Pflugers Arch - Eur J Physiol (2007) 453:675–684
DOI 10.1007/s00424-006-0114-1

INVITED REVIEW

ABCC10, ABCC11, and ABCC12

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Received: 23 May 2006 / Accepted: 6 June 2006 / Published online: 26 July 2006

Abstract Multidrug resistance protein (MRP)7, MRP8, and MRP9 (gene symbols ABCC10, ABCC11, and ABCC12) are recently identified members of the MRP family that are at relatively early stages of investigation. Of these proteins, a physiological function has only been established for MRP8, for which a single nucleotide polymorphism determines wet vs dry earwax type. MRP7 and MRP8 are lipophilic anion pumps that are able to confer resistance to chemotherapeutic agents. MRP7 is competent in the transport of the glucuronide $E_2$17βG, and its resistance profile, which includes several natural product anticancer agents, is distinguished by the taxane docetaxel. MRP8 is able to transport a diverse range of lipophilic anions, including cyclic nucleotides, $E_2$17βG, steroid sulfates such as dehydroepiandrosterone (DHEAS) and E_1S, glutathione conjugates such as leukotriene C4 and dinitrophenyl-S-glutathione, and monoanionic bile acids. However, the constituent of earwax that is susceptible to transport by MRP8 has not been identified. MRP8 has complex interactions with its substrates, as indicated by the nonreciprocal ability of DHEAS to stimulate $E_2$17βG transport. Similar to the case for other MRPs that possess only two membrane spanning domains (MRP4 and MRP5), MRP8 is a cyclic nucleotide efflux pump that is able to confer resistance to nucleoside-based agents, such as PMEA and 5FU. The functional characteristics of MRP9 are currently unknown.

Keywords Docetaxel · cAMP · Earwax · DHEAS · Resistance

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Introduction: structure and expression

The multidrug resistance protein (MRP) family, a subset of the C family of ATP-binding cassette (ABC) transporters, is composed of nine members [1]. In this review, MRP7, MRP8, and MRP9 (gene symbols ABCC10, ABCC11, and ABCC12), the last three members of the MRP family to be identified, will be discussed. Another gene sequence, ABCC13, does not appear to encode a functional ABC transporter [2, 3]. MRP7, MRP8, and MRP9 are at early stages of investigation. However, functional properties of MRP7 and MRP8 have been reported, and a surprising physiological function has recently been determined for MRP8 [4–8]. At the time of writing, studies on MRP9 are limited to descriptions of its structure and expression pattern, and it will not be discussed beyond the introduction. The properties of MRP7, MRP8, and MRP9 are summarized in Table 1.

The cDNA sequence of MRP7, a product of a large scale sequencing project, was identified in a data base search we performed in an attempt to flesh out the MRP family [9]. The topology of MRP7 is similar to that of MRP1, MRP2, MRP3, and MRP6, in that it possesses three membrane spanning domains and two nucleotide-binding domains (Fig. 1). However, on the basis of amino acid comparisons, MRP7 is about as related to the CFTR chloride channel as it is to other MRPs. The evolutionary divergence of MRP7 from other MRPs is also reflected in the genomic organiza-

Table 1 Properties of ABCC10, ABCC11, and ABCC12

Human gene name	Protein name	Physiological substrates	Drug resistance profile	Tissue distribution (transcript, RT/PCR)	Protein expression	Established physiological functions	Human gene locus	Murine orthologue	Splice variants
ABCC10	MRP7	$E_2$17βG; LTC4	Docetaxel; paclitaxel; vinblastine; vincristine	Wide?; difficult to detect by northern analysis	?	?	6p12	Abcc10	Yes
ABCC11	MRP8	$E_2$17βG; cAMP; cGMP, LTC4; glycocholate; taurocholate; DHEAS, $E_1$3S; folic acid	PMEA; 5-FU; ddC	Wide?	Axons; apical in transfected LLC–PK1 cells	Single nucleotide polymorphism; (180Gly→Arg) determines wet vs dry earwax type	16q12	No	Yes
ABCC12	MRP9	?	?	Wide?	?	?	16q12	Abcc12	Yes

