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COMMENTARY

New Insights into the Biology and Pharmacology of the Multidrug Resistance Protein (MRP) from Gene Knockout Models

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ABSTRACT. Growing interest in the *MRP* (multidrug resistance protein) gene stems from its importance in multidrug resistance to chemotherapy, its possible use in gene therapy, and its relationship with the glutathione system. The recent generation of *mrp* gene knockout models *in vitro* and *in vivo* is providing information on the mechanism of action and the physiological function(s) of *mrp*. The importance of *mrp* in protection of normal tissues from the toxicity of the anticancer agent etoposide has been established. A total block of *mrp* has been found to be compatible with life, suggesting that MRP inhibitors can be safely used for treating cancer patients. In some sub-classes of leukocytes, *mrp* contributes to the transport of leukotriene C_4, an endogenous glutathione-*S*-conjugate. However, the baseline expression of *mrp* does not appear to contribute to the export of glutathione-*S*-conjugates of alkylating agents, and thus does not exert a protective role against their toxicity. Besides being capable of exporting certain glutathione-*S*-conjugates, mrp also catalyzes the co-transport of GSH and drug and, presumably, a presently unknown endogenous metabolite(s). BIOCHEM PHARMACOL **58**;4: 557–562, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. multidrug resistance; MRP; knockout mice; etoposide; glutathione; co-transport

The *MRP*§ gene is a member of the superfamily of ABC-type cassette transporters [1]. To date, only two members of this superfamily, the 190-kDa MRP protein and the *MDR1*-encoded 170-kDa P-glycoprotein, have been clearly shown to cause multidrug resistance, i.e. resistance to a spectrum of drugs with different structures and cellular targets [2]. Due to the importance of inherent and acquired multidrug resistance as obstacles to the success of anticancer chemotherapy for the treatment of disseminated cancers, a great effort has been made to understand the mechanism of action and the physiological function(s) of these proteins, as well as to discover clinically effective inhibitors. Several inhibitors of the P-glycoprotein and MRP have been studied in preclinical models, and some have recently entered clinical trials [3]. Knowledge of the physiological functions of the MRP and P-glycoprotein is essential to anticipate and to prevent possible side-effects of their respective inhibitors.

Recently, the use of these drug resistance proteins in gene therapy has been proposed as a means to protect normal sensitive tissues, such as the bone marrow, from the toxic effects of chemotherapy [4–6]. This would be accomplished by the use of safety-modified, replication-incompetent retroviral vectors to deliver such genes to human hematopoietic progenitors in patients with advanced cancer. Making the bone marrow resistant to the toxicity of a chemotherapeutic agent could allow dose amplification, hopefully leading to eradication of the neoplastic clone. Clinical trials with MDR1-expressing retroviral vectors are currently ongoing [7–10], and MRP-expressing retroviral vectors are being evaluated in preclinical models [6]. Another interesting approach is to employ *MDR1* or *MRP* as selectable markers to increase the expression of passenger genes in various gene therapy protocols, including those for the treatment of hereditary diseases. Thus, the selection and persistence in the body of cells transduced with a potentially curative gene could be determined by the co-expression of a simultaneously transduced drug resistance gene [11].

To assist in the understanding of the mechanism of action and especially of the physiological function(s) of proteins, the generation of gene knockout models is often very useful. This strategy has been informative with respect to the P-glycoprotein [12]. Two functional homologues of the *MDR1* gene exist in mice, *mdr1a* and *mdr1b*. Borst and his colleagues [13] have obtained mice with a disruption of each of these two genes and also a double knockout in which both *mdr1a* and *mdr1b* are disrupted. The results obtained in these model systems have been summarized recently [14], the main findings being the recognition of the P-glycoprotein as an important component of the blood–

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§ *Abbreviations*: MRP, multidrug resistance protein; and MDR, multidrug resistance.

brain barrier and its involvement in the absorption and elimination of several clinically used drugs.

Although much progress has been made in the past 2 years on the biology of MRP, the physiological role(s) of MRP needs to be further clarified. The fact that MRP is ubiquitously expressed, although at very low levels in the liver and some other organs [1, 15, 16], suggests that it exerts a common function(s). Alternatively, MRP may exert different tissue-specific functions, determined by tissue-specific promoters, cooperation with other tissue-specific proteins, differential splicing, or post-translational modifications. Based on the similarity between MRP and other members of the same superfamily of transporters, as well as on indirect laboratory evidence, several physiological functions of MRP have been suggested: (a) protection against environmentally present heavy metal oxyanions, (b) modulation of the activity of ion channels, (c) transport of leukotriene C_4 and other glutathione, glucuronate and sulfate conjugates, and (d) transport of GSH through a co-transport mechanism.

