Several membrane proteins mediate the ATP-dependent export of endogenous substances, carcinogens, toxins, and cytotoxic drugs as well as their conjugates from cells into the extracellular space. Studies in the Division of Tumor Biochemistry resulted in the discovery of the molecular identity of ATP-dependent export pumps for lipophilic compounds conjugated with glutathione, glucuronate, sulfate, or other anionic residues. Overexpression of these transport proteins, including the multidrug resistance protein 1 (MRP1, transporter symbol ABCC1) and the multidrug resistance protein 2 (MRP2, symbol ABCC2) confers drug resistance to tumor cells (for review see ref. 13). Proteins of the MRP family (MRP1-MRP6 as well as additional isoforms) are very different in their sequence and substrate specificity from MDR1 P-glycoprotein which also confers multidrug resistance [2]. Inhibitors of MRP1, MRP2, and other members of the MRP family are of considerable interest since they may overcome clinical multidrug resistance conferred by these ATP-dependent export pumps. Physiological substrates of the conjugate export pumps of the MRP family include glutathione S-conjugates, such as leukotriene C_{4}, as well as glucuronate conjugates of bilirubin, bile salts, and steroid hormones [5, 6, 10, 13, 16]. Our studies on the expression of the apical conjugate export pump MRP2 in liver demonstrated that the human Dubin-Johnson syndrome is a consequence of the absence of the MRP2 protein from the apical membrane of hepatocytes which, therefore, leads to the characteristic conjugated hyperbilirubinemia in patients with this syndrome [1, 6, 7, 13, 25]. A strong expression of MRP2 has been demonstrated in primary hepatocellular carcinoma and in clear cell renal carcinoma which is the most frequent type of this chemoresistant malignant tumor in kidney [4]. The isoform MRP5 has been recognized as an ATP-dependent export pump for cyclic nucleotides with a high affinity for the ATP-dependent export of 3', 5'-cyclic guanosine monophosphate [24].

The effective intracellular concentration of chemotherapeutic drugs is not only determined by metabolism and ATP-dependent export from cells but also by transport proteins mediating the uptake of drugs and endogenous substances into malignant as well as normal cells. We have cloned and characterized several novel organic anion transporters of the OATP family (transporter symbol SLC21A) and localized them to the basolateral membrane of human hepatocytes [15, 22]. Expression of these uptake transporters may play an important role in the entry of anionic cytotoxic substances into normal and malignant cells and influence the targeting of drugs to selected cell types.

Information via Internet:
http://www.dkfz.de/tumorbiochem/
Multidrug resistance mediated by members of the MRP family

Y. Cui, J. König, G. Jedlitschky, I. Leier, D. Keppler

The resistance of malignant tumors to chemotherapeutic agents is an important problem in clinical chemotherapy. Drug resistance of tumors to structurally diverse cytotoxic agents (multidrug resistance) has been frequently related to overexpression of the MDR1 P-glycoprotein. In 1992 an additional membrane glycoprotein associated with multidrug resistance has been cloned by Susan Cole and Roger Deeley (Science 258: 1650-1654, 1992). We have elucidated the function of this protein termed MRP1 (Jedlitschky et al., Cancer Res. 54: 4833-4836, 1994; Leier et al., J. Biol. Chem. 269: 27807-27810, 1994) and demonstrated that the ATP-dependent export pump overexpressed in human HEK and canine MDCK cells increased the resistance to etoposide (4-fold), cisplatin (10-fold), doxorubicin (7.8-fold), and epirubicin (5-fold) [5]. These studies directly demonstrated that overexpression of MRP2 confers multidrug resistance [5]. Since MRP2 has been detected in hepatocellular carcinoma and renal cell carcinoma, this ATP-dependent export pump may contribute to the well-known multidrug resistance of these tumors [4]. Inhibitors of MRP2-mediated transport of cytotoxic drugs may serve to counteract the multidrug resistance. They also may be of particular interest in solid tumors frequently associated with an overexpression of MRP2.

Substrate specificity of the conjugate export pumps MRP1 (ABCC1), MRP2 (ABCC2), MRP3 (ABCC3), and MRP5 (ABCC5)