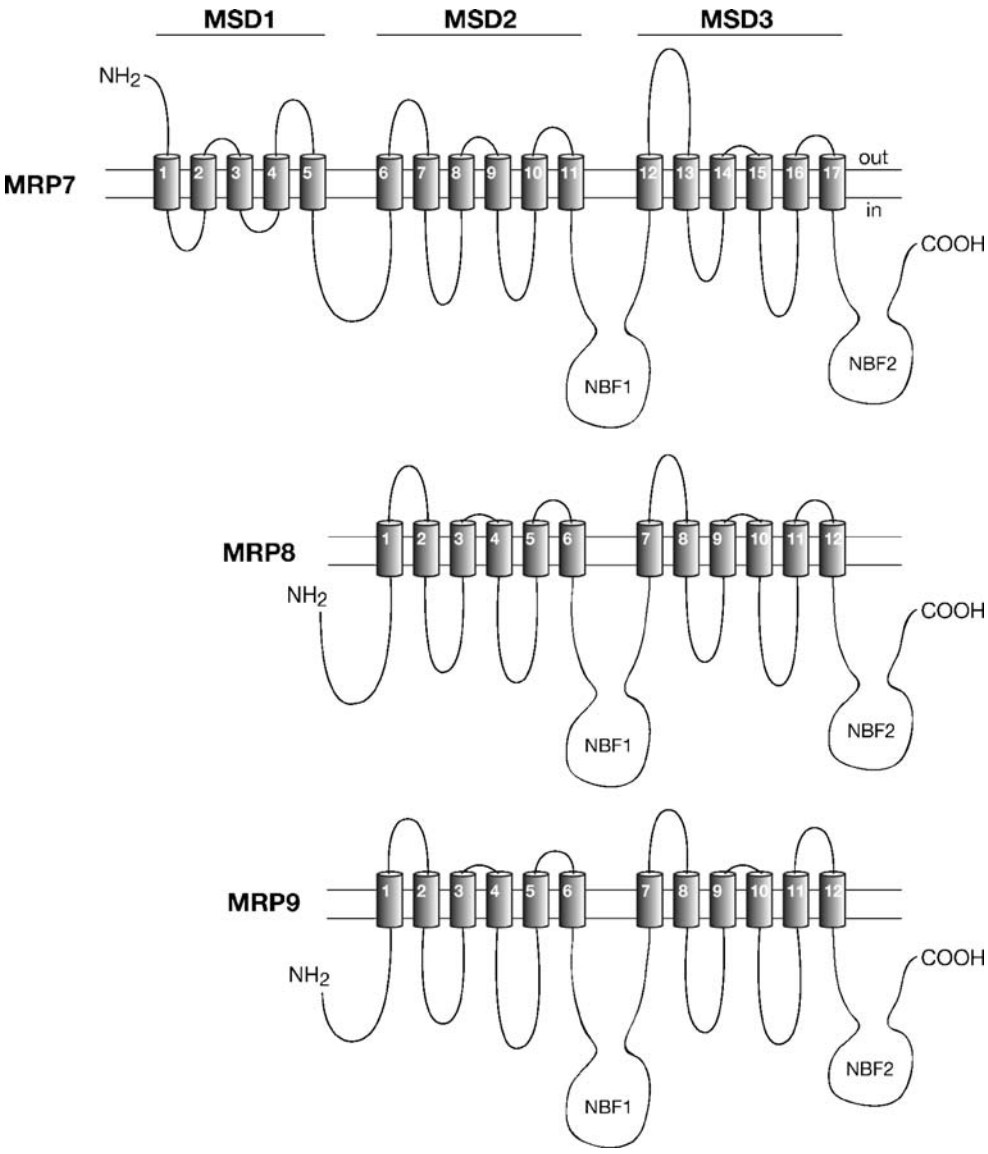

Fig. 1 Schematic showing the structures of MRP7, MRP8, and MRP9. MRP7, like MRP1, MRP2, MRP3, and MRP6, is composed of three membrane-spanning domains and two nucleotide-binding folds. MRP8 and MRP9 have two membrane-spanning domains, and therefore resemble MRP4 and MRP5 in this regard. The predicted structures of MRP7, MRP8, and MRP9 illustrated in the schematic have not as yet been subjected to experimental analysis. *MSD*, membrane-spanning domain; *NBF*, nucleotide-binding fold

tion of its introns and exons. The amino terminal third of the MRP7 protein is encoded by just three exons, whereas the analogous portions of other MRPs that harbor three membrane-spanning domains are encoded by 11–12 exons [10]. Studies on the expression of MRP7 protein in tissues have not yet appeared in the literature. Using reverse transcriptase polymerase chain reaction, we found MRP7 transcript expression in testis, skin, stomach, spleen, colon, kidney, heart, and brain. However, transcript was difficult to detect by Northern analysis, suggesting that its levels in many tissues are low. Another report described highest MRP7 transcript expression in pancreas, followed by liver, placenta, lung, kidney, brain, ovary, lymph node, spleen, heart, leukocytes, and colon [9, 11]. A splice variant of MRP7 that is truncated at its N terminus and has a 15-amino acid deletion between MSD2 and the second nucleotide-binding domain has been described [10]. Murine Mrp7 is ~84% identical to MRP7, and its transcript is expressed in many tissues, with highest levels in testis, ovary, gut, kidney, and lung [12, 13]. Rat Mrp7 transcript was reported to be expressed in liver, kidney, and ileum [14].