DEVELOPMENT OF *MRP* KNOCKOUT MODELS

To assist in the understanding of the physiological mechanism(s) of action of MRP, we and others have generated *in vitro* and *in vivo mrp* knockout models [17–20]. Human and murine MRPs have 88% amino acid identity, and they both can induce multidrug resistance when their respective cDNAs are transfected into drug-sensitive cells [21–24]. Therefore, it is conceivable that the results obtained in the murine *mrp* knockout systems can be extrapolated to humans. The strategy we have used to disrupt both *mrp* alleles in murine embryonic stem cells *in vitro* is based on the method of Mortensen *et al.* [25]. A single construct targeted to the second ATP-binding domain was used to disrupt one *mrp* allele in embryonic stem cells by homologous recombination. Subsequently, *mrp* single knockout clones were exposed to high concentrations of G-418 to select for *mrp* double knockout clones. It is not yet clear whether the conversion to homozygosity in high G-418 concentrations is due to mitotic recombination, gene conversion, or homologous recombination with episomally maintained construct DNA. However, the complete abrogation of *mrp* expression, measured by immunoblotting and reverse transcription-polymerase chain reaction (RT-PCR), resulted in hypersensitivity of two separate embryonic stem cell clones *in vitro* to many natural product toxins, including the epipodophyllotoxin derivatives etoposide and teniposide, sodium arsenite, sodium arsenate, antimony potassium tartrate, vincristine, and the anthracyclines doxorubicin and daunorubicin, with single *mrp* knockout cells displaying an intermediate level of sensitivity [17, 18]. This pattern of hypersensitivity resembled the pattern of resistance expressed by cell lines transfected with *MRP* cDNA or that overexpressed endogenous *MRP* [21–24, 26–29]. We and others have also developed *mrp* double knockout mice from two separate embryonic stem cell clones [19, 20]. In acute toxicity experiments, using lethality as an end-point, *mrp* knockout mice were found to be 2-fold hypersensitive to etoposide and etoposide phosphate, a water-soluble prodrug of etoposide [19, 20]. In particular, etoposide phosphate exerted a differential toxicity to the bone marrow of *mrp* (+/+) and *mrp* (−/−) mice [19]. It is important to stress that a hypersensitivity of 2-fold can be extremely important clinically, where even a small increase in the dosage of a chemotherapeutic agent, such as etoposide, may result in toxicity to normal tissues. These results have established for the first time the importance of *mrp* in protecting normal tissues from the toxicity of anticancer agents. The development of *mrp* knockout mice has also shown that this mutation does not interfere with viability or fertility, and that *mrp* is dispensable in early development. The heterozygous and homozygous mice were not distinguishable from wild-type animals in appearance or mortality rate up to 1 year of age [19, 20]. The levels of a range of serum enzymes, proteins, electrolytes, and other components, as well as of hematological parameters also did not differ between *mrp* double knockout and wild-type mice [19, 20]. The fact that a total block of *mrp* is compatible with life suggests that MRP inhibitors can be safely used for treating cancer patients. Thus, it is conceivable that MRP-overexpressing tumors could lose their advantage over normal tissues and re-acquire sensitivity to chemotherapeutic agents, without major effects on human physiology. However, greater host toxicity exerted by some chemotherapeutic agents, e.g. etoposide, particularly at the level of the bone marrow, should be expected.

TRANSPORT OF HEAVY METAL OXYANIONS

Protection of organisms from natural toxins in the environment, and particularly from heavy metal oxyanions, could constitute a physiological role for MRP. Human beings are exposed to inorganic as well as to organic arsenicals in the environment. In fact, drinking water may contain variable levels of both trivalent and pentavalent arsenic [30]. We have found hypersensitivity in two separate *mrp* (−/−) cell clones to sodium arsenite (3-fold), sodium arsenate (2-fold), and antimony potassium tartrate (5- to 6-fold), but not to cadmium chloride [18]. These findings are consistent with resistance patterns in *MRP*-transfected cell lines [29]. Although *MRP* has a high degree of homology with the yeast YCF-1 cadmium resistance gene, the observed pattern of hypersensitivity is not surprising, given the fact that *MRP*-transfected cells acquire resistance to arsenicals and antimony-containing compounds, but not to cadmium [29]. To ascertain whether the baseline expression of *mrp* protects mice from the toxic effects of heavy metal oxyanions, limited toxicity tests have been conducted with two of these compounds. Sodium arsenite, injected i.p. as a single dose, was equivalent in toxicity to *mrp* (+/+) and *mrp* (−/−) mice [19]. Also for antimony potassium tartrate, to which *mrp* double knockout cell lines displayed the greatest level of *in vitro* hypersensitivity, no difference was observed