I. Leier, G. Jedlitschky, Y. Cui, A. Nies, T. Komisako, D. Keppler

The substrate specificity of recombinant human MRP1 was further elucidated in inside-out membrane vesicles from MRP1-transfected cells [13,17,21]. The substrate efficiency determined by the $V_{max}/K_m$ ratio is as follows: leukotriene $C_4$ and $D_4$ ($LTC_4$, $LTD_4$) $>$ S-(2,4-dinitrophenyl)glutathione $>$ 17$\beta$-glucuronosylestradiol $>$ monoglucuronosyl bilirubin $>$ 3α-sulfatoxycholyltaurine $>$ glutathione disulfide. The substrate specificity of recombinant human MRP2 was considered to be similar as MRP1, however, we have demonstrated significant kinetic differences [5]. Cloning and expression of recombinant MRP2 has now enabled a direct comparison of the kinetic properties of MRP2 and MRP1 [5]. Accordingly, the $K_m$ values of MRP2 for LTC4 and 17$\beta$-glucuronosyl estradiol are 10- and 5-fold higher than the ones of MRP1 [5]. On the other hand, the apical isoform MRP2 has a remarkably high affinity for the endogenous substrates monoglucuronosyl bilirubin and bisglucuronosyl bilirubin with $K_m$ values of 0.7 and 0.9 µM, respectively [6]. Glutathione S-conjugates of the bifunctional alkylating agents chlorambucil and melphalan are substrates for MRP1 [13]. This suggests that MRP1 also contributes to the drug resistance of malignant tumors to alkylating agents. Moreover, we have demonstrated that the fluorescent amphiphilic pentaaean fluo-3 is a good substrate for MRP1 and for MRP2 [10,13,16]. This substance is useful for studies on MRP functions in intact cells [16]. MRP3 preferentially pumps glucuronate conjugates such as monoglucuronosyl bilirubin [21]. MRP5 has been identified as an ATP-dependent export pump for cyclic nucleotides [24].

Expression and localization of members of the MRP family in normal and malignant tissues

A. Nies, J. König, D. Post, W. Hagmann, D. Keppler

In cooperation with: Prof. Dr. Jürgen Kartenbeck, Division of Cell Biology (Immunofluorescence and electron microscopy of MRP proteins); Dr. Herbert Spring, Biomedical Structure Analysis Group (Confocal laser scanning microscopy); Dr. Walter Hofmann, Department of Pathology, University of Heidelberg (MRP family members in hepatocellular carcinoma)

Isoform-specific antibodies were developed on the basis of the sequence information for the cloned human MRP2 and MRP3 cDNAs and used for the detection of the transport proteins in distinct membrane domains of polarized cells (Figure 1). The expression of MRP2 in liver, kidney, and intestine has been analyzed by Northern blotting. Immunofluorescence microscopy served to localize MRP2 to the apical membrane domain of hepatocytes [1,7] and to the brush border membrane of kidney proximal tubules [4]. In addition, MRP2 has been detected in hepatocellular carcinoma cells and human liver tumors (A. Nies, W. Hofmann et al., unpublished) and in clear cell renal carcinoma [4]. The isoform MRP3 (ABCC3) is composed of 1527 amino acids and represents another integral membrane protein of the MRP family with an amino acid identity of 58 % and 48 % when compared to MRP1 and MRP2, respectively [1,13]. MRP3 expression, as analyzed by Northern blotting, was detected in human liver, colon, pancreas, and kidney [1]. Most recent analyses demonstrate a pronounced expression of MRP3 in human hepatocellular carcinoma (A. Nies et al., unpublished). The genes encoding MRP1, MRP2, and MRP3 are localized to different chromosomes, however, the predicted proteins have a similar membrane topology. Peptide antibodies directed against MRP3 allowed for the localization of MRP3 to the basolateral membrane of human hepatocytes [1] and additional polarized cells as well as to the lateral membrane domain of MRP3-transfected MDCK cells [13]. A particularly marked expression of MRP3 has been observed in the basolateral membrane of hepatocytes from patients with Dubin-Johnson syndrome [1] who lack the apical isoform MRP2 because of mutations in the MRP2 gene [7]. This indicates that MRP3 is upregulated under conditions where the apical secretion of anionic conjugates by MRP2 is impaired. Further studies deal with the function and substrate specificity of MRP3. A possible multidrug resistance conferred by overexpression of MRP3 may play a role in hepatocellular carcinoma which is intrinsically resis-
tant to chemotherapy. The domain-specific localization of MRP2 and MRP3 in polarized cells under in vivo conditions [1,13] differs from the one of MRP1 which has been localized to the plasma membrane of many tissues and unpolarized cells such as human erythrocytes and mast cells.

