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Review Article

Theme: Transporters and Cancer Therapy
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Multidrug Resistance Proteins (MRPs) and Cancer Therapy

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Abstract. The ATP-binding cassette (ABC) transporters are members of a protein superfamily that are known to translocate various substrates across membranes, including metabolic products, lipids and sterols, and xenobiotic drugs. Multidrug resistance proteins (MRPs) belong to the subfamily C in the ABC transporter superfamily. MRPs have been implicated in mediating multidrug resistance by actively extruding chemotherapeutic substrates. Moreover, some MRPs are known to be essential in physiological excretory or regulatory pathways. The importance of MRPs in cancer therapy is also implied by their clinical insights. Modulating the function of MRPs to re-sensitize chemotherapeutic agents in cancer therapy shows great promise in cancer therapy; thus, multiple MRP inhibitors have been developed recently. This review article summarizes the structure, distribution, and physiological as well as pharmacological function of MRP1–MRP9 in cancer chemotherapy. Several novel modulators targeting MRPs in cancer therapy are also discussed.

KEY WORDS: ABC transporters; cancer chemotherapy; multidrug resistance; multidrug resistance proteins.

INTRODUCTION

ATP-binding cassette (ABC) transporters utilize the energy from ATP hydrolysis to transport substrates across the membranes. Genetic studies have divided the ABC transporter superfamily into seven subfamilies based on sequence similarities (1). Nine human multidrug resistance proteins (MRPs) are generally members of subfamily C in the ABC superfamily. Similar to other members belonging to the ABC superfamily, MRPs share several common structural features including multiple transmembrane (TM) α helices arranged in membrane-spanning domains (MSDs), as well as nucleotide-binding domains (NBDs) at the intracellular site for ATP binding and hydrolysis (2). A four-domain arrangement of two MSDs and two NBDs is commonly observed in the ABC superfamily and also in MRP4, 5, 8, and 9 (named as "short MRPs"). However, MRP1, 2, 3, 6, and 7 have an additional NH_2-proximal MSD0 and therefore named as "long MRPs" (3) (Fig. 1).

Though structurally different, nine MRPs share a similar ATP-driven transport mechanism (2,4). A wide range of endo- and xenobiotics can be transported by MRPs and different MRPs may have similar substrate specificity (4). Although most MRPs are initially cloned from tumors, they are also broadly found in normal tissues. In humans, functions of MRPs may range from tissue defense to resistance of cancer chemotherapy based on different locations, expression levels, and activities (5) (Table I). Understanding and modulating MRPs would be favorable in cancer therapy. In this review, the structure, function, and clinical insights of MRPs and recent MRP modulators in cancer therapy will be discussed (Tables I and II).

STRUCTURE AND FUNCTION OF MRPS

MRP1

Multidrug resistant protein 1 (MRP1, length 1531) was first cloned from doxorubicin-selected lung cancer cell line H69AR. The low-resolution crystal structure (22 Å) suggested that MRP1 contained two MSDs and two NBDs as well as a third MSD with an extra-cytosolic NH_2 terminus (6). In the absence of high-resolution structural data, multiple indirect attempts had been performed in order to elucidate the transport mechanism. Homology model of four domains of human MRP1 was built based on Sav1866 (Fig. 2a) from *Staphylococcus aureus*, and residue Tyr324 showed great functional importance in this model (7). Moreover, multiple residues, which play roles in determining substrates specificity and selectivity, were located in MSD1 and MSD2 by mutagenesis studies, photo-labeling studies, structure–activity relationship (SAR) studies or *in silico* attempts (8,9). Besides MSDs, it has been previously illustrated that the seventh cytoplasmic loop (CL7) of human MRP1 is responsible for mediating signaling between the NBDs and substrate-binding sites in MSDs (10). Recent mutation-based study suggests that four charged residue (Lys513, Lys516, Glu521, and

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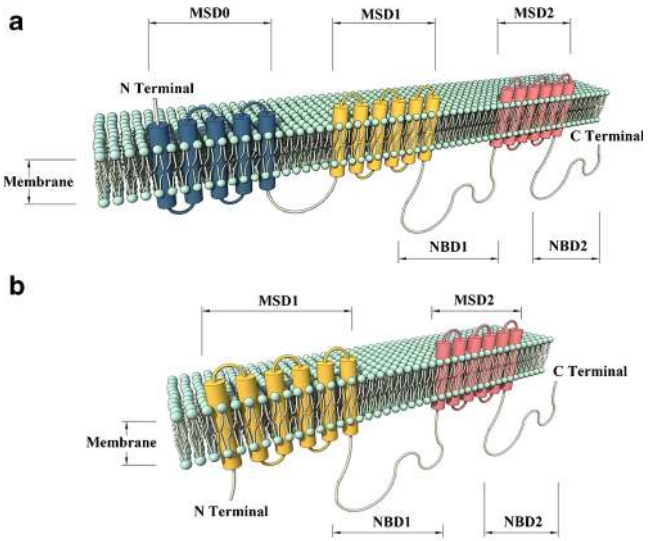

Fig. 1. Schematic view of the predicted topology of long and short MRPs. Protein backbone is illustrated as thin tubes while transmembrane helices are shown as cylinders. The *upper panel* is a schematic of the predicted topology of the MSDs and NBDs of long MRPs (MRP1, 2, 3, 6, 7). The N-terminal of MRP2 has also been shown to be extracellular by some *in silico* prediction algorithms. The *lower panel* shows a carton of the predicted arrangement of short MRPs (MRP4, 5, 8, 9). The *coloring* of various regions of the protein is consistent between *upper and lower panels*

Glu535) in the fifth cytoplasmic loop (CL5) participate in important inter-domain interactions that are crucial for proper folding and assembly of MRP1 (9). However, the exact mechanism of MRP1 still remains unclear.