in mice treated with 15–60 mg/kg as a single i.p. dose (Rappa *et al.*, unpublished observations). The discrepancy between *in vitro* and *in vivo* experimental results can be reconciled if the dose-limiting organ(s) target of acute arsenite or antimony toxicity does not express significant levels of mrp. In fact, the liver, which expresses very low levels of MRP [1, 15, 16], is a major target of arsenite or antimony toxicity [31, 32]. Alternatively, other proteins can substitute *in vivo* for the mrp detoxification function. Further studies will be needed to determine whether or not MRP has a physiological role in protecting host tissues from heavy metal oxyanions.

REGULATION OF K^+ CHANNELS

In addition to its function as a transporter, MRP may be involved in the regulation of endogenous ion channels. Thus, ABC transporters have been shown to modulate the activity of ion channels, including outwardly rectifying Cl^- channels (CFTR), volume-regulated Cl^- channels (P-glycoprotein), and inwardly rectifying K^+ channels (SUR) [33–37]. In particular, Hardy *et al.* [35] have found recently that protein kinase C-mediated phosphorylation of the P-glycoprotein regulates an endogenous, cell volume-activated Cl^- channel. Furthermore, the overexpression of MRP was accompanied by increases in both K^+ channel and volume-regulated Cl^- channel currents [38]. Changes in the level of MRP expression were also closely correlated with the appearance of an inwardly rectifying K^+ channel in resistant H69AR cells and its subsequent loss in revertant H69PR cells [38]. If baseline MRP expression resulted in inwardly rectifying K^+ channel activity, this activity would be expected to disappear in *mrp* knockout cell lines. Instead, only an outwardly rectifying (delayed) K^+ channel activity of similar intensity was detected both in parental and in two *mrp* knockout embryonic stem cell lines (Lorico *et al.*, unpublished observations), using experimental conditions analogous to those described by Jirsch *et al.* [38]. This finding suggests that physiological expression of *mrp* does not determine K^+ channel activity. However, it is possible that ion channel regulation in mice is different than in humans. It remains also to be determined whether mrp regulates Cl^- channel currents.

GS-X PUMP AND EXPORT OF LEUKOTRIENE C_4

In membrane vesicles isolated from *MRP*-transfected or -overexpressing cell lines, MRP has been reported to be a high-affinity transporter of cysteinyl leukotrienes, and to transport many other glutathione and glucuronide conjugates, including alkylating agents, steroids, prostaglandin A_2, bile salt derivatives, as well as glutathione disulfide [39–45]. Borst and colleagues [20] have found recently that the high-affinity pump for 2,4-dinitrophenyl *S*-glutathione was abolished in erythrocytes from *mrp* knockout mice. Furthermore, (a) mast cells from *mrp* knockout mice have a partial defect in the secretion of leukotriene C_4, a derivative of arachidonic acid, and (b) ear swelling induced by topical application of arachidonic acid is reduced in the *mrp* knockout mice [20]. Leukotriene C_4 is involved in the control of smooth muscle contraction and vascular permeability and is synthesized from leukotriene A_4 by conjugation with GSH. A physiological role for mrp as transporter of leukotriene C_4 in leukotriene-synthesizing leukocytes suggests that MRP inhibitors might have a role against certain inflammatory processes [20].

Since secretion of leukotriene C_4 occurs mainly in some subclasses of leukocytes, it remains to be established whether export of additional glutathione-*S*-conjugates occurs *in vivo* under physiological conditions. Although it had been proposed earlier that MRP could be the "GS-X pump," a previously known glutathione-*S*-conjugate export carrier, it now seems clear that many GS-X pumps, including several MRP homologs, exist in nature, whose molecular structure and function have been conserved throughout evolution [46–48].

