A new aspect on glutathione-associated biological function of *MRP/GS-X* pump and its gene expression

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Abstract

The biological function as well as gene expression of the *MRP/GS-X* pump is closely linked with cellular GSH metabolism. This article describes two important aspects, i.e., 1) a role of the *MRP/GS-X* pump in the modulation of cell cycle arrest induced by anticancer prostaglandins; 2) coordinated up-regulation of γ -glutamylcysteine synthetase (γ -GCS) and MRP1 genes. The A and J series of prostaglandins (PGs) accumulate in the nuclei to suppress the proliferation of cancer cells. Δ^7 -Prostaglandin A₁ (Δ^7 -PGA₁) methyl ester, a synthetic anticancer PG, increased the mRNA level of the cyclin-dependent kinase inhibitor p21^{Sdi1/CIP1/WAF1} in human leukemia HL-60 cells. The induction of p21^{Sdi1/CIP1/WAF1} was associated with the accumulation of hypophosphorylated retinoblastoma protein (pRB) and the suppression of *c-myc* gene expression. Unlike HL-60 cells, cisplatin-resistant HL-60/R-CP cells were insensitive to Δ^7 -PGA₁ methyl ester. While *c-myc* expression was transiently suppressed, neither G1 arrest nor hypophosphorylation of pRB was observed with the anticancer PG. Plasma membrane vesicles from HL-60/R-CP cells showed an enhanced level of GS-X pump activity toward the glutathione S-conjugate of Δ^7 -PGA₁ methyl ester. GIF-0019, a potent inhibitor of the GS-X pump, dose-dependently enhanced the cellular sensitivity of HL-60/R-CP cells to Δ^7 -PGA₁ methyl ester, resulting in G1 arrest. The GS-X pump is suggested to play a pivotal role in modulating the biological action of the anticancer PG. The expression of MRP1 and γ -GCS genes can be coordinately up-regulated by cisplatin, 1-[5-(4-amino-2-methylpyrimidyl)methyl-3-(2-chloroethyl)-3-nitrosourea (ACNU), and heavy metals in human cancer cells. For the up-regulation of these genes, both transcriptional and posttranscriptional regulations are considered to be involved.

Introduction

Drug resistance is a biological response of tumor cells to antitumor agents, representing a constitution of different biochemical and genetic determinants. Identification and modulation of such determinants are important for understanding the biological nature of drug resistance and solving the associated problems in human cancer chemotherapy. Recent studies on the multidrug resistance phenotype of tumor cells have led to the discovery of two different types

of ATP-binding cassette (ABC) transporters, i.e., P-glycoprotein (MDR) (Gottesman and Pastan, 1988; Endicott and Ling, 1989) and multidrug resistance-associated protein (MRP) (Cole *et al.*, 1992; Zaman *et al.*, 1994), that mediate the efflux of certain anticancer drugs from cells. The overexpression of one of these export pumps in tumor cells *in vitro* has been shown to be closely associated with certain multidrug resistance phenotypes. The substrate specificities of P-glycoprotein and MRP appears to be different. While the exact mechanisms of MRP-

mediated drug resistance remain to be determined, many recent studies strongly suggest that MRP may transport a wide range of organic anions, including glutathione disulfide (GSSG), glutathione-metal complexes, glutathione conjugates, cysteinyl leukotrienes as well as glucuronate and sulfate conjugates (Müller *et al.*, 1994; Leier *et al.*, 1994; Jedlitschuk *et al.*, 1996; Loe *et al.*, 1996a, b). These findings are consistent with the idea that the MRP gene product is the previously described *GS-X* pump, a member of the phase III drug metabolizing system (Ishikawa, 1992).