Physiologically, MRP1 mediated the active efflux of a broad range of glucuronide, glutathione, and sulfate conjugates (5). Consistent with this transport preference, there is a strong correlation between substrates topological polar surface area (TPSA) and MRP1 transport properties (24). Generally, MRP1 is mainly found to be expressed in physiology barriers such as blood–brain barrier and may limit drug uptake and retention (25). According to its broad substrate range and ubiquitous tissue distribution, MRP1 is now believed to serve a broader role in etiology. MRP1 may be involved in many physiological and pathophysiological processes, including inflammatory responses and oxidative stress defense (26). Physiological substrates of MRP1 also includes cobalamin (vitamin B12), which is confirmed by vesicular transport experimentation and gene silencing studies (27).

In cancer cells, MRP1 can mediate resistance to a wide variety of anticancer drugs, including doxorubicin, methotrexate (MTX), vincristine, and etoposide (28–30). Besides the known MRP1 inhibitor MK-571, new approaches against MRP1 are discussed in Section 4.

MRP2/cMOAT

MRP2 was first identified and cloned from rat liver cells as homologue of MRP1 and then named hepatocellular canalicular multiple organic anion transporter (cMOAT). Similarly with MRP1 as a long MRP, MRP2 has three MSD and two NBD characteristics. Basic residues in TM helices especially in TM6, TM9, TM16, and TM17 are proven to be essential for substrates recognition and binding by mutation-based methods (31). Moreover, transport pattern of MRP2 substrates revealed that the substrate translocation binding site of MRP2 can be allosterically altered by another site outside the substrate translocation site (32).

MRP2 is expressed in various tissues including hepatocytes, kidney proximal tubules, intestine, nerves, bladder, placenta, and $CD4^+$ lymphocytes. *MRP2* knockdown animal models were used to illustrate the physiological roles of MRP2. $MRP2^{-/-}$ mice showed decreased hepatobiliary excretion of drugs and toxins as well as their metabolites (33). Also, in MRP2-deficient rats, biliary excretion of

Table I. Classification, Subcellular Localization, and Substrate Specificity of the MRPs

Name	Amino acid sequences length	Classification	Subcellular localization	Substrates Specificity
MRP1	1531	Long MRP	Basolateral	GSH conjugates, glucuronide conjugates, sulfate conjugates, anthracyclines, epipodophyliotoxins, vinca alkaloids, folic acid, and MTX
MRP2	1545	Long MRP	Apical, basolateral	GSH conjugates, glucuronide conjugates, anthracyclines, epipodophyliotoxins, vinca alkaloids, cisplatin, and MTX
MRP3	1527	Long MRP	Basolateral	Glucuronide conjugates, MTX, etoposide, and teniposide
MRP4	1325	Short MRP	Apical, basolateral	Nucleotide analogues, glucuronide conjugates, MTX, and bile acids
MRP5	1437	Short MRP	Basolateral	Nucleotide/nucleoside analogues and GSH conjugates
MRP6	1503	Long MRP	Basolateral	GSH conjugates, anthracyclines, epipodophyliotoxins, and cisplatin
MRP7	1492	Long MRP	?	Glucuronide conjugates, GSH conjugates, anthracycline, *vinca* alkaloids, cisplatin, and taxanes
MRP8	1382	Short MRP	Apical, basolateral	Glucuronides, bile acids, steroid sulfates, 5-FU, and MTX
MRP9	1356	Short MRP	?	Unknown

The presence of both locations indicates cell type specific differences in subcellular localization; the question mark (?) indicates that the subcellular localization is not known
GSH glutathione, *MTX* methotrexate *MRP* multidrug resistance protein

Table II. Summary of Novel Modulators Against MRPs

Name	Target	Drug classification	Effects on target MRPs		Mechanism of inhibition on target MRPs		
			In vitro		*In vivo*	Transcription	
Expression	Function						
Ibrutinib	MRP1	Bruton's tyrosine kinase inhibitor	+(11)	+(11)	↔[a]	↔	↓[b]
Nilotinib	MRP7	BCR-Abl inhibitor	+(12)	+(12)	N/A	↔	↓
Imatinib	MRP7	BCR-Abl inhibitor	+(12)	N/A	N/A	↔	↓
Erlotinib	MRP7	EGFR and HER2 inhibitor	+(13,14)	N/A	N/A	↔	↓
Lapatinib	MRP7	EGFR inhibitor	+(14)	N/A	N/A	↔	↓
Tandutinib	MRP7	FLT3 inhibitor	+(15)	N/A	N/A	↔	↓
Masitinib	MRP7	c-Kit inhibitor, PDGFR inhibitor	+(16)	+(16)	N/A	↔	↓
Sildenafil and vardenafil	MRP7	PDE5 inhibitor	+(17)	N/A	N/A	↔	↓
Tariquidar	MRP7	P-gp (ABCB1) inhibitor	+(18)	N/A	↔	↓ (>24 h treatment)	↓
NVP-BHG712	MRP7	EphB4 inhibitor	+(19)	+(19)	N/A	↔	↓
GSH-conjugated catechol metabolite	MRP1 and MRP2	GSH-conjugated catechol metabolites	+(20)	N/A	N/A	N/A	↓
3ATA	MRP1-4, preferred selectivity for MRP1	Triterpene	+(21)	N/A	N/A	N/A	↓
miR-326	MRP1	miRNA	+(22)	N/A	↓	↓	↓
miR-297	MRP2	miRNA	+(23)	+(23)	↓	↓	↓