Glutathione-*S*-conjugation of alkylating agents is a well known detoxification pathway. Increases in expression of glutathione-*S*-transferase (GST) π, Ya, or Yb_1 following transfection of the respective GST cDNAs impart resistance to many alkylating agents, including chlorambucil, cisplatin, and melphalan [49]. The export of the conjugated drugs by the GS-X pump is presumed to be a crucial step in this detoxification pathway [50]. Although in systems of membrane vesicles monochloro-monoglutathionyl melphalan has been reported to be a substrate for MRP [43], we have not found a difference between parental and two *mrp* knockout embryonic stem cell lines in their sensitivity to melphalan, chlorambucil, and cisplatin [18]. This finding is in agreement with the observation that in *MRP*-transfected cells no cross-resistance to alkylating agents occurs [29]. *In vivo* studies, although preliminary, seem to confirm these findings; thus, the acute toxicity of cisplatin, injected i.p. as a single dose, was equivalent in *mrp* (+/+) and *mrp* (−/−) mice [19]. It is conceivable that glutathione-*S*-conjugation of alkylating agents results in their complete detoxification, and consequently their export from cells does not confer additional protection from cytotoxicity. The physiological importance of the export of glutathione-*S*-conjugates may be emphasized in cases where the GSH conjugation results in bioactivation as opposed to detoxification [50]. However, no difference in sensitivity existed between parental and two *mrp* knockout embryonic stem cell lines for the haloethanes, 1,2-dichloroethane and 1-chloro-2-iodoethane, which are *activated* to DNA-reactive electrophiles by GSH conjugation via glutathione-*S*-transferase [18]. Also, no differences in the rate of efflux of glutathione-bimane conjugates were found. These findings collectively demonstrate that baseline *mrp* expression does not appear to exert a protective role against the toxicity of alkylating agents.

MRP-MEDIATED CO-TRANSPORT OF GSH AND DRUGS (OR ENDOGENOUS METABOLITES)

It is clear that, under physiological conditions, *in vivo*, etoposide is a substrate for the mrp pump. However, etoposide constitutes a major obstacle to the hypothesis that MRP is solely a transporter of glutathione-*S*-conjugates, since GSH conjugates of etoposide are not known to exist. Furthermore, although MRP-mediated transport has been observed for glucuronosyl-etoposide in membrane vesicles from *MRP*-transfected cells [43], glucuronide conjugation of etoposide seems to take place mainly in the liver, and MRP is poorly expressed or not expressed in this tissue [19]. In addition, doxorubicin and vincristine are not known to undergo major modification in cells, at least not modifications that would turn these agents into substrates for a GS-X pump. Interestingly, vincristine transport by MRP-enriched membrane vesicles was demonstrable only in the presence of GSH, and the ability of vincristine and vinblastine to inhibit leukotriene C_4 transport was enhanced by GSH [40]. An ATP-dependent transport of aflatoxin B_1 by MRP-enriched membrane vesicles in the presence of GSH has been found recently, even though glutathione-*S*-conjugates of aflatoxin B_1 were not formed by the vesicles [51]. Regulation by GSH of drug transport in MRP-overexpressing tumor cell lines was observed by Versantvoort *et al.* [52].

In 1995, Zaman and his colleagues [53] reported that MRP-transfected cells export two times more GSH into the extracellular medium than parental cells. Export of GSH from eukaryotic cells in culture has been documented extensively in studies carried out in the presence of D,L-buthionine sulfoximine (BSO), an inhibitor of γ–glutamyl cysteine synthetase (γ-GCS) [54, 55]. The multiple essential functions of intracellular GSH make it extremely important for every cell in the body to maintain high intracellular levels of this peptide (0.1 to 10 mM). Since a considerable amount of energy is required to keep most of the cellular GSH in the reduced state, the release of GSH from cells must involve an important function. MRP-mediated GSH export may contribute to fueling the production of cysteine by extracellular enzymatic hydrolysis of GSH. In fact, a variety of cells in the body have an absolute requirement for a constant supply of cysteine, an amino acid that is exceedingly toxic when present in cells at high concentrations [55]. GSH export may also be of general importance in protecting the surface of cells from oxidants [55].

We have utilized the *mrp* gene knockout systems to clarify the relationship between mrp and GSH. When parental embryonic stem cells, which express baseline levels of mrp, were depleted of GSH to 10% of their normal level, and subsequently incubated in medium containing radiolabeled etoposide, the steady-state intracellular concentration of etoposide doubled, whereas the steady-state concentration of etoposide in *mrp* knockout embryonic stem cells was not affected by GSH depletion [18]. Consistent with these findings, BSO-induced GSH depletion reversed the multidrug resistant phenotype in cells overexpressing MRP [52, 53] and enhanced the therapeutic efficacy of doxorubicin *in vivo* against MRP-overexpressing tumors [56]. Furthermore, a direct interaction between GSH and MRP has been demonstrated recently by the observation that etoposide, vincristine, and GSH stimulated the vanadate-induced trapping of 8-azido ATP by MRP [57]. A possible interpretation of these findings is that MRP co-transports GSH and drug(s).