MRP8 and MRP9 are closely related proteins (47% identity) whose genes are located in close proximity and in a head to tail orientation at 16q12 [15–17]. Phylogenetic analysis indicates that ABCC11 and ABCC12 arose recently by gene duplication. Computer hydropathy analysis and comparisons with other MRPs suggest that MRP8 and MRP9 harbor 12 transmembrane helices arranged in two membrane spanning domains, but other configurations are also possible (Fig. 1). Hence, their topologies are similar to those of MRP4 and MRP5, which also lack the 3rd (N-terminal) membrane-spanning domain that is present in the larger MRPs [18, 19]. MRP8 and MRP9 are closely related to MRP5, with the three proteins residing in a common subcluster. Transcript analysis reported by two groups suggests that MRP8 is widely expressed in tissues [16, 17]. Another group found more limited expression, with highest levels in liver, brain, and placenta, followed by breast and testis [20]. MRP9 transcript expression was reported for testis, ovary, brain, prostate, and breast [15, 17]. However, a more widespread expression pattern has also been reported [16]. Alternative splice forms of MRP8 and MRP9 have been described [15, 16]. Protein expression studies are obviously needed for clarification of the tissue expression of MRP8 and MRP9, and also for determining the significance of alternative splice forms. A murine homologue of MRP9 has been reported (85% identical to MRP9; 48% identical to MRP8), whereas an orthologue of MRP8 is absent from the mouse genome [21]. Mrp9 transcript is abundantly expressed in testis, with very modest expression in other tissues [12, 14]. In testis, the transcript has been localized to sertoli cells of the seminiferous tubules, with lower levels in leydig cells.

MRP7 [ABCC10]

Drug resistance capabilities

The physiological functions of MRP7 are currently unknown. However, its functional characteristics were described in two reports from our laboratory [4, 5]. These studies established that MRP7 is a lipophilic anion transporter that has the facility for conferring resistance to some natural product anticancer agents. The drug resistance capabilities of MRP7 were determined using HEK293 cells in which the protein was ectopically expressed. Like other MRPs that possess three membrane-spanning domains, MRP7 is able to confer resistance to several natural product anticancer agents [1]. A distinctive feature of MRP7 is that it is capable of conferring high levels of resistance (9–13-fold) to docetaxel, a microtubule stabilizing agent. In addition, three- to fourfold levels of resistance were observed for another taxane, paclitaxel, as well as for the microtubule destabilizing agents vincristine and vinblastine. With the exception of MRP2, for which resistance towards paclitaxel is discernable under conditions in which endogenous P-glycoprotein activity is pharmacologically suppressed [22], no other MRP family member has been reported to confer resistance towards taxanes (Fig. 2). In this respect, MRP7 is similar to P-glycoprotein (ABCB1) and ABCG2, both of which have activity towards this class of agents.

Whether MRP7 contributes significantly to the in vivo sensitivity of normal tissues or tumors is unknown, but it is reasonable to speculate that it may play a role in the intrinsic sensitivity of tissues and tumors in which it is expressed. Limited information is currently available on MRP7 expression in tumors. MRP7 transcript was detected in the HepG2 liver cancer cell line and in two prostate cancer cell lines, CWR22Rv1 and TSU-PR1 [23]. In

	Agent								
Transporter	DOX	DNR	VCR	VBL	ETOP	DOC	PAC	CPT11	SN38
MRP1	+	+	+	+	+	nr	−	+	+
MRP2	+	nr	+	nr	+	nr	+	+	+
MRP3	−	−	−	−	+	nr	−	−	−
MRP6	+	+	−	−	+	nr	−	−	nr
MRP7	+	−	+	+	−	+	+	nr	−

Fig. 2 Natural product resistance profiles of MRP1, MRP2, MRP3, MRP6, and MRP7. +, >fivefold resistance; −, ≤fivefold resistance; *nr*, not reported; *DOX*, doxorubicin; *DNR*, daunorubicin; *VCR*, vincristine; *VBL*, vinblastine; *ETOP*, etoposide; *DOC*, docetaxel; *PAC*, paclitaxel; *CPT11*, irinotecan; *SN38*, active metabolite of irinotecan. Not shown are MRP4, MRP5, MRP8, and MRP9. MRP4 confers resistance to camptothecins, a capability that has not been reported for MRP5 and MRP8. The resistance profile of MRP9 is currently unknown. Adapted from Hopper-Borge et al. [5]

addition, transcript was expressed in eight tumor specimens, including breast, lung, colon, prostate, ovary, and pancreas [11]. It will be of particular interest to determine whether MRP7 is frequently expressed in cancers of the breast, lung, and ovary, as this finding would suggest that the pump may contribute to intrinsic sensitivity in the setting of cancers for which taxanes are mainstays of treatment.