The fact that the MRP1 gene encodes a human *GS-X* pump supports an important scenario that glutathione (GSH) metabolism may play a pivotal role in overall MRP-mediated drug resistance. Accumulating evidence suggests that GSH-mediated drug metabolism and disposition are major mechanisms of tumor drug resistance. It has been reported that depletion of intracellular GSH by buthionine sulfoximine (BSO) resulted in a complete reversal of drug resistance in MRP1 cDNA-transfected lung carcinoma cells (Zaman *et al.*, 1995). ATP-dependent transport of certain anticancer drugs, *e.g.* vincristine, can be enhanced by the presence of GSH in membrane vesicles prepared from MRP1-overexpressing cells (Loe *et al.*, 1996a). Furthermore, we have demonstrated increases of intracellular GSH level in the MRP1-overexpressing human cisplatin-resistant HL-60 leukemia cell line, HL-60/R-CP (Ishikawa *et al.*, 1994). Steady state levels of MRP1 and γ -GCS catalytic heavy subunit mRNA were found to be elevated in HL-60/R-CP cells as compared to those in the parental HL-60 cells (Ishikawa *et al.*, 1996). Coordinated overexpression of these genes frequently occurs in human colorectal cancers (Kuo *et al.*, 1996). Likewise, a coordinated increase of MRP1 and γ -GCS mRNA was also observed in ethacrynic acid-resistant HT29 human colon cancer cells (Ciaccio, 1996). These observations strongly suggest that the biological function of the *GS-X* pump encoded by the MRP1 gene is closely linked with cellular GSH metabolism.

This review article presents a new aspect on the role of the *GS-X* pump encoded by the MRP1 gene in GSH-associated drug resistance of cancer cell, in particular, the modulation of cell cycle arrest induced by anticancer prostaglandins (PGs) that has most recently been studied in our laboratory. In addition, we will discuss transcriptional and posttranscriptional regulations as potential molecular mechanisms involved in the induction of the *MRP/GS-X* pump.

***GS-X* Pump Family**

Cancer cells, as well as normal cells in animals and plants, eliminate a broad range of lipophilic toxins from the cytosol after their conjugation with GSH (Ishikawa, 1992; Martinoia *et al.*, 1993; Li *et al.*, 1995). This transport process is mediated by a *GS-X* pump, an organic anion-transporting Mg^{2+} -ATPase. The term of '*GS-X* pump' was originally proposed based on its transport activity and high affinity toward glutathione S-conjugates (GS-conjugates), GSSG, and cysteinyl leukotrienes (Ishikawa, 1992). The kinetic properties and substrate specificity of the *GS-X* pump have been intensively studied using plasma membrane vesicles from different biological sources. The *GS-X* pump has a broad substrate specificity toward different types of organic anions and thereby plays a physiologically important role in inflammation, oxidative stress, xenobiotics metabolism, and tumor drug resistance. In plants, the *GS-X* pump is competent in the transport of GS-conjugates of the chloroacetanilide and triazine herbicides, metolachlor and simetryn, respectively, as well as GSSG and GS-conjugates of 1-chloro-2,4-dinitrobenzene (CDNB) and monochlorobimane (Martinoia *et al.*, 1993; Li *et al.*, 1995). It has recently been suggested that the *GS-X* pump is involved in the anthocyanin biosynthetic pathway (Marrs *et al.*, 1995).

During past five years, there was a remarkable progress in our understanding of the molecular nature of the *GS-X* pumps. Overexpression of the MRP1 gene in human cancer cells has been first reported to result in increased ATP-dependent GS-conjugate transport, thus demonstrating that the MRP1 gene encodes a human *GS-X* pump (Müller *et al.*, 1994; Leier *et al.*, 1994). A liver specific *GS-X* pump, named *cMOAT*, has also been cloned from rat liver cDNA libraries and it exhibited an extensive homology with human *MRP1* (Paulusma *et al.*, 1996; Büchler *et al.*, 1996; Ito *et al.*, 1997). Mutation of the *cMOAT* gene is implicated to be a cause of hereditary hyperbilirubinemia associated with defective function of the hepatic *GS-X* pump. The yeast cadmium factor (*YCF1*) gene from *Saccharomyces cerevisiae* has been identified on the basis of its ability to confer cadmium resistance (Szczypka *et al.*, 1994). The *YCF1* gene encodes an ABC protein homologous to human *MRP1*, and the protein has recently been identified as a vacuolar *GS-X* pump in yeast cells (Li *et al.*, 1996). Furthermore, two cDNAs, *i.e.* *AtMRP1* and *AtMRP2*, encoding *GS-X* pump in a plant *Arabidopsis thaliana* have been cloned and they

Figure 1. Schematic illustration of the biosynthesis of GSH, conjugation of GSH with electrophilic drugs (X), inhibition of GSH transferase by GSH conjugate (GS-X), and ATP-dependent export of GS-X from cells via the GS-X pump.

also show extensive sequence homologies to *MRP1*, *cMOAT* and *YCF1* (Lu, *et al.*, 1997). *GS-X* pumps comprise a multigene family in both the animal and plant kingdoms (Ishikawa *et al.*, 1997; Keppler and König, 1997; Kool *et al.*, 1997).