We have reported that in the absence of drugs, parental embryonic stem cells exported GSH into the extracellular medium at approximately double the rate that occurred in two *mrp* knockout embryonic stem cell lines [18]. Thus, the expression of the *mrp* gene appears to account for more than one-half of the baseline GSH export. The fact that, in the absence of the mrp protein, the two knockout clones exhibit a basal level of GSH release suggests that there is at least one other mrp-independent system responsible for GSH release into the extracellular milieu. In purified membrane vesicles, GSH alone does not appear to be a substrate for the MRP pump [38]. Thus, it is conceivable that the mrp-mediated basal release of GSH occurs in association with a presently unknown endogenous metabolite(s).

In *mrp* knockout mice, tissue levels of GSH were increased markedly [19], especially in tissues such as lung, kidney, and muscle, where *mrp* is highly expressed in normal mice [16]. Tissue levels of GSH were unchanged in the small intestine and liver, which are known to express very little, if any, *mrp* [19]. The fact that most tissues of *mrp* (−/−) mice displayed an increased level of GSH is consistent with the abrogation of mrp-mediated GSH export in knockout animals. This observation reinforces the hypothesis that MRP is a physiological co-transporter of GSH and a presently unknown endogenous compound(s). Exposure of parental embryonic stem cells to etoposide or sodium arsenite resulted in a great increase in GSH export and a simultaneous equivalent decrease in intracellular GSH, whereas, in contrast, no change in GSH concentration was observed in two *mrp* knockout embryonic stem cell lines [18]. Consistent with these findings, Zaman *et al.* [53] reported that exposure of *MRP*-transfected lung cancer cells to sodium arsenite increased the rate of export of GSH compared with the parental counterpart. It is not clear at this time whether a molecular interaction between drug and GSH is required, but if this is the case, the complex must be easily dissociable, since the exported GSH is present in the extracellular medium as free GSH [18]. An association between GSH synthesis and MRP expression has been observed by Kuo *et al.* [58], who have reported that MRP and γ-GCS, the rate-limiting enzyme in GSH biosynthesis, are coordinately overexpressed in both untreated and drug-resistant tumor cells, as well as in normal tissues. Thus, our finding of a baseline MRP-mediated export of GSH provides an explanation for the observed coordinated up-regulation of MRP and γ-GCS. Elevated γ-GCS activity may be needed to supply sufficient GSH for overall MRP-mediated drug transport.

In conclusion, studies of *mrp* knockout models strongly suggest that MRP, besides being capable of exporting certain glutathione-*S*-conjugates, also catalyzes the co-transport of GSH and drugs or endogenous metabolites. The existence of a link between two of the most studied mechanisms of resistance to anticancer agents, the ATP-dependent membrane export of drugs and the GSH detoxification pathway, is clearly intriguing. A systematic assessment of the co-transport properties of ABC-type transporters may open a new chapter in the search for physiological functions of the GSH system.