Substrate selectivity

Analysis of the substrate selectivity of MRP7 using membrane vesicles prepared from MRP7-transfected HEK293 cells revealed that $E_2$17βG (Fig. 3), a prototypical transport substrate of several MRPs, is a good substrate of MRP7 (K_m=57.8 μM), and that the pump has modest activity towards leukotriene C4 (LTC4) [5]. However, a variety of other compounds that are substrates of other MRP family members, including glycocholic acid, taurocholic acid, methotrexate, folic acid, cAMP, and cGMP were not detected as transport substrates. Hence, this study established that MRP7 possesses a cardinal biochemical feature of MRPs—the ability to transport lipophilic anions—but also showed that its substrate selectivity is relatively limited, at least in terms of the types of compounds that are frequently used as probes to study MRP family members.

Additional insights into the transport characteristics of MRP7 were provided by inhibition studies. In accord with the results of the membrane vesicle transport and drug resistance assays, and with the inference derived from these studies that the substrate binding pocket of MRP7 has sites for both lipophilic and negatively charged ligands, MRP7-mediated transport of $E_2$17βG was susceptible to inhibition by LTC4 ($K_{i(app)}$=1.5 μM), which was the single best inhibitor identified in a panel of test compounds, as well as by the natural product anticancer agents towards which the pump confers resistance. In addition, similar to the case reported for MRP2 and MRP3 [24, 25], cyclosporine A ($K_{i(app)}$=14.4 μM) but not verapamil or PSC833, was a good inhibitor of MRP7, a finding that may be of consequence in understanding the impact of P-glycoprotein inhibitors on cellular resistance conferred by MRP7. Finally, MK571, an LTD4 antagonist, was a good inhibitor of MRP7($K_{i(app)}$=28.5 μM), as was glycolithocholate-3-sulfate ($K_{i(app)}$=34.2 μM), although the inhibitions exerted by these compounds were ~30-fold lower than that of LTC4.

Regulation of expression

Factors that influence MRP7 expression are largely unexplored. However, induction of MRP7 was described in MCF7 cells treated with doxorubicin [11]. In addition, two

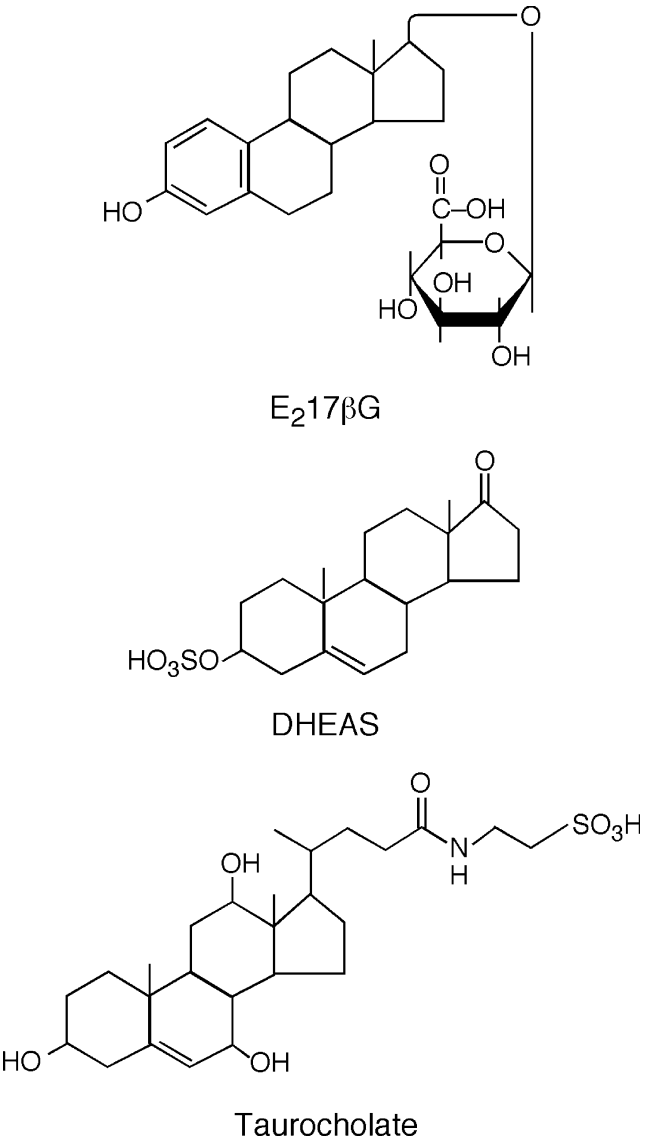

Fig. 3 Structures of selected compounds mentioned in the text

Sp1 sites and an E2F site located within the 169 bp segment 5′ of the ATG, but downstream of the transcriptional start point, were inferred to be involved in basal MRP7 promoter activity based upon functional deletion analysis, in situ mutagenesis and electrophoretic mobility shift assays [23]. In another report, it was concluded that sequences located at −605–1068 and −1744–2237 (relative to the ATG) were involved in basal promoter activity [10]. In addition, this group found that the activity of a reporter construct bearing ~2 kb of MRP7 flanking region could be induced by the alkylating agent 2-acetylaminofluorene.