N/A study not conducted, *MRP* multidrug resistance proteins, *EGFR* epidermal growth factor receptor, *PDGFR* platelet-derived growth factor, *PDE5* phosphodiesterase type 5, *GSH* glutathione
(+): this compound shows reversal activity on the targeted MRPs, superscript number showing the relevant reference
[a] (↔): no significant alterations
[b] (↓) downregulation or inhibition

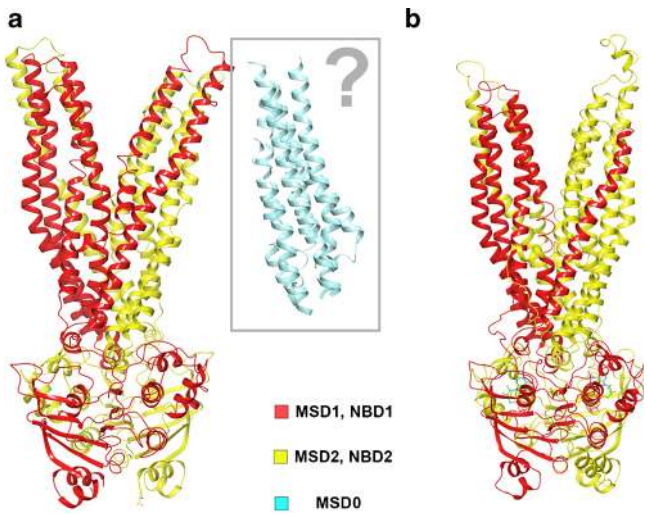

Fig. 2. Three-dimensional homology model of MRP1 and MRP4. Protein structures are shown in α-carbon backbone ribbon representation, as viewed from the plane perpendicular to the membrane. MSD1-NBD1 region is colored as *red*, MSD2-NBD2 region is colored as *yellow*. *Panel* ***a*** is the homology model of the second and third MSDs and the two NBDs of MRP1 based on Sav1866 by DeGorter *et al.* (7). The five TM helices of MSD0 are depicted in *light green*. However, the tertiary structure of MSD0 and the manner in which the three MSDs are arranged is unknown. *Panel* ***b*** is the homology model of MRP4 provided by Dr. Koenderink

glucuronide conjugates of sets of xenobiotics such as acetaminophen, were significantly eliminated as compared to normal rats (34). These studies suggest that MRP2 may play a role in eliminating endogenous and xenobiotic metabolites. Previously, deficiency of MRP2 has been related to Dubin–Johnson syndrome. Additionally, numerous studies recently suggested that SNPs greatly influence the function of MRP2, such as substrate effluxing and miRNA recognizing (35).

In cancerous cell lines, MRP2 messenger RNA (mRNA) levels have been related to resistance to vincristine, cisplatin, and doxorubicin. Vesicle transport assays also suggested that substrates of MRP2 include vincristine, cisplatin, irinotecan, paclitaxel, and MTX (36).

MRP3

MRP3 shares the similar topology structure as MRP1. In humans, the expression of MRP3 was found on the basolateral membrane of hepatocytes and cholangiocytes (37,38). Compensatory increase of MRP3 mRNA can be observed in MRP2-deficient rats (EHBR) as well as in normal rats with ligated bile ducts, indicating that there may be an overlap between MRP2 and MRP3 excretory functions (39). More details about the function of MRP3 as a basolateral excretory system were studied by the *MRP3*$^{-/-}$ mice model. These mice showed increased concentration of glucuronide

conjugates of bile acids in liver and bile, and also simultaneously decreased serum concentration (40).

Over expression of MRP3 has been detected in human hepatocellular carcinomas, primary ovarian cancer, adult acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) (41–44). Moreover, MRP3 has been reported to be induced through the progression of various forms of cancer, and recently the functional Nrf2 response element (involved in MRP3 induction) was identified within *MRP3* gene (45). Substrate drugs of MRP3 in cancer chemotherapy includes etoposide, MTX and teniposide (46).