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References

1. Cole SPC, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AMV and Deeley RG, Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* **258:** 1650–1654, 1992.
2. Lautier D, Canitrot Y, Deeley RG and Cole SPC, Multidrug resistance mediated by the multidrug resistance protein (*MRP*) gene. *Biochem Pharmacol* **52:** 967–977, 1996.
3. Fisher GA and Sikic BI, Clinical studies with modulators of multidrug resistance. *Hematol Oncol Clin North Am* **9:** 363–382, 1995.
4. Hanania EG and Deisseroth AB, Serial transplantation shows that early hematopoietic precursor cells are transduced by MDR-1 retroviral vector in a mouse gene therapy model. *Cancer Gene Ther* 1: 21–25, 1994.
5. Sorrentino BP, McDonagh KT, Woods D and Orlic D, Expression of retroviral vectors containing the human multidrug resistance 1 cDNA in hematopoietic cells of transplanted mice. *Blood* 86: 491–501, 1995.
6. D'Hondt V, Caruso M and Bank A, Retrovirus-mediated gene transfer of the multidrug resistance-associated protein (MRP) cDNA protects cells from chemotherapeutic agents. *Hum Gene Ther* 8: 1745–1751, 1997.
7. Deisseroth AB, Kavanagh J and Champlin R, Use of safety-modified retroviruses to introduce chemotherapy resistance sequences into normal hematopoietic cells for chemoprotection during the therapy of ovarian cancer: A pilot trial. *Hum Gene Ther* 5: 1507–1522, 1994.
8. Deisseroth AB, Holmes F, Hortobagyi G and Champlin R, Use of safety-modified retroviruses to introduce chemotherapy resistance sequences into normal hematopoietic cells for chemoprotection during the therapy of breast cancer: A pilot trial. *Hum Gene Ther* 7: 401–416, 1996.
9. O'Shaughnessy JA, Cowan KH, Nienhuis AW, McDonagh KT, Sorrentino BP, Dunbar CE, Chiang Y, Wilson W, Goldspiel B, Kohler D, Cottler-Fox M, Leitman S, Gottesman M, Pastan I, Denicoff A, Noone M and Gress R, Retroviral mediated transfer of the human multidrug resistance gene (MDR-1) into hematopoietic stem cells during autologous transplantation after intensive chemotherapy for metastatic breast cancer. *Hum Gene Ther* 5: 891–911, 1994.
10. Hesdorffer C, Antman K, Bank A, Fetell M, Mears G and Begg M, Human MDR gene transfer in patients with advanced cancer. *Hum Gene Ther* 5: 1151–1160, 1994.
11. Aran JM, Gottesman MM and Pastan I, Construction and characterization of bicistronic retroviral vectors encoding the multidrug transporter and beta-galactosidase or green fluorescent protein. *Cancer Gene Ther* 5: 195–206, 1998.
12. Schinkel AH, Smit JJM, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol CA, van der Valk MA, Robanus-Maandag EC, te Riele HPJ, Berns AJM and Borst P, Disruption of the mouse *mdr1a* P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* 77: 491–502, 1994.
13. Schinkel AH, Mayer U, Wagenaar E, Mol CA, van Deemter L, Smit JJ, van der Valk MA, Voordouw AC, Spits H, van Tellingen O, Zijlmans JM, Fibbe WE and Borst P, Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. *Proc Natl Acad Sci USA* 94: 4028–4033, 1997.
14. Borst P and Schinkel AH, What have we learnt thus far from mice with disrupted P-glycoprotein genes? *Eur J Cancer* 32A: 985–990, 1996.
15. Nooter K, Westerman AM, Flens MJ, Zaman GJR, Scheper RJ, van Wingerden KE, Burger H, Oostrum R, Boersma T, Sonneveld P, Gratama JW, Kok T, Eggermont AMM, Bosman FT and Stoter G, Expression of the multidrug resistance-associated protein (*MRP*) gene in human cancers. *Clin Cancer Res* **1**: 1301–1310, 1995.
16. Stride BD, Valdimarsson G, Gerlach JH, Wilson G, Cole SPC and Deeley RG, Structure and expression of the messenger RNA encoding the murine multidrug resistance protein, an ATP-binding cassette transporter. *Mol Pharmacol* **49:** 962–971, 1996.
17. Lorico A, Rappa G, Flavell RA and Sartorelli AC, Double knockout of the *MRP* gene leads to increased drug sensitivity *in vitro*. *Cancer Res* 56: 5351–5355, 1996.
18. Rappa G, Lorico A, Flavell RA and Sartorelli AC, Evidence that the multidrug resistance protein (MRP) functions as a co-transporter of glutathione and natural product toxins. *Cancer Res* 57: 5232–5237, 1997.
19. Lorico A, Rappa G, Finch RA, Yang D, Flavell RA and Sartorelli AC, Disruption of the murine *MRP* (multidrug resistance protein) gene leads to increased sensitivity to etoposide (VP-16) and increased levels of glutathione. *Cancer Res* 57: 5238–5242, 1997.
20. Wijnholds J, Evers R, van Leusden MR, Mol CA, Zaman GJ, Mayer U, Beijnen JH, van der Valk M, Krimpenfort P and Borst P, Increased sensitivity to anticancer drugs and decreased inflammatory response in mice lacking the multidrug resistance-associated protein. *Nat Med* **3:** 1275–1279, 1997.
21. Stride BD, Grant CE, Loe DW, Hipfner DR, Cole SPC and Deeley RG, Pharmacological characterization of the murine and human orthologs of multidrug-resistance protein in transfected human embryonic kidney cells. *Mol Pharmacol* 52: 344–353, 1997.
22. Zaman GJR, Flens MJ, van Leusden MR, de Haas M, Mülder HS, Lankelma J, Pinedo HM, Scheper RJ, Baas F, Broxterman HJ and Borst P, The human multidrug resistance-associated protein MRP is a plasma membrane drug-efflux pump. *Proc Natl Acad Sci USA* 91: 8822–8826, 1994.
23. Grant CE, Valdimarsson G, Hipfner DR, Almquist KC, Cole SPC and Deeley RG, Overexpression of multidrug resistance-associated protein (MRP) increases resistance to natural product drugs. *Cancer Res* 54: 357–361, 1994.
24. Breuninger LM, Paul S, Gaughan K, Miki T, Chan A, Aaronson SA and Kruh GD, Expression of multidrug resistance-associated protein in NIH/3T3 cells confers multidrug resistance associated with increased drug efflux and altered intracellular drug distribution. *Cancer Res* 55: 5342–5347, 1995.
25. Mortensen RM, Conner DA, Chao S, Geisterfer-Lowrance AAT and Seidman JG, Production of homozygous mutant ES