Physiological functions

The physiological functions of MRP7 have not been determined. However, an interesting finding concerning MRP7 relates to its potential involvement in suppression of

natural killer (NK)-mediated lysis [26]. Human histocompatibility leukocyte antigen-E (HLA-E) binds in a highly specific fashion to a limited set of peptides originating from MHC class Ia leader sequences. Presentation of this cell surface complex to the CD94/NKG2 family of receptors found on NK and T cells suppresses cell-mediated cytotoxicity mediated by these effector cells. To identify HLA-E binding peptides other than those derived from MHC class Ia, HLA-E-associated peptides were analyzed in an MHC class Ia-null cell line. Using this approach, a peptide derived from amino acids 496–504 (ALALVRMLI) of the first transmembrane domain of MRP7 was identified. This association was confirmed in experiments showing that the MRP7 peptide was able to suppress NK-mediated lysis as efficiently as class Ia leader derived peptides. The authors of this study point out that while the connection between HLA-E and MRP7 is currently unclear, it is possible that this mechanism may suppress cytolytic activity of CD94/NKG2-bearing cells during periods of stress, when class Ia peptides may be limiting.

Information on the protein expression pattern of MRP7, as well as its subcellular localization in polarized cells, are needed to begin to understand how the pump's ability to transport glucuronides may relate to its potential physiological functions. An Mrp7 null mouse we generated by homologous recombination is fertile and appears healthy, indicating that Mrp7 is dispensable for life (E.H.-B. and G.D.K., unpublished).

MRP8 [ABCC11]

Drug resistance capabilities

The drug resistance properties and substrate selectivity of MRP8 were analyzed in two reports from our laboratory [4, 7]. In the first of these studies, the drug resistance profile of MRP8 was determined using LLC-PK1 cells in which the pump was ectopically expressed. We found that MRP8 confers significant levels of resistance to several nucleoside-based analogs. By contrast, resistance activity was not observed for a variety of natural product anticancer agents. Agents to which MRP8 confers resistance include 9′-(2′-phosphonylmethoxyethyl)adenine (PMEA, the active metabolite of the prodrug Adefovir dipivoxil), an acyclic nucleoside phosphonate employed in the treatment of hepatitis B, 2′,3′-dideoxycytidine (ddC, Zalcitabine), an anti-AIDS drug, and 5-fluorouracil (5-FU), an antimetabolite used in the treatment of a variety of cancers, including breast and colon. Thus, the drug resistance capabilities of MRP8 resemble those of MRP4 and MRP5, which it topologically resembles, in that the latter pumps also confer resistance to nucleoside-based agents [27–29].

In contrast to the nucleotide analog PMEA, which is an amphipathic anion that is susceptible to direct transport by lipophilic anion transporters, ddC and 5-FU are uncharged nucleobase analogs that are metabolized to anionic nucleotide analogs in the cell. Insights into the substrate selectivity of MRP8, and the mechanism by which it confers resistance to nucleobase analogs, were provided by analyzing the ability of MRP8 to transport 5-FU, 5-fluorodeoxyuridine (5′-FdURD) and 5-fluorodeoxyuridine monophosphate (5′-FdUMP) in membrane vesicle assays. These experiments showed that 5-FdUMP, the cytotoxic intracellular metabolite of 5-FU, was susceptible to MRP8-mediated transport, but not the parent compound or its nucleoside metabolite, as would be expected for a lipophilic anion transporter (Fig. 4). Similar findings concerning resistance to nucleobase analogs have been reported for MRP4 and MRP5. MRP5 confers resistance to 5-FU by transporting 5′-FdUMP, and MRP4 and MRP5 confer resistance to 6-mercaptopurine and 6-thioguanine by transporting thionucleoside monophosphates [30, 31]. We did not observe resistance to thiopurines in MRP8-transfected LLC-PK1 cells. In addition, resistance towards camptothecins, which is a capability of MRP4, was not detected [32, 33]. These potential differences require further analysis in the context of other types of cell lines in which MRP8 is ectopically expressed.