MRP4/MOAT-B

MRP4 (MOAT-B) is a lipophilic anion efflux pump that is able to confer resistance to broad range of substrates including nucleotide analogues, MTX, and glutathione (GSH) (47). This 1325 amino acids ABC transporter was predicted to contain only two MSDs and two NBDs (Fig. 2b). Categorized as a short MRP, MRP4 has similar membrane topologies as MRP5, 8, and 9. MRP4 may be involved in physiological regulation pathways through transport of cyclic nucleotide and nucleotide analogues. For example, enhanced cyclic adenosine monophosphate (cAMP) level may upregulate MRP4 expression and result in increased cAMP efflux. MRP4 was found at the basolateral site of prostate membrane, as well as the apical membrane of renal proximal tubules (48,49). The tissue specific localization may suggest the involvement of MRP4 in multiple functions. Basolateral location of MRP4 indicated its protective role as *MRP4*$^{-/-}$ mice showed increased cholestatic injury (50). Similarly, MRP4 deficient mice showed increased accumulation of topotecan (substrate of MRP4) in brain, also suggesting the protective role of MRP4 as an efflux pump (51). Moreover, in MRP2-deficient mice, compensated upregulation of MRP4 was observed in apical membrane of renal proximal tubules, suggesting its excretory role as an anion pump (52).

In cancer chemotherapy, MRP4 may cause treatment failure by effluxing anionic phosphate metabolites of chemotherapy agents. Recent evidences also suggested that overexpression of MRP4 in lung cancer may be related to COX-2 upregulation *via* PGE2-dependent pathway (53).

MRP5

MRP5 was first identified as homologues of MRP1 by database screening of expressed sequence tags. MRP5 was predicted to be a short MRP, lacking the additional MSD0. The detailed structural knowledge of MRP5 is limited in lack of x-ray structure and only the homology model is currently available. MRP5 was identified to transport cAMP, cyclic guanosine monophosphate (cGMP), and antiretroviral compound PMEA (54). Therefore, MRP5 may also be involved in the regulation of physiological pathways (55). MRP5 was able to confer some resistance against mercaptopurines by actively transporting thiopurine metabolites (56). However the affinity of MRP5 for nucleotide-based substrate was suggested to be low (57). Further results also recognize MRP5 as a folate exporter (58). Expression of MRP5 was detected broadly in human tissues such as liver, placenta, and cornea, and also in carcinomas. MRP5 expression level was also associated with exposure to cisplatin (59). Several *in vitro* studies suggested that MRP5 would transport several anticancer drugs including cisplatin, MTX, purine analogues, and pyrimidine analogues (56,60,61). However, the exact role of MRP5 in mediating resistance still needs to be elucidated.

MRP6

MRP6 gene is located next to *MRP1* gene on chromosome 16, and encodes a 1503 amino acids protein (62). MRP6 presents three MSDs and two NBDs as other long MRPs; however, it is structurally poorly characterized. Only two NBDs structures of MRP6 were characterized by NMR or CD spectra (63). As predicted, these two NBDs have cooperative function in binding and hydrolyzing ATP for the transmembrane transport. MRP6 was first found to be highly expressed in liver and kidney in a co-amplified pattern with MRP1 (62). Furthermore, immunohistochemistry studies have confirmed that MRP6 has widespread tissue distribution in various epithelial cells of exocrine and endocrine tissues, such as acinar cells in the pancreas, mucosal cells of intestine, and follicular epithelial cells of the thyroid, as well as at the lateral and canalicular plasma membrane of hepatocytes (64,65). Previous mutation studies had linked *MRP6* gene mutations or MRP6 deficiency to pseudoxanthoma elasticum (PXE), which is characterized by the deposition of aberrant elastic fibers (66,67). Furthermore, membrane vesicles transport studies illustrated that MRP6 is a glutathione conjugate anion pump and may bring low levels of resistance to certain anticancer agents (68). However, so far, there are no further evidences supporting the role of MRP6 in multidrug resistance (MDR).

MRP7

MRP7 was firstly identified and nominated through complementary DNA (cDNA) library search in 2001 (69). Structure analysis of this 1492 amino acids sequence showed that the TM helices in MRP7 are arranged into three MSDs (69). Specialty of MRP7 included its low sequence identity compared to other members and its absence of N-linked glycosylation in the N-terminus (69).

MRP7 gene expression has been proven to be in pancreas, colon, skin, and testes (69,70). Membrane vesicles illustrated that glucuronide conjugates such as $E_2 17\beta G$ and GSH conjugates such as LTC_4 can be transported by MRP7 (71). Besides physiological substrates, MRP7 had been greatly linked to MDR. MRP7-transfected HEK293 cells are used to determine drug resistance profile and the results show MRP7 may exhibit resistance towards docetaxel, paclitaxel, vincristine, vinorelbine, and vinblastine as a lipophilic anion pump (72,73). SiRNA study and *in vivo MRP7* knockout null mouse model also confirmed that MRP7 conferred paclitaxel resistance (74). Cepharanthine is the earliest discovered MRP7 inhibitor (75), and more recent approaches towards MRP7 inhibition will be discussed in section 4.

MRP8

Identified in 2003, MRP8 is an amphipathic anion transporter that is functional to efflux purine and pyrimidine

nucleotide analogs including cAMP and cGMP (76). MRP8 was predicted to be a short MRP with 12 TM helices. In the absence of MRP8 crystal structure, two putative binding sites were predicted inside homology model of MRP8. Unlike the mechanism of MRP2, MD study predicted that substrates of MRP8 may first bind intracellularly, then pass through the second site to release along with conformational changes of MRP8 (77).