GS-X Pump and GSH Metabolism

The function of the *GS-X* pump is closely linked with cellular GSH metabolism. Glutathionation provides negative charges to compounds and thereby enables the substrate recognition by the *GS-X* pump. GSH bears two important features in the structure, namely, the γ -glutamate linkage and the SH-group, both of which are intimately linked to its intracellular stability and biological functions. The intracellular concentration of GSH in mammalian cells is 1–10 mM,

which is even higher than the intracellular concentration of ATP. In many cells, GSH accounts for more than 90% of the total nonprotein sulfur. Such high intracellular concentration of GSH is made possible by the γ -Glu linkage structure, which protects the GSH molecule from protease cleavage. The SH-group of GSH is strongly nucleophilic and confers to the molecule the unique ability to react with a wide variety of agents including free radicals, reactive oxygen species, heavy metals, and cytotoxic electrophilic compounds, thereby playing a critical role of detoxification in living cells.

The biosynthesis of GSH takes place in the cytosol. The reaction consists of two steps catalyzed by γ -glutamylcysteine synthetase and by GSH synthetase, where each step requires one molecule of ATP (Figure 1). γ -GCS catalyzes the first step (Reaction 1) in which an amide linkage is formed between the amino

Figure 2. Effect of Δ^7 -PGA₁ methyl ester (inset) on cell proliferation of human leukemia HL-60 (●) and HL-60/R-CP cells (○) incubated with 5 μ M Δ^7 -PGA₁ methyl ester. Data are expressed as mean $\pm$ S.D. of triplicate experiments.

group of cysteine and the γ -carboxyl group of glutamate. In the second step, GSH synthetase catalyzes the reaction between glycine and the cysteine carboxyl group of γ -glutamylcysteine:

The reaction catalyzed by γ -GCS is the rate-limiting step of GSH biosynthesis and is controlled by negative feedback from its end product, GSH, via non-allosteric competitive inhibition. As described below, coordinated up-regulation of the γ -GCS gene together with the *MRP1/GS-X* pump gene has been frequently observed in many of human cancer cells.

The pathway of GSH-mediated drug inactivation is a biologically 'expensive' mechanism. The synthesis of GSH and the export of GS-conjugates from the cytosol require at least three molecules of ATP in order to metabolize 1 mol of the drug molecule. This compares unfavorably with the glycolytic pathway, in which only two molecules of ATP are gained from one molecule of glucose. Nevertheless, the fact that the *GS-X* pump is ubiquitously distributed in the plant and animal kingdoms strongly suggests that the GSH-associated metabolism and transport pathway are fundamentally important for the survival of living cells.

Figure 3. Effect of Δ^7 -PGA₁ methyl ester on *c-myc* and β -actin mRNA levels. HL-60 and HL-60/R-CP cells were incubated with 5 μ M Δ^7 -PGA₁ methyl ester. mRNA levels were detected by northern blot hybridization.

Anticancer Prostaglandins and their Biological Action

Regulation of cell growth and proliferation is of fundamental biological interest. We here provide evidence that GSH and the GS-X pump are critically involved in the modulation of cell cycle arrest induced by anticancer prostaglandins (PGs). Accumulating evidence suggests that arachidonic acid and its metabolites constitute a novel class of intracellular second messengers. Of the different types of PGs, the A and J series PGs are unique in exerting their biological activity via direct interaction with putative nuclear proteins rather than with plasma membrane receptors. The A and J series of PG (PGA and PGJ) suppress proliferation of tumor cells *in vivo* as well as *in vitro* without affecting intracellular cAMP levels (Narumiya and Fukushima, 1989). These antitumor PGs are actively transported into tumor cells (Narumiya and Fukushima, 1986) and their nuclear accumulation correlates closely with cell growth inhibition (Narumiya *et al.*, 1986) and cell-cycle arrest in the G1 phase (Ohno *et al.*, 1988).