MRP8 transcript expression was reported for 10 of 12 breast cancer samples, and an analysis of MRP8 ESTs in data bases indicated that 17 of 29 entries were from breast cancers [17, 20]. It is interesting to note that MRP9 transcript also appears to be frequently expressed in breast cancers [15]. MRP8 transcript has also been detected in breast, lung, colon, prostate, ovary, and pancreas tumor cell lines [16]. Expression of MRP8 in breast and colon cancers would be of particular interest because fluoropyrimidines are mainstays in treating these tumors. However, it is important to bear in mind that the ability of efflux pumps to affect in vivo sensitivity towards nucleoside-based agents has not been established. Additional studies, including protein expression studies in tumors, will help to clarify the potential contribution of MRP8 to the chemosensitivity of these cancers.

Substrate selectivity

The substrate selectivity of MRP8 was characterized in our laboratory using intact cells and in vitro transport assays [7]. Among characterized MRPs, a distinguishing feature of MRP4 and MRP5 is their facility for transporting cyclic nucleotides [34–36]. The ability of MRP8 to transport these second messengers was analyzed in MRP8-transfected LLC-PK1 cells. Under basal and stimulated conditions, expression of MRP8 resulted in decreased intracellular

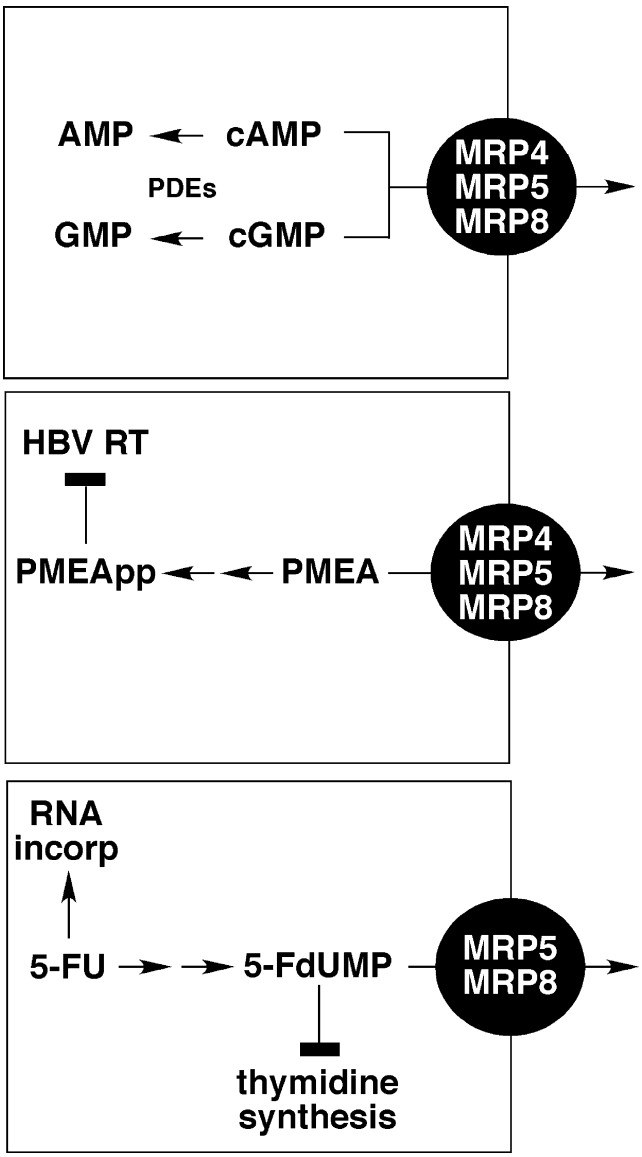

Fig. 4 Involvement of MRPs in the transport of cyclic nucleotides, PMEA, and 5-fluorouracil. *Top panel* Attenuation of signaling mediated by cyclic nucleotides is accomplished by phosphodiesterases. MRP4, MRP5, and MRP8 are cellular efflux pumps for cAMP and cGMP. However, the physiological circumstances in which they may impact intracellular cyclic nucleotide levels have not been defined. It is also possible that these pumps may function in the provision of extracellular cyclic nucleotides involved in intercellular signaling. *Middle panel* PMEA, an acyclic phosphonate, is metabolized to its active triphosphate form, which inhibits hepatitis B reverse transcriptase. MRP4, MRP5, and MRP8 confer resistance to PMEA by directly extruding the unmetabolized parent compound. *Bottom panel* MRP5 and MRP8 confer resistance to fluoropyrimidines by effluxing 5′-dUMP, the active nucleoside monophosphate metabolite of 5-FU

concentrations and increased extracellular concentrations of cAMP and cGMP, consistent with the operation of a cyclic nucleotide efflux pump. Membrane vesicle transport experiments showed that, in addition to cyclic nucleotides, MRP8 is able to transport a range of lipophilic anions including the natural and synthetic glutathione conjugates LTC4 and dinitrophenyl-S-glutathione (DNP-SG), $E_2$17βG, the monoanionic bile acids glycocholate and taurocholate, the steroid sulfates dehydroepiandrosterone (DHEAS) and estrone 3-sulfate ($E_1$3S), folic acid, and the folic acid analog methotrexate. $E_2$17βG and DHEAS were transported with good affinities (K_m=63 and 13 μM, respectively). The susceptibility of DHEAS to MRP8-mediated transport has also been demonstrated by another group who employed membrane vesicles prepared from transfected MDCK cells [8].