MRP8 is also located on the axons of human CNS and PNS (78). Membrane vesicle studies determine that MRP8 might be involved in physiological processes related to bile acids, steroid sulfates, and glucuronides (79). Physiologically, MRP8 is associated with axillary odor and secretion of cerumen (earwax) (80). There has been an association between breast cancer risk and wet earwax observed in Japanese women (81). However, the effect of MRP8 genotypes on breast cancer is likely to be more complex since there are studies showing that the association could not be observed in Caucasian (82). Although there is a report showing a decrease in MRP8 level in breast cancer (83), high level of MRP8 was reported in breast cancer and gastric cancer cell lines by other groups (84,85). Other substrates of MRP8 include MTX (79), 5-FU, and pemetrexed (86,87). Further studies are needed to eliminate the controversy of MRP8's role in clinical MDR.

MRP9

MRP9 gene is cloned, identified, and mapped on the human chromosome 16q12.1 in a tail-to-head orientation (88). Transcripts of *MRP9* gene are detected in various tissues, including liver, lung, kidney, and fetal tissues (88). Multiple transcripts of *MRP9* existed and encoded different sized proteins (89). Identified variants of MRP9 showed unusual fragmentary topology as compared to other ABC family members, which may indicate different function of MRP9 (89). However, until now, it is still uncertain that substrates of MRP9 can be demonstrated and therefore the functional characteristics of MRP9 are currently unknown.

CLINICAL STATUSES OF MRPS IN CANCER THERAPY

Recently, Akimitsu *et al.* showed that in the aggressive breast carcinoma subtypes, there is an overexpression of MRP1 and MRP8 (85). In another study, it has been shown that MRP1, MRP2, and MRP3 are expressed in lung cancer patients, with differences in the lung cancer subtypes (90). A higher expression of MRP1, MRP2, and MRP3 was found in non-small cell lung cancer (NSCLC) than in small cell lung cancer (SCLC) cell lines, with MRP3 mRNA levels being the highest, suggesting its specificity towards developing resistance in NSCLC (90). Furthermore, an overexpression of MRP1 and MRP3 is responsible for decrease in drug sensitivity towards vincristine, etoposide, doxorubicin, and cisplatin in lung cancer patients (90).

For colorectal carcinoma, an involvement of various MRPs has been shown (91). Levels of MRP1 and MRP2 are found to be higher in patients with colorectal tumors as compared to the control group (91). In patients not responding to chemotherapy, there was a higher expression of MRP6 and MRP8 in contrast to the responders (91). Also, MRP8 was established as a predictive biomarker for the effectiveness of 5-FU therapy as it was found to be overexpressed in colorectal tumors prior to 5-FU therapy (91). Northern blotting and RT-PCR analysis showed the presence of MRP1 in prostate cancer cell lines resistant to doxorubicin thus confirming that MRP1 expression may lead to resistance in patients with prostate cancer (92). MRP1 mRNA overexpression has also been reported and confirmed by immunohistochemistry in pancreatic carcinoma cell lines and pancreatic tumor sections (93). An association of MRP1 levels with poor chemotherapeutic response in patients with ALL has also been shown although no significant effect of MRP1 levels in AML therapy has been reported (94). Immunohistochemistry studies have confirmed a significant higher expression of MRP1 in renal cell carcinoma patients (95). Studies conducted on the role of MRP1 in neuroblastoma reveal that MRP1 expression is responsible for decreased prognosis and poor patient survival (96). Moreover, a recent *ex vivo* study also demonstrated that MRP7 was highly expressed in $Her2^+$ and ER^+ breast cancers and relationship with MRP7 and docetaxel-treatment failure was confirmed (97). Inhibition of MRP7 was suggested to have clinical potential to reverse multidrug resistance in chemotherapy.

Overall, although the role of some MRPs is not fully understood, the importance and the influence of MRPs on the strategy and outcome of clinical cancer treatment cannot be neglected. Therefore, developing modulators to block drug efflux function of MRPs is a viable strategy especially for the patients selected with high expression level of MRPs.

RECENT APPROACHES FOR MRP MODULATORS IN CANCER THERAPY

In recent years, multiple attempts have been performed in order to reverse MRP-mediated MDR. Off-target small molecular inhibitors, especially tyrosine kinase inhibitors, derivatives of endogenous or natural products, and miRNA-based therapy are three main research focuses in developing novel MRPs modulators. These potential modulators will be reviewed in this section (Table II).

Off-Target Small Molecular Inhibitors as Modulators of MRPs

Ibrutinib as Modulator of MRP1

Bruton's tyrosine kinase (BTK) is a fundamental cytoplasmic protein tyrosine kinase in B cell receptor signaling pathway. In addition, ibrutinib received approval for the cancer therapy of chronic lymphocytic leukemia (CLL) or mantle cell lymphoma by the US FDA. It has been found that ibrutinib (1 and 5 μM) could significantly sensitize HL60/Adr leukemia cells and transfected HEK293/MRP1 to the substrates of MRP1. This reversal activity of ibrutinib was stronger than that of MK-571 as positive control (11). It was also reported that ibrutinib potently blocked the efflux of [^{3}H]-vinblastine in HEK293/MRP1 cells and doxorubicin in HL60/Adr cells, increasing intracellular [^{3}H]-vinblastine accumulation. However, 5 μM ibrutinib (72 h) did not significantly alter the expression levels of mRNA or protein

