the SR3A cell line (○, ●) and its transfectant clone 14 (■) were used to measure LTC₄ uptake in the presence (○, ■) and absence (●) of ATP. B, similar experiments were performed using membrane vesicles prepared from the H-4-II-E cell line (○, ●) and its transfectant H9 (■) measured in the presence (○, ■) and absence (●) of ATP.
g-GCSh expression. GSH is a well known antioxidant that directly reacts with free radicals and serves as a cofactor for GSH peroxidase to reduce hydrogen peroxide, resulting in reduction of the oxidative cellular environment (38). Using the fluorescent probe DHR123, we demonstrated that reduced ROI levels indeed occurred in several independently established cell lines containing elevated GSH levels (Fig. 6). These results, together with the finding of no alteration of MRP1 and g-GCSh mRNA levels in the BSO-treated cells in which only marginal increases of ROIs were found, are consistent with the hypothesis that expression of MRP1 and g-GCSh is regulated by intracellular redox status. To the best of our knowledge, this is the first report describing that the redox regulatory mechanism is associated with the expression of the MRP1 gene.

The reasons why depleting >65% of intracellular GSH levels by BSO failed to induce appreciable increases in ROI and MRP1 and g-GCSh mRNA levels are unknown. GSH is among the most abundant thiol-containing small peptide in living organisms (1–10 mM). Such abundant levels may preserve a capacity that can afford substantial loss without tipping the balance of intracellular redox conditions. Previous studies (34–36) and the present results (Fig. 8) suggest that there is a BSO-resistant intracellular GSH pool (34–36). Although the nature of this residual GSH pool has not been fully elucidated, it has been suggested that this may represent a compartmentalized GSH pool, located primarily in mitochondria and nuclei (Fig. 8). The mitochondrial GSH pool involved in the regulation of intramitochondrial redox status has been demonstrated in many cell types (35, 36). Although this mitochondrial GSH is sensitive to depletion by many antitumor agents, it is relatively insensitive to BSO depletion. Likewise, many reports have suggested that GSH pools in nuclei are more resistant than those in the cytoplasm to BSO depletion (42–46). However, since our knowledge is rather limited at this point, it is speculated that these compartmentalized GSH levels may play a dominant role in controlling intracellular ROIs and gene regulation compared with those in the cytoplasmic compartment. This hypothesis requires further experimental demonstration.

A previous study (34) demonstrated moderate levels (1.7–2.5-fold) of increases in g-GCSh transcript in BSO-treated HBT5 and HBT28 brain tumor cells under conditions in which 95% of intracellular GSH was depleted. These results differ from those presented in this study. Although the basis for this discrepancy is unclear, it may reflect the differential response of different cell lines to BSO. For example, treating HBT5 and HBT28 cells with 100 μM BSO depleted 95% of the GSH content in these cells, whereas under the same conditions, only 85 and 65% depletions were seen in SR3A and SCLC cells, respectively. Significant heterogeneity in the effects of BSO on g-GCSh expression has indeed been reported in these HBT cells.
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expression reveals a previously unreported feedback inhibitory mechanism of gene expression. Furthermore, because GSH levels are involved in many important cellular signaling pathways (52–54), the present findings may have a broad implication beyond the presently described MRP1 and γ-GCSh expression. Finally, our present study may have clinical relevance when modulators of MRP1 and GSH function are considered in combination cancer chemotherapy (10).

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