The significance of anticancer PGs as novel bio-

chemical probes has been strengthened by a newly developed method called the 'three-component coupling synthesis', which has made it possible to generate large quantities of structurally modified PGs (Suzuki *et al.*, 1990; Noyori and Suzuki, 1993). Δ^7 -PGA₁ methyl ester, a synthetic antitumor PG, exhibits remarkable biological properties. At a concentration of 1.5 μ M, it suppressed the proliferation of HL-60 cells by 50%, 3- to 5-fold more effectively than PGA₂, a natural antitumor PG. 5 μ M Δ^7 -PGA₁ methyl ester suppressed the proliferation of HL-60 cells over 100 h (Figure 2), where the cell cycle was blocked in G1 phase. *c-myc* mRNA level decreased within 2 h and the suppression of *c-myc* expression lasted over 36 h (Figure 3; Akimaru *et al.*, 1996).

The inhibition of cell proliferation, cell-cycle arrest, and suppression of *c-myc* gene expression were closely related to accumulation of hypophosphorylated retinoblastoma gene product (pRB) (Figure 4). pRB plays a critical role in cell-cycle progression by virtue of its potency of inhibiting the activity of the E2F family of transcription factors for *c-myc*, B-myb, dihydrofolate reductase, and DNA polymerase α .

Figure 4. Effect of Δ^7 -PGA₁ methyl ester on the phosphorylation state of pRB. HL-60 and HL-60/R-CP cells were incubated with 5 μ M Δ^7 -PGA₁ methyl ester. pRB was detected by western blotting using pRB-specific antibody.

Figure 5. Effect of Δ^7 -PGA₁ methyl ester on the expression of p21 in HL-60 cells. Cells were treated with 5 μ M Δ^7 -PGA₁ methyl ester for the specified time periods (0, 1, 3, 6, 9, 12 and 24 h). mRNA levels of p21 at 2.1 kb were detected by northern blot hybridization.

Hypophosphorylated pRB associates and inactivates E2F-1, thereby suppressing *c-myc* gene expression. Since *c-myc* expression is required for cell proliferation, especially cell-cycle entry from G1 into S phase, the suppression of *c-myc* expression is considered to be one of the major mechanisms of anti-proliferatory action of Δ^7 -PGA₁ methyl ester.

Hitherto at least five different cycline-dependent kinase inhibitors (CKIs) were identified. Figure 5 shows that one such inhibitor, p21^{Sdi1/CIP1/WAF1}, was specifically induced by Δ^7 -PGA₁ methyl ester in HL-60 cells (Figure 5). This induction was well correlated with the growth inhibition, the accumulation of hypophosphorylated pRB and cell cycle arrest. No induction was detected without the antitumor PG. Moreover, mRNA levels of other CKIs, p27^{Kip1}, p16^{INK4A} and p18^{INK4C}, remained unchanged, and no p15^{INK4B} expression was detected

in the presence or absence of Δ^7 -PGA₁ methyl ester in HL-60 cells. These observations strongly suggest that the synthetic antitumor PG specifically induces p21^{Sdi1/CIP1/WAF1}.

The p21^{Sdi1/CIP1/WAF1} protein binds CDKs and inhibits phosphorylation of pRB by cyclin-CDK complexes, resulting in accumulation of hypophosphorylated pRB (Harper *et al.*, 1993; Xiong *et al.*, 1993). p21^{Sdi1/CIP1/WAF1} was shown to be involved in *p53*-mediated G1 arrest which is related to DNA repair processes after γ -irradiation (El-Dairy *et al.*, 1994; Dulic *et al.*, 1994). However, since the *p53* gene is deleted in HL-60 cells, we conclude that the induction of p21^{Sdi1/CIP1/WAF1} by the antitumor PG is mediated by a novel process independent of the *p53* transcription factor. Moreover, p21^{Sdi1/CIP1/WAF1} has recently been identified as a DNA synthesis inhibitor in senescent cells. Thus,