of MRP1 (11). Interestingly, *in vivo* xenograft MDR model suggested that the mice group with coadministration of ibrutinib (30 mg/kg p.o.) and vincristine (0.4 mg/kg i.p.) showed a great inhibitory effect on the tumor growth, as compared to mice administered with vehicle, vincristine, or ibrutinib alone. Also, there were no significant visible toxicities or phenotypic changes between different treatment groups (11). Moreover, ibrutinib (5 μM) can strengthen the effect of vincristine to the leukemia blast cells in three patient samples, which exhibited detectable levels of MRP1 expression (11). Collectively, ibrutinib could increase the antitumor activity of MRP1 substrate anticancer drugs in MRP1 overexpressing cells *in vitro*, *in vivo*, and *ex vivo*. These studies revealed high clinical values for the coadministration of ibrutinib and MRP1 substrate chemotherapeutical agents in cancer patients.

Nilotinib, Imatinib, Erlotinib, Lapatinib, and Tandutinib as Modulators of MRP7

Nilotinib, imatinib, erlotinib, lapatinib, and tandutinib are inhibitors for different receptors in tyrosine kinase family. In recent years, it has been reported that these modulators could reverse MRP7-mediated MDR in transfected HEK/MRP7 cells (12–15,98,99). Further mechanism studies (radiolabeled accumulation and efflux assays, immunoblotting assay) revealed that these drugs could reverse MRP7-mediated MDR *via* blocking the drug efflux function of MRP7 (12–15,98,99). If all these findings could be clinically translated, these potential inhibitors could be used in combination with paclitaxel or other MRP7 substrates to overcome MRP7-mediated MDR in certain types of carcinomas.

Masitinib as Modulator of MRP7

Masitinib, a novel phenyl-aminothiazole derivative, is an inhibitor of the class III receptor tyrosine kinase stem-cell growth factor receptor (c-Kit), platelet-derived growth factor receptor α and β, and the nonreceptor tyrosine kinase Lyn (100). Masitinib, at 0.625, 1.25, and 2.5 μM, significantly reduced the drug resistance of paclitaxel, docetaxel, vincristine, and vinblastine to HEK293/MRP7 cell line, as compared to the HEK293/pcDNA3.1 cell line. The treatment of HEK293/MRP7 cells with masitinib (2.5 μM) greatly enhanced the accumulation of BODIPY-paclitaxel in MRP7 overexpressing cells, as compared to HEK293/pcDNA3.1 cells (16). Moreover, masitinib induced an obvious concentration-dependent enhancement in the intracellular accumulation of [^{3}H]-paclitaxel in HEK293/MRP7 as compared to the controls. Through a period of time (0, 30, 60, 120 min), masitinib (2.5 μM) greatly inhibited the efflux of [^{3}H]-paclitaxel in HEK293/MRP7 cells. In addition, no significant change was observed in the expression levels of MRP7, c-Kit and p-c-Kit when HEK293/MRP7 cells were exposed to masitinib for 72 h (16).

Interestingly, the combination of masitinib (12.5 mg/kg, p.o.) and paclitaxel (15 mg/kg, i.p.) greatly reduced the sizes, weights, and tumor volumes of the tumor expressing MRP7 transporter during a period of 18 days, as compared to animals treated with vehicle, masitinib, or paclitaxel alone. The pharmacokinetic data showed that coadministration of masitinib and paclitaxel produced a transient increase in the plasma levels of paclitaxel after 10 min of administration, but not at subsequent time points. The combination of masitinib and paclitaxel significantly increased the intratumoral concentration of paclitaxel (69.93±14.15 ng/mL) as compared with paclitaxel administration alone (16.31±6.45 ng/mL, $p<0.05$) after 240 min of administration. Therefore, the combination of masitinib and paclitaxel has the potential to be a novel therapy to circumvent the MRP7-mediated MDR (16).

PDE5 Inhibitors, Sildenafil, and Vardenafil, as Modulators of MRP7

Sildenafil and vardenafil are cGMP-specific phosphodiesterase type 5 (PDE5) competitive inhibitors that can prevent cGMP degradation. They are widely used in the treatment of male erectile dysfunction and pulmonary hypertension. Sildenafil and vardenafil concentration dependently decreased the IC_{50} values of different MRP7 substrates in HEK293/MRP7 cells. Sildenafil or vardenafil (5 μM) greatly enhanced the intracellular accumulation of [^{3}H]-paclitaxel in HEK293/MRP7 cells, which is comparable to cepharanthine as a known MRP7 inhibitor (17). Furthermore, sildenafil or vardenafil significantly inhibit the efflux of intracellular [^{3}H]-paclitaxel from HEK293/MRP7. Additionally, Western blot and immunofluorescence assay indicated sildenafil and vardenafil did not significantly change the protein expression and translocation of MRP7 (17). Collectively, these PDE5 inhibitors could serve as a new and promising adjuvant chemotherapeutic drugs in clinical practice.