cells with a single targeting construct. *Mol Cell Biol* 12: 2391–2395, 1992.
26. Zaman GJR, Versantvoort CHM, Smit JJM, Eijdems EWHM, de Haas M, Smith AJ, Broxterman HJ, Mulder HS, de Vries EGE, Baas F and Borst P, Analysis of the expression of *MRP*, the gene for a new putative transmembrane drug transporter, in human multidrug resistant lung cancer cell lines. *Cancer Res* **53:** 1747–1750, 1993.
27. Barrand MA, Heppell-Parton AC, Wright KA, Rabbitts PH and Twentyman PR, A 190-kilodalton protein overexpressed in non-P-glycoprotein-containing multidrug-resistance cells and its relationship to the MRP gene. *J Natl Cancer Inst* **86:** 110–117, 1994.
28. Lorico A, Rappa G, Srimatkandada S, Catapano CV, Fernandes DJ, Germino JF and Sartorelli AC, Increased rate of adenosine triphosphate-dependent etoposide (VP-16) efflux in a murine leukemia cell line overexpressing the multidrug resistance-associated protein (*MRP*) gene. *Cancer Res* **55:** 4352–4360, 1995.
29. Cole SPC, Sparks KE, Fraser K, Loe DW, Grant CE, Wilson GM and Deeley RG, Pharmacological characterization of multidrug resistant MRP-transfected human tumor cells. *Cancer Res* **54:** 5902–5910, 1994.
30. Braman RS and Foreback CC, Methylated forms of arsenic in the environment. *Science* **182:** 1247–1249, 1973.
31. Tripathi N and Flora SJ, Effects of some thiol chelators on enzymatic activities in blood, liver and kidneys of acute arsenic (III) exposed mice. *Biomed Environ Sci* **11**: 38–45, 1998.
32. Saleh S and Khayyal MT, Effect of cysteine on the hepatic toxicity and antischistosomial activity of antimonyl potassium tartrate. *Bull World Health Organ* **53**: 379–384, 1976.
33. Jovov B, Ismailov II and Benos DJ, Cystic fibrosis transmembrane conductance regulator is required for protein kinase A activation of an outwardly rectified anion channel purified from bovine tracheal epithelia. *J Biol Chem* **270:** 1521–1528, 1995.
34. Gabriel SE, Clarke LL, Boucher RC and Stutts MJ, CFTR and outwardly rectifying chloride channels are distinct proteins with a regulatory relationship. *Nature* **363:** 263–266, 1993.
35. Hardy SP, Goodfellow HR, Valverde MA, Gill DR, Sepulveda FV and Higgins CF, Protein kinase C-mediated phosphorylation of the human multidrug resistance P-glycoprotein regulates cell volume-activated chloride channels. *EMBO J* **14:** 68–75, 1995.
36. Higgins CF, Volume-activated chloride currents associated with the multidrug resistance P-glycoprotein. *J Physiol (Lond)* **482:** 31S–36S, 1995.
37. Inagaki N, Gonoi T, Clement JP 4th, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S and Bryan J, Reconstitution of IKATP: An inward rectifier subunit plus the sulfonylurea receptor. *Science* **270:** 1166–1170, 1995.
38. Jirsch J, Deeley RG, Cole SPC, Stewart AJ and Fedida D, Inwardly rectifying K^+ channels and volume-regulated anion channels in multidrug-resistant small cell lung cancer cells. *Cancer Res* **53:** 4156–4160, 1993.
39. Leier I, Jedlitschky G, Buchholz U, Cole SPC, Deeley RG and Keppler D, The *MRP* gene encodes an ATP-dependent export pump for leukotriene C_4 and structurally related conjugates. *J Biol Chem* **269:** 27807–27810, 1994.
40. Müller M, Meijer C, Zaman GJR, Borst P, Scheper RJ, Mulder NH, de Vries EGE and Jansen PLM, Overexpression of the gene encoding the multidrug resistance-associated protein results in increased ATP-dependent glutathione *S*-conjugate transport. *Proc Natl Acad Sci USA* **91:** 13033–13037, 1994.
41. Loe DW, Almquist KC, Cole SPC and Deeley RG, ATP-dependent 17β-estradiol 17-(β-D-glucuronide) transport by multidrug resistance protein (MRP). Inhibition by cholestatic steroids. *J Biol Chem* **271:** 9683–9689, 1996.