Figure 6. Putative pathways of hypophosphorylation of pRB and suppression of *c-myc* expression triggered by Δ^7 -PGA₁-mediated induction of p21, as well as GSH conjugation and the subsequent elimination by *GS-X* pump. The anticancer PG induces *de novo* synthesis of p21, which is followed by the inhibition of Cdk2, 4 and 6 and the association of hypophosphorylated pRB with E2F-1, a transcription factor of the *c-myc* gene. Cyc, cyclin; Cdk, cyclin-dependent kinase.

induction of p21^{Sdi1/CIP1/WAF1} appears to play a central role in the cell growth inhibition by the anti-tumor PG (Figure 6). Our results are consistent with recent observations reported by Holbrook's research group (Gorospe and Holbrook, 1996; Gorospe *et al.*, 1996) as well as Stacy and his collaborators (Hitomi *et al.*, 1996).

Role of *MRP/GS-X* pump in Cellular Resistance to Antitumor PG

Cell growth inhibition by antitumor PGs is reversible (Narumiya *et al.*, 1986) and the reversibility of anti-tumor action is considered to be due to loss of the reactivity of PGs in the cell (Narumiya and Fukushima, 1986). The α,β -unsaturated carbonyl group of antitumor PGs is electrophilic and readily reacts with thiol groups of cellular constituents, such as GSH, cysteine, and proteins (Honn and Marnett, 1985). Anticancer PGs with a simple enone group, such as PGA₂, react

with GSH, however the equilibrium favors the GSH adduct formation (Suzuki *et al.*, 1997). The simple enone PGs are therefore expected to be present in cytoplasm predominantly as biologically inactive GSH adducts. On the other hand, Δ^7 -PGA₁ that possesses a cross-conjugated dienone structure reacts with GSH in a reversible manner but the equilibrium shifts to the free PG to a considerable extent (Suzuki *et al.*, 1997). In such a case, increased GSH levels are considered to be a major factor to shift the equilibrium of the reaction of Δ^7 -PGA₁ with GSH toward adduct formation. In addition, it is also hypothesized that the resulting GS-PG conjugate is readily exported from cells by the *GS-X* pump. Overexpression of the *MRP/GS-X* pump has been found in cisplatin-resistant human leukemia HL-60/R-CP cells (Ishikawa *et al.*, 1996). Using HL-60/R-CP cells, we have examined a potential role of the *MRP/GS-X* pump in cellular resistance to the antitumor PG.

As shown in Figure 2, proliferation of HL-60/R-CP cells was little affected by 5 μ M Δ^7 -PGA₁ methyl

Figure 7. Inhibition of GS-X pump by GIF-0017. GIF-0019 undergoes hydrolysis catalyzed by esterase in the cell. GIF-0017, thus formed, is considered to inhibit the GS-X pump and thereby to enhance cellular accumulation of the anticancer PG, Δ^7 -PGA₁.

ester. No G1-arrest was observed in the cisplatin-resistant cells. *c-myc* suppression, hypophosphorylation of pRB, and p21^{Sdi1/CIP1/WAF1} induction in these cells were detected for only a short period (<6 h). Thus, these results suggest that HL-60/R-CP cells, which overexpress the MRP/GS-X pump, are insensitive or resistant to the antitumor PG.

To gain further insight in the role of the MRP/GS-X pump in the cellular resistance, we first examined the reaction of Δ^7 -PGA₁ methyl ester with GSH. The reaction product was identified by reverse-phase high performance liquid chromatography (RP-HPLC) as well as by reverse-phase thin-layer chromatography and NMR. Furthermore, the GSH conjugate of Δ^7 -PGA₁ methyl ester was purified by RP-HPLC, and ATP-dependent transport of the compound was examined using plasma membrane vesicles prepared from HL-60/R-CP cells. ATP-dependent transport of leukotriene C₄, an endogenous substrate of the MRP/GS-X pump, was competitively inhibited by the GSH conjugate of Δ^7 -PGA₁ methyl ester (IC₅₀ = 0.75 μ M) (Akimaru *et al.*, 1996), suggesting that

the GS-conjugate of the antitumor PG is a good substrate for the MRP/GS-X pump. Indeed, plasma membrane vesicles from HL-60/R-CP cells exhibited ATP-dependent transport of the GSH adduct of Δ^7 -PGA₁ methyl ester with an apparent K_m value of 110 nM. The K_m value is comparable to that for leukotriene C₄.