Exon-intron organization of the human MRP2 gene mutated in Dubin-Johnson Syndrome

J. König, H. Tajiri, A. Nies, V. Keitel, D. Rose, B. Stöckel, D. Keppler

In cooperation with: Prof. Dr. Ulrich Leuschner, Department of Medicine, University of Frankfurt

Elucidation of the exon-intron boundaries of the human MRP2 gene has provided the basis for the analysis of mutations in the coding sequence of this gene [7]. Moreover, comparison of the splice junction sites of the MRP2 gene with the genomic organization of MRP1 and MRP3 has given a better insight into the relationship of these genes located on different chromosomes. The human MRP2 gene comprises 32 exons and is approximately 65 kb in length [7]. It shares 21 identical splice junctions with MRP1 and MRP3. We have identified two mutations in the MRP2 gene in patients with Dubin-Johnson syndrome, one leading to a premature termination-codon in the linker region and another one leading to the loss of two amino acids in the carboxy-proximal nucleotide-binding domain of the MRP2 protein [7,13,23,25]. Both mutations are associated with an absence of the MRP2 protein from the apical membrane of hepatocytes. The mutation leading to the deletion of arginine 1392 and methionine 1393 in the second nucleotide-binding domain causes an impaired maturation and trafficking of the protein from the endoplasmic reticulum to the Golgi complex [25]. Inhibition of proteasome function resulted in a paranuclear accumulation of the mutant protein suggesting that proteasomes are involved in the degradation of mutant MRP2 protein [25]. Our studies have elucidated the molecular basis of the Dubin-Johnson syndrome which is associated with a conjugated hyperbilirubinemia. This is in line with our studies on the substrate specificity of recombinant MRP2 as an ATP-dependent export pump for bilirubin conjugates [6].

Purification and functional characterization of human MRP2

W. Hagmann, J. König, A. Nies, S. Borgers, D. Keppler

In cooperation with: Dr. M. Frey and PD Dr. H. Zentgraf, Applied Tumorvirology Program, and Dr. M. Schmölzer and Dr. T. Kempf, Central Protein Analysis Group, DKFZ

Availability of purified MRP2 protein is essential for the detailed functional characterization of this member of the family of ATP-binding cassette transporters. We have stably expressed MRP2 containing an added carboxy-terminal (His)₆-tag [9]. Membrane vesicles from transfected cell clones demonstrated ATP-dependent transport of leukotriene C₄. MRP2-(His)₆ was solubilized from membranes of these transfected cells and was purified to homogeneity for the first time in a three-step procedure using immobilized metal affinity chromatography, desalting, and immunoaffinity chromatography [9]. The purified MRP2-(His)₆ glycoprotein was reconstituted into proteoliposomes and showed functional activity as ATPase in a protein-dependent manner. This ATPase activity was substrate-stimulated by oxidized and reduced glutathione and by S-decyl-glutathione [9].

Characterization of multidrug resistance protein 5 (ABCC5) as an ATP-dependent export pump for cyclic nucleotides

G. Jedlitschky, D. Keppler

In cooperation with: Professor Brian Burchell, University of Dundee, Scotland, UK.

Cellular export of cyclic nucleotides has been observed in a variety of tissues and may represent an elimination pathway for these signaling molecules. An important physiological transport function of recombinant MRPs, expressed in V79 hamster lung fibroblasts, was identified for the first time as an ATP-dependent export of 3', 5'-cyclic guanosine monophosphate with a Kₘ value of 2.1 µM [24]. MRPs-mediated transport was also detected for 3', 5'-cyclic adenosine monophosphate at a lower affinity, with a Kₘ value of 379 µM. A potent inhibition of MRPs-mediated transport was observed with several substances known as phosphodiesterase modifiers, including trequinsin, with a Kᵢ value of 250 nM, and sildenafil, with a Kᵢ value of 267 nM [24]. Thus, cyclic nucleotides are physiological substrates for MRPs. Moreover, MRPs may represent a novel pharmacological target for the enhancement of tissue levels of cyclic guanosine monophosphate [24].

Expression and functional characterization of the human uptake transporters OATP2 (SLC21A6) and OATP8 (SLC21A8)

J. König, Y. Qiu, A. Nies, D. Keppler

Uptake transporters belonging to the organic anion transporter family (OATP, symbol SLC21A) are expressed in a variety of tissues. Based on sequence homologies we cloned human OATP2 and OATP8 [15,22]. Both are preferentially expressed in human liver as demonstrated by Northern blot analysis. Specific antibodies were raised to detect OATP2 and OATP8 both in human liver and in stably transfected cells. Immunofluorescence microscopy localized OATP2 [15] and OATP8 [22] to the basolateral membrane of human hepatocytes and to the lateral membrane of polarized transfected MDCK cells. Using stable transfectants, several organic anions including sulfobromophthalein, 17]-glucuronosyl estradiol, and dehydroepiandrosterone sulfate were demonstrated to be substrates for both OATP2 and OATP8 [15,22,26]. However, OATP2 and OATP8 differ by their ability to transport bile salts and unconjugated bilirubin [26]. At physiological concentrations, several bile salts were taken up by OATP2-expressing cells, but not by OATP8-expressing cells. Furthermore,
unconjugated bilirubin is taken up in the presence of albumin by OATP2-expressing cells, but not by OATP8-expressing cells [26]. Thus, OATP2 was identified as a high-affinity uptake transporter for unconjugated bilirubin in human liver [26].

Publications (* = co-author)