42. Loe DW, Almquist KC, Deeley RG and Cole SPC, Multidrug resistance protein (MRP)-mediated transport of leukotriene C_4 and chemotherapeutic agents in membrane vesicles. Demonstration of glutathione-dependent vincristine transport. *J Biol Chem* **271:** 9675–9682, 1996.
43. Jedlitschky G, Leier I, Buchholz U, Barnouin K, Kurz G and Keppler D, Transport of glutathione, glucuronate, and sulfate conjugates by the *MRP* gene-encoded conjugate export pump. *Cancer Res* **56:** 988–994, 1996.
44. Evers R, Cnubben NH, Wijnholds J, van Deemter L, van Bladeren PJ and Borst P, Transport of glutathione prostaglandin A conjugates by the multidrug resistance protein 1. *FEBS Lett* **419**: 112–116, 1997.
45. Leier I, Jedlitschky G, Buchholz U, Center M, Cole SPC, Deeley RG and Keppler D, ATP-dependent glutathione disulphide transport mediated by the *MRP* gene-encoded conjugate export pump. *Biochem J* **314**: 433–437, 1996.
46. Ishikawa T, Li ZS, Lu YP and Rea PA, The GS-X pump in plant, yeast and animal cells: Structure, function, and gene expression. *Biosci Rep* **17:** 189–207, 1997.
47. Keppler D, Konig J and Buchler M, The canalicular multidrug resistance protein, cMRP/MRP2, a novel conjugate export pump expressed in the apical membrane of hepatocytes. *Adv Enzyme Regul* **37**: 321–333, 1997.
48. Kool M, de Haas M, Scheffer GL, Scheper RJ, van Eijk MJ, Juijn JA, Baas F and Borst P, Analysis of expression of *cMOAT* (*MRP2*), *MRP3*, *MRP4*, and *MRP5*, homologues of the multidrug resistance-associated protein gene (*MRP1*), in human cancer cell lines. *Cancer Res* **57**: 3537–3547, 1997.
49. Puchalski RB and Fahl WE, Expression of recombinant glutathione *S*-transferase π, Ya, or Yb_1 confers resistance to alkylating agents. *Proc Natl Acad Sci USA* **87:** 2443–2447, 1990.
50. Ishikawa T, The ATP-dependent glutathione S-conjugate export pump, *Trends Biochem Sci* **17:** 463–468, 1992.
51. Loe DW, Stewart RK, Massey TE, Deeley RG and Cole SPC, ATP-dependent transport of aflatoxin B_1 and its glutathione conjugates by the product of the multidrug resistance protein (*MRP*) gene. *Mol Pharmacol* **51**: 1034–1041, 1997.
52. Versantvoort CH, Broxterman HJ, Bagrij T, Scheper RJ and Twentyman PR, Regulation by glutathione of drug transport in multidrug-resistant human lung tumour cell lines overexpressing multidrug resistance-associated protein. *Br J Cancer* **72:** 82–89, 1995.
53. Zaman GJR, Lankelma J, van Tellingen O, Beijnen J, Dekker H, Paulusma C, Oude Elferink RPJ, Baas F and Borst P, Role of glutathione in the export of compounds from cells by the multidrug-resistance-associated protein. *Proc Natl Acad Sci USA* **92:** 7690–7694, 1995.
54. Meister A, Methods for the selective modification of glutathione metabolism and study of glutathione transport. *Methods Enzymol* **113**: 571–585, 1985.
55. Kaplowitz N, Fernandez-Checa JC, Kannan R, Garcia-Ruiz C, Ookhtens M and Yi JR, GSH transporters: Molecular characterization and role in GSH homeostasis. *Biol Chem Hoppe Seyler* **377:** 267–273, 1996.
56. Vanhoefer U, Cao S, Minderman H, Toth K, Skenderis BS, Slovak ML and Rustum YM, D,L-Buthionine-(*S*,*R*)-sulfoximine potentiates *in vivo* the therapeutic efficacy of doxorubicin against multidrug resistance protein-expressing tumors. *Clin Cancer Res* **2:** 1961–1968, 1996.
57. Taguchi Y, Yoshida A, Takada Y, Komano T and Ueda K, Anti-cancer drugs and glutathione stimulate vanadate-induced trapping of nucleotide in multidrug resistance-associated protein (MRP). *FEBS Lett* **401:** 11–14, 1997.
58. Kuo MT, Bao J, Furuichi M, Yamane Y, Gomi A, Savaraj N, Masuzawa T and Ishikawa T, Frequent coexpression of MRP/GS-X pump and γ-glutamylcysteine synthetase mRNA in drug-resistant cells, untreated tumor cells, and normal mouse tissues. *Biochem Pharmacol* **55:** 605–615, 1998.