GIF-0017, a potent GS-X pump inhibitor synthesized in our group (Furuta, Ishikawa, and Suzuki, manuscript in preparation), effectively inhibited the transport of the GS-PG conjugate in plasma membrane vesicles prepared from HL-60/R-CP cells. GIF-0019, a derivative of GIF-0017 modified to penetrate cell membrane, elicited cellular sensitivity of HL-60/R-CP cells to Δ^7 -PGA₁ methyl ester. 10 μ M GIF-0019 inhibited cell proliferation of HL-60/R-CP cells in the presence of Δ^7 -PGA₁ methyl ester over 100 h. This suggests that GIF-0019 readily undergoes hydrolysis catalyzed by esterase in the cell to form GIF-0017, which blocks the GS-X pump for the extrusion of the GS-PG conjugate from the cell (Figure 7). Because of the reversible reaction between GSH and Δ^7 -PGA₁, the free active form of the PG can be formed from its

Figure 8. Detection of MRP1 and γ -GCS mRNA in different cancer cell lines by RNase protection assay. Experimental details and methods are described in Kuo *et al.*, 1997.

GSH adduct. The *GS-X* pump is considered to play a significant role in reducing accumulation of Δ^7 -PGA₁ in the cell, thereby modulating the antiproliferative activity of the anticancer PG.

Coordinated Expression of γ -GCS and *MRP/GS-X* Pump

At present, little is known about the regulation of the expression of the *MRP/GS-X* pump gene. Our group has recently demonstrated that MRP1 and γ -GCS genes are coordinately induced by cisplatin and heavy metals, such as arsenite, cadmium, and zinc in cisplatin-resistant HL-60/R-CP cells (Ishikawa *et al.*, 1996). To extend the study, we analyzed the levels of MRP and γ -GCS mRNA in

small-cell lung cancer cells and their drug-resistant variants, *i.e.*, SR2 (cisplatin-resistant) as well as SR2A and SR3A (doxorubicin-resistant). There was a clear correlation between the levels of MRP1 and γ -GCS mRNA expression (Kuo *et al.*, 1997). Furthermore, human glioma A172 cells pretreated with 1-[5-(4-amino-2-methyl)pyrimidyl]methyl-3-(2-chloroethyl)-3-nitrosourea (ACNU) for 24 h enhanced mRNA levels of both MRP1 and γ -GCS by 3- to 5-fold (Gomi *et al.*, 1997), suggesting that expression of MRP1 gene is closely related with cellular GSH biosynthesis and that certain common factor(s) may regulate the expression of both MRP1 and γ -GCS genes.

We further examined coordinated expression of MRP1 and γ -GCS mRNA in drug-sensitive tumor cell lines (Figure 8). We randomly chose 17 cell lines: three breast cancer (MDA-MB-468, MDA-MB-453, and MDA-MB-361), three glioma (U251, D54, and T98), three small-cell lung cancer (H-226, H-460, SCOG), two pancreas cancer (PANC3 and BXP3), two leukemia (pre-B and T-ALL), one prostate cancer (PC3), one ovarian cancer (SK-OV-3), one hepatoma (HepG2) and one colon cancer (HT29). Substantial variations in MRP1 and γ -GCS mRNA levels were observed in those tumor cell lines. Cell lines expressing low levels of MRP1 and γ -GCS mRNA include PC3, H-226, T-ALL and MDA-MB-468; and those with high levels include PAN3, HT-29, BXP3, T98, MDA-MB361, and pre-B cells. When the levels of MRP1 mRNA were plotted against those of γ -GCS mRNA, a correlation coefficient of 0.861 was obtained (Kuo *et al.*, 1997). These results suggest that, even in drug-sensitive tumor cells, there was a good correlation between the levels of MRP1 and γ -GCS mRNA expression.