The experiments on MRP8 described above, and others showing that the prostaglandins PGE1 and PGE2 are not transported by MRP8, suggest that the selectivity of the pump with respect to physiological compounds differs from that of other MRPs that are competent in the transport of cyclic nucleotides. MRP4 is also able to transport LTC4, $E_2$17βG, steroid sulfates and folates, but is also able to transport prostaglandins, and requires glutathione for bile acid transport, and MRP5 only appears to be competent in the transport of folates [34, 35, 37–40].

MRP8 and the methotrexate efflux system

A crucial property of methotrexate, a potent dihydrofolate reductase inhibitor, is its susceptibility to polyglutamylation by the same enzyme that polyglutamylates physiological folates. By comparison with the parent molecule, a monoglutamate that is effluxed from the cell via an energy dependent process, polyglutamylated methotrexate is poorly extruded. The prolonged intracellular retention of polyglutamylated methotrexate is fundamental to its potency. In previous studies, we showed that the transport characteristics of MRP1, MRP2, MRP3, and MRP4 [37, 41] are precisely those that would be expected for components of the methotrexate efflux system. These pumps are high capacity, low affinity transporters of methotrexate, but are unable to transport methotrexate species having as little as one addition glutamyl residue (i.e., methotrexate diglutamate). Analysis of the transport properties of MRP8 indicates that its characteristics are quite similar to MRPs 1–4. MRP8 has high capacity and low affinity for methotrexate (K_m=0.96 mM), and its activity towards methotrexate diglutamate is severely attenuated (Fig. 5) [7]. We have not formally demonstrated that MRP8 is able to confer resistance to methotrexate, as is the case for other MRPs that have the facility for transporting methotrexate [28, 42, 43], but we anticipate that this will be the case. MRP5 and ABCG2 are also components of the methotrexate efflux system, but their selectivities for lower polyglutamyl species differ from the other pumps shown in Fig. 5 [44–46]. MRP5 and ABCG2

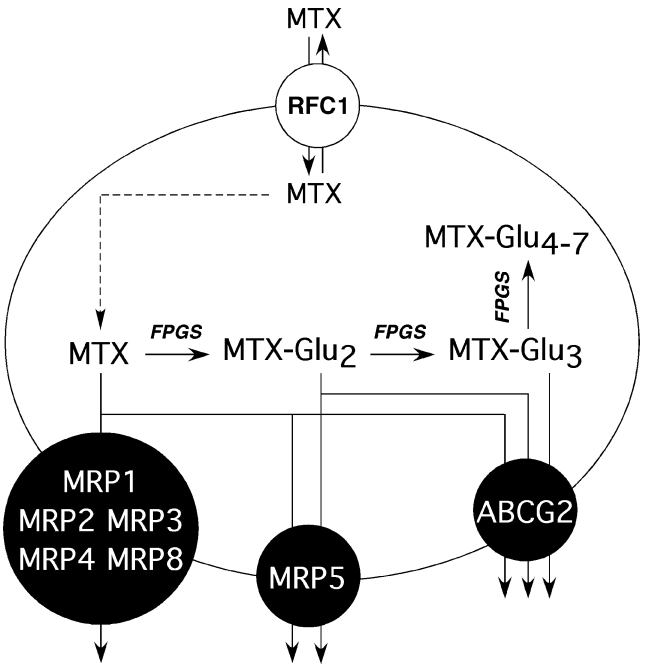

Fig. 5 MRP8 and the methotrexate efflux system. Methotrexate (MTX) uptake is mediated by the reduced folate receptor (RFC1). Once inside the cell, methotrexate is converted to methotrexate polyglutamates (MTX-Glu_n) by folyl-poly-γ-glutamate synthetase (FPGS). Free methotrexate is effluxed by MRP1, MRP2, MRP3, MRP4, MRP5, MRP8, and ABCG2, whereas MTX-Glu_2 is extruded by MRP5 and ABCG2. MTX-Glu3 is subject to efflux by ABCG2. Adapted from Kruh and Belinsky [1]

are able to transport methotrexate diglutamate, and ABCG2 is able to transport methotrexate triglutamate.