Tariquidar as Modulator of MRP7

Tariquidar is a third-generation P-gp inhibitor with high affinity, low toxicity, and enhanced selectivity. Tariquidar (0.1, 0.3 μM) greatly reduced the IC_{50} values of paclitaxel, docetaxel, vincristine, and vinblastine. Immunoblotting revealed that incubation of HEK293/MRP7 cells with tariquidar for more than 24 h greatly decreased the MRP7 protein expression in a time- and concentration-dependent manner. However, mRNA levels of MRP7 did not significantly alter in the treatment of tariquidar for more than 72 h (18). Thus, tariquidar may downregulate MRP7 expression at the post-transcriptional level. Tariquidar significantly increased the intracellular [^{3}H]-paclitaxel accumulation in HEK293/MRP7 cells. Similarly, the intracellular accumulation of BODIPY-paclitaxel was greatly increased after the treatment of tariquidar for 72 h. Moreover, tariquidar was able to reduce the efflux of paclitaxel by MRP7, which induced higher intracellular accumulation of paclitaxel (18). Hence, tariquidar may have incredible potential as an anticancer drug in the clinical chemotherapy of MRP7-resistant cancer patients.

NVP-BHG712 as Modulator of MRP7

NVP-BHG712 is a specific EphB4 receptor (receptor cloned from erythropoietin-producing hepatocellular

carcinoma) inhibitor that blocks vascular endothelial growth factor mediated angiogenesis *in vivo*. NVP-BHG712 (0.25, 0.5 μM) significantly sensitized HEK293/MRP7 cells to paclitaxel, docetaxel and vinblastine in comparison with the control HEK293/pcDNA3.1 cells *in vitro*. Importantly, the combination of NVP-BHG712 (25 mg/kg, p.o.) greatly reduced the sizes and weights of mice xenograft tumors that expressed MRP7 during a period of 18 days, in comparison with groups administered with vehicle, NVP-BHG712, or paclitaxel alone. In the meantime, no significant weight loss or phenotypic changes were observed in any groups (19). Furthermore, the pharmacokinetic studies in tumor-carrying nude mice model indicated that combination of NVP-BHG712 and paclitaxel significantly increased the intratumoral concentration of paclitaxel (434.58±124.49 ng/mL) as compared to paclitaxel administration alone (160.13 ±41.12 ng/mL, $p<0.01$). And the combination of NVP-BHG712 and paclitaxel had no significant effect on plasma levels of paclitaxel (19). Hence, the combination of NVP-BHG712 and paclitaxel would become a novel and effective therapy to overcome the MRP7-mediated MDR.

Derivatives of Endogenous or Natural Products as Modulators for MRPs

Glutathione-Conjugated Catechol Metabolites as Modulators of MRP1 and MRP2

In order to determine if some structurally distinct GSH-conjugated catechol metabolites are inhibitors of MRP1 and MRP2, one study investigated the ATP-dependent vesicular transport of LTC_4 and $E_2$17βG mediated by the MRP1 and MRP2 transporters in the presence of six potential modulators from three different classes of GSH-conjugated catechol metabolites: the ecstasy metabolite 5-(glutathion-*S*-yl)-*N*-methyl-α-methyldopamine (5-GS-*N*-Me-α-MeDA), the caffeic acid metabolite 2-(glutathion-*S*-yl)-caffeic acid (2-GS-CA), and four GSH conjugates of 2-hydroxy (OH) and 4-OH estrogens (GS estrogens) (20). The methylenedioxymethamphetamine (MDMA) and caffeic acid (CA) inhibited both $E_2$17βG and LTC_4 uptake by MRP1 in a concentration-dependent manner with IC_{50} values ranging from 3 to 137 μM. Moreover, four GS estrogens could inhibit both $E_2$17βG and LTC_4 uptake by MRP1 in a concentration-dependent manner with IC_{50} values less than 2 μM. Similarly, the MDMA and CA metabolites inhibit MRP2-mediated $E_2$17βG uptake in a concentration-dependent fashion, with IC_{50} values ranging from 10 to 145 μM. Furthermore, the 2-OH-1-GS-E_2 and 2-OH-1-GS-E_1 metabolites potently inhibited $E_2$17βG uptake by MRP2 with IC_{50} values of 2.1 and 1.6 μM, respectively, whereas the 2-OH-4-GS-E_2 and 4-OH-2-GS-E_2 conjugates were approximately 50- and 300-fold less potent (IC_{50} values of approximately 95 and 580 μM) (20).

In general, the six GSH conjugates were stronger in inhibiting MRP1-mediated $E_2$17βG transport than LTC_4 transport and they were potent in inhibiting MRP1- than MRP2-mediated $E_2$17βG transport. MRP1-mediated $E_2$17βG transport was inhibited in a competitive manner with a relative order of potency of GS estrogens (IC_{50}<1 μM)>2-GS-CA (IC_{50} 3 μM)>5-GS-*N*-Me-α-MeDA (IC_{50} 31 μM). MRP2-mediated transport was inhibited with a similar order of potency, except the 2-OH-4-GS-E_2 and 4-OH-2-GS-E_2 were approximately 50- and 300-fold less potent (20). Further studies have been initiated to test if these metabolites are only the mediators of MRP1 and MRP2 or they are the substrates. Therefore, it is essential to clarify the therapeutic effect of these metabolites and their whole therapeutic properties.