The fact that MRP1 and γ -GCS can be coordinately induced by cisplatin, ACNU, and heavy metals provides important information to our understanding of how drug resistance genes are acutely induced upon drug treatments. Since *cis*-regulatory elements including AP-1 binding sites have been identified in the promoter regions of human γ -GCS and MRP1 genes (Mulcahy and Gipp, 1995; Yao *et al.*, 1995; Zhu and Center, 1994) (Figure 9), it is extremely interesting to examine whether AP-1 is responsible for the transcriptional regulation of the expression of MRP1 and γ -GCS genes in cancer cells. Unlike cell culture studies where drug-resistant variants are usually obtained through continuous drug exposure, such transient induction of drug-resistance gene expression

Figure 9. Potential regulation sites in the promoter regions of human MRP1 and γ -GCS genes. CRE, cyclic AMP response element; ERE, estrogen response element; GRE glucocorticoid response element, XRE, xenobiotic response elements, EpRE, Electrophile response element; CAT box, CCAAT box. Data from Zhu and Center, 1994; Yao *et al.*, 1995; Mulcahy and Gipp, 1995.

is more directly related to cancer chemotherapeutic protocols. Interestingly, human colorectal cancers frequently overexpress MRP1 and γ -GCS genes (Kuo *et al.*, 1996). Because patients involved in the study had not been treated with chemotherapeutic agents, it is suggested that up-regulation of different drug resistance genes is associated with different human cancers and, more importantly, development of drug resistance in these cancers is an intrinsic mechanism. Because intrinsic drug resistance is the major factor controlling efficacy of chemotherapy in cancer treatment, a better understanding of how different drug-resistance genes are regulated in different tumor systems is of great importance for development of effective strategies to circumvent drug resistance in cancer chemotherapy.

Importance of Posttranscriptional Regulation

Emerging evidence in the recent years has indicated that posttranscriptional regulation plays an important role in the modulation of drug-resistance gene expression in cells treated with various cytotoxic substances and under various physiological conditions (Chin *et al.*, 1990; Chaudhary and Roninson, 1993). For example, steady state levels of MDR mRNA encoding

P-glycoprotein can be transiently induced in cultured cells by many chemotherapeutic agents (Chin *et al.*, 1990; Chaudhary and Roninson, 1993). Although transcriptional regulation has been noted, posttranscriptional regulation is primarily responsible for the observed increases of MDR mRNA. Inhibition of protein synthesis by cycloheximide could also enhance the steady state level of MDR mRNA in cultured cells (Gant *et al.*, 1992; Fardel *et al.*, 1993). Although an initial report suggested that the enhancement was due to increased transcriptional rate (Gant *et al.*, 1992), recent reports have demonstrated that posttranscriptional control is primarily responsible (Schuetz *et al.*, 1995; Kren and Steer, 1996). In experimental animal models, posttranscriptional regulation was observed for the upregulation of *mdr* gene expression during partial hepatectomy (Chin *et al.*, 1990) and in the uterine epithelial cells during pregnancy (Kuo *et al.*, 1995). Thus, there may be overlapping mechanisms that control P-glycoprotein and MRP/GS-X pump expression. It would be of interest to determine whether similar mechanisms are involved in the increased mRNA levels of MRP1 and γ -GCS *in vivo* as well as *in vitro*.

It remains critically important to determine whether MRP1 and γ -GCS mRNA levels are transiently elevated during chemotherapy in clinical set-

tings, and if so, whether the induction is controlled by posttranscriptional or transcriptional events. Our most recent study has demonstrated that MRP1 and γ -GCS were transiently induced by ACNU and cycloheximide, and that the upregulation of the expression of these genes was controlled at the posttranscriptional levels (Gomi *et al.*, 1997). While *cis*-acting elements for transcriptional regulation of MRP1 (Zhu and Center, 1994) and γ -GCS (Mulcahy *et al.*, 1997) have recently been identified, little is known about molecular details regarding posttranscriptional control for these genes. A better understanding of how MRP and γ -GCS genes are upregulated under various environmental influences as well as how γ -GCS is actually involved in the MRP-mediated drug resistance, might provide valuable insights into molecular mechanisms underlying the clinical problems of drug resistance in cancer chemotherapy.

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