Constructive interactions between some MRP8 substrates

By analyzing the effects of transport substrates on each other, we found that MRP8 has complex interactions with its substrates [7]. Notably, DHEAS stimulates transport of $E_2$17βG. By contrast, $E_2$17βG exerts an inhibitory effect on DHEAS transport, taurocholate inhibits transport of DHEAS and $E_2$17βG, and both DHEAS and $E_2$17βG inhibit taurocholate transport. Of the two potential mechanisms that could account for the constructive interaction between DHEAS and $E_2$17βG, namely, cotransport of DHEAS and $E_2$17βG from a single substrate binding site or that DHEAS stimulates $E_2$17βG transport by binding to a topologically distinct site on the protein, we favor the latter possibility based upon the observations that the stimulatory interaction between DHEAS and $E_2$17βG is not reciprocal and that stimulation of $E_2$17βG transport was attenuated at high DHEAS concentrations. The latter finding suggests that at lower concentrations, DHEAS stimulates $E_2$17βG transport by binding to a distinct site, whereas at higher concentrations it competes with $E_2$17βG binding at the transport site. The notion that MRP8 has a distinct binding site that affects the activity of a separate transport site is supported by reports showing that the activities of other MRPs are also susceptible to stimulation. This has been demonstrated for the plant MRP AtMRP2, for which mutual stimulation of DNP-SG and $E_2$17βG was described, for MRP1, for which transport of $E_1$3S is stimulated by glutathione, for MRP2, for which transport of $E_2$17βG and AIDS protease inhibitors is stimulated by inhibitors of organic anion transporters, and for MRP3, for which transport of $E_2$17βG is stimulated by the sulfate conjugates of ethinylestradiol and E3040 [47–53]. Of note is that in some but not all of these examples, the stimulating compound has been demonstrated not to be a transport substrate, which tends to support the notion that these constructive interactions are mediated by a binding site distinct from the transport site.

Physiological functions

A surprising physiological function of MRP8 was recently revealed by an elegant analysis of the genetics of earwax type [54]. Earwax (cerumen) is secreted by ceruminous apocrine glands. Individuals have either wet earwax, which is common in populations of European and African origin, or dry earwax which lacks cerumen and is common in East Asians. Earwax type is a Mendelian trait, with wet earwax dominant to the dry type. Yoshiura et al. determined that the basis of this trait is a single nucleotide polymorphism at nucleotide 538 (538G→A; 180Gly→Arg) of the ABCC11 gene. Individuals whose genotypes are either GG or GA have wet earwax, whereas homozygous AA individuals have dry earwax. In accord with the notion that dry earwax is attributable to a deficiency of MRP8 transport activity, MRP8 180R was unable to transport cGMP in membrane vesicle assays by comparison with the robust activity of 180G, and amino acid 180 is located within the first transmembrane helix of MRP8, a position where the introduction of a charged residue would be expected to perturb the protein's conformation. The authors speculate that MRP8 may be involved in the secretion of the aliphatic or aromatic hydrocarbon constituents of earwax, and also point out that because earwax type may play a role in axillary odor and possibly in breast cancer susceptibility, the MRP8 SNP may also be relevant to these processes. The ability of MRP8 to transport lipophilic compounds is consistent with the former possibility, and while the relationship between earwax type and breast cancer is controversial [55–57], it is worth mentioning in passing that breast cancer is a hormonally responsive tumor and MRP8 is able to transport steroid sulfates.

The involvement of MRP8 in other physiological processes associated with its diverse substrates remains to be determined. However, a potential physiological function

was suggested by a recent study indicating that the transporter is expressed in axons of the central and peripheral nervous system [8]. On the basis of this localization, and the ability of neurosteroids such as DHEAS to function as neuromodulators by interacting with neurotransmitter receptors on the plasma membrane, the authors propose that axonal MRP8 may be involved in the provision of extracellular DHEAS, and thereby affect neuronal excitability. It is interesting to note in connection with this possibility, that the gene for paroxysmal kinesigenic choreoarthetosis (PKC) has been mapped close to the chromosomal position where the genes for ABCC11 and ABCC12 are located [58, 59]. However, as yet there is no evidence linking ABCC11 or ABCC12 to PKC. This study also showed that MRP8 assumes apical localization in transfected MDCK cells, and the authors point out that this is consistent with the axonal localization of MRP8 because of the existence of common trafficking motifs for polarized subcellular targeting in epithelial cells and neurons [60].

Acknowledgements This work was supported by NIH Grants CA73728 (G.D.K.) and CA06927 (Fox Chase Cancer Center), and by an appropriation of the Commonwealth of Pennsylvania.

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