3β-Acethyl Tormentic Acid (3ATA) as Modulator of MRP1-4

A recent study reports that 3ATA, a triterpene isolated from *Cecropia lyratiloba*, can induce apoptosis in an MDR leukemia cell line overexpressing P-gp without interfering with ABCB1 expression or activity. It has also been reported that 3ATA significantly blocks the efflux activity of MRP1 in murine melanoma B16F10 and monkey epithelial Ma104 cell lines that express MRP1. Nonetheless, 3ATA exhibits a weaker effect in the A549 lung cancer cell line expressing various members of the MRP subfamily, indicating that this triterpene might have a preferred selectivity for MRP1 (21). Furthermore, 3ATA could inhibit the efflux activity of the MRP1-4, and the inhibition ratio of MRP1 was significantly higher than that of MRP2, MRP3, or MRP4. In conclusion, 3ATA is a new and potent modulator of MRP1-4 with preferred selectivity for MRP1. 3ATA could be considered as a promising lead compound to assist in designing more potent MRPs modulators (21).

miRNAs as Modulators of MRPs

miR-326 as Modulator of MRP1

In VP-16-resistant breast cancer cell line MCF-7/VP, the mRNA and protein levels of MRP1 are overexpressed. Utilizing a microarray comprising 463 human mature miRNA probes, 17 of miRNAs were diversely expressed between MCF-7/VP and MCF-7 cells. Most of the miRNAs tested showed increased expression levels; however, miR-326, miR-429, miR-187, miR-7, and miR-92-2 exhibited decreased expression (22). In consistency with the microarray results, quantitative RT-PCR analysis revealed the 3.3-fold decreased expression of miR-326 in MCF-7/VP as compared to MCF-7, which is the highest downregulated expression among the four downregulated miRNAs. Consistently, advanced breast cancer tissues expressed the lowest levels of miR-326 while the highest levels of miR-326 were observed in normal breast tissues. The expression level of miR-326 mRNA corresponds inversely with MRP1 mRNA in tissues (22).

Using RT-PCR and Western blot, it was observed that the expression levels of MRP1 mRNA and protein were reduced in miR-326 miRIDIAN mimic-transfected MCF-7/VP cells in comparison with control cells. The IC_{50} of resistant MCF-7/VP cells to VP-16 priory was 15.3 times higher than parental MCF-7 cells. The IC_{50} of miR-326-transfected MCF-7/VP cells to VP-16 was 7.1 times lower than MCF-7/VP cells transfected with control oligonucleotide and only 2.1 times higher than MCF-7 cells (22). All these findings suggested that miR-326 could strengthen the cytotoxic effect of VP-16 and doxorubicin on MCF-7/VP cells through the blockade of MRP1. Importantly, it would trigger

more research of designing personalized treatment for patients with MDR breast cancer.

miR-297 as Modulator of MRP2

Similarly, microarray comprising 873 human mature miRNA probes was instrumented to analyze the miRNA expression in oxaliplatin-resistant human colorectal carcinoma cell line HCT116/L-OHP and parental HCT116 cells. Interestingly, 16 miRNAs were diversely expressed in HCT116/L-OHP and HCT116 cells. Consistent with microarray data, quantitative RT-PCR analysis showed that miR-297 expression was downregulated in HCT116/L-OHP and HCT-8/VCR cells as compared to their parental cells (23).

There is a significantly negative correlation between miR-297 and MRP2 expression (mRNA levels) in CRC. MiR-297 directly inhibited MRP-2 expression at the post-transcriptional level through its 3′-UTR. Using MTT assay, HCT116 cells transfected with miR-297 show significantly decreased sensitivity to vincristine, doxorubicin, and oxaliplatin (L-OHP). Consistent data were also obtained in human ileocaecal colorectal adenocarcinoma cell line HCT-8 and its MDR cell line HCT-8/VCR. Furthermore, miR-297 mimics could also inhibit doxorubicin efflux by reducing the MRP2 expression. Analyzed by flow cytometry, an obvious enhancement in apoptosis was found in miR-297 mimic-transfected HCT116/L-OHP cells after L-OHP treatment in comparison with the control oligonucleotide-transfected cells. Importantly, miR-297 exhibited no effect on human MDR CRC growth, however miR-297 was able to significantly inhibit the tumor growth by downregulating MRP2 expression and potentiating the efficacy of L-OHP to the human MDR CRC *in vivo* (23). All these findings would further help us understand the modulation of MRP2 in cancer patients.

SUMMARY

As discussed, nine MRPs have different patterns in topological structure, subcellular localization, tissue expression, substrate specificities, as well as their functions. Most members of the MRP family are clearly involved in protection of various tissues and organs against a vast array of endogenous and xenobiotic substrates and their metabolites. In cancer therapy, MRPs have been illustrated to confer resistance to several broadly used anticancer drugs. Many valuable strategies against MRPs, ranging from small molecular inhibitors to miRNA gene mute techniques, have been shown to be effective on target MRPs. Successful preclinical studies reveal the promising prospects of adjuvant chemotherapeutic in clinical cancer therapy practice. Moreover, crystallographic and mechanism studies at the molecular level are still needed for the purpose of better understanding and developing effective MRP modulators.

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Conflict of Interest The authors declare no conflict of interest.

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