Minireview

Drug Transporters and Their Role in Multidrug Resistance of Neoplastic Cells

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Abstract. Multidrug resistance (MDR) of neoplastic cells, i e resistance towards large groups of unrelated drugs, represents the phenomenon that dramatically depresses the effectiveness of cancer chemotherapy Membrane transport of ATPases from ABC superfamily plays an important role in MDR In the present paper we are aiming to compare two members of this family P-glycoprotein (PGP products of *mdr* genes) and multidrug resistance-associated protein (MRP, products of *mrp* genes) and their impact for MDR of neoplastic cells

Key words: Multidrug resistance — ABC transporters — P-glycoprotein — MRP protein

Multidrug resistance of neoplastic cells

Multidrug resistance (MDR) of neoplastic cells describes the phenomenon when cells become resistant to unrelated drugs with different chemical structures (Endicott and Ling 1989, Gottesman and Pastan 1993) Studies with cell lines and transplantable tumors have shown that MDR can develop rapidly and development of MDR represents a serious obstacle to successful treatment by cancer chemotherapy (Simon and Schindler 1994, Ling 1997) Resistant cells were found to be cross-resistant to a broad but well defined spectrum of structurally unrelated cytotoxic drugs like athracyclines (i e , doxorubicin – inhibitor of topoisomerase II, DNA intercalating substance), mitomycin C – DNA interacting substance, actinomycin D – antibiotics, *Vinca* alkaloids – inhibition of mitotic spindle formation (Biedler et al 1975, Bech-Hansen et al 1976) MDR can be intrinsic or acquired, depending on the time of its occurrence, either at diagnosis or during the chemotherapy Investigations of molecular mechanisms involved in MDR of neoplastic tissue during the last two decades resulted in identification of the following mechanisms

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- Reduced level of drug accumulation mediated by drug-efflux activity of P-glycoprotein (PGP; 170 kDa product of *mdr1*, 2 or 3 genes; Childs and Ling 1994) or MDR-associated protein (MRP; 190 kDa product of *mrp* gene; Cole et al. 1992). Both peptides are the members of ABC (ATP-binding cassette) transporters superfamily and are widely distributed in all kingdoms of life.
- Enhanced level of drug detoxification induced by increases in activities of enzymes of cell detoxification pathways like glutathione-S-transferase, glutathion reductase, glutathion peroxidase, cytochrome P450 etc. (Morrow and Cowan 1990).
- Reduced content of drug target proteins such as topoisomerase II isoenzymes (Beck et al. 1993).
- Alterations in drug-induced apoptosis that involve genes in the Bcl-2 pathway (Reed 1995).

The present review paper is focused on MDR associated predominantly with PGP or MRP overexpression.

ABC transporters

Distribution and Transport Functions

The ABC transporter superfamily includes proteins, which are capable of a multitude of active membrane transport functions (Henikoff et al. 1997). More than 130 representatives have been identified in species ranging from archaebacteria to humans (Higgins 1992; Volkl et al. 1996). ABC transporters in prokaryotes and eukaryotes participate in the transport of a wide range of distinct substrates: alkaloids, lipids, peptides, steroids, sugars, inorganic anions and heavy metal chelates. With the exception of inorganic anions, the transport of all these substrates is directly energized by MgATP consumption.

Structure

All ABC transporters are constituted of one or two copies containing two basic hydrophobic and hydrophilic structural elements. Hydrophobic, membrane spanning domain (MSD) contains multiple (usually four or six) transmembrane helices (TM). Hydrophilic, cytoplasmically oriented nucleotide-binding domain (NBD) binds and hydrolyzes ATP (Hyde et al. 1990; Higgins 1992).

The MSD spans the membrane to form the pathway for movement of substrates across the lipidic bilayer (or between bilayer leaflets) and also determines the specificity (selectivity) of the transporter. The NBDs and MSDs can be expressed from one to four genes as separate polypeptides, or they may be fused together in one of several alternative arrangements. In *E. coli*, the histidine permease is encoded by four separated genes, one for each membrane-bound domain and NBD, whereas the eukaryotic TAP 1/2 peptide transporter is encoded by two genes (Kelly et al. 1992).

The NBDs, highly conserved units, are 30–40% identical over a span of about 200 amino acid residues between family members, and each NBD encompasses

one copy of each of three idiotypic sequence motifs These motifs are a Walker A box and a Walker B box (Walker et al 1982) and a C motif (or an ABC signature), situated between the two Walker boxes Walker motifs were found in several nucleotide-binding proteins, for example cation ATPases, myosin, adenylate kinase, phosphofructokinase Contrary to Walker boxes, the C motif is unique to the NBDs of ABC transporters (Cole and Deeley 1998)

Multidrug resistance-associated proteins (MRP)

Mammalian MRP proteins are members of phylogenetically discrete subgroup of ABC transporters which includes human proteins MRP1, MRP2 (multispecific organic anion transporter – cMOAT, Paulusma et al 1996) and the more recently identified members MRP3, MRP4, MRP5, and MRP6 (Allikmets et al 1996, Kool et al 1997), the yeast cadmium resistance factor 1 (YCF1, Szczypka et al 1994), the yeast oligomycin resistance I protein (YORI, Katzmann et al 1995), the Leishmania tarentolae P glycoprotein A (associated with resistance to arsenicals and antimony, Callahan et al 1991), and the mammalian sulfonylurea receptors (SUR1 and SUR2, Inagaki et al 1996)

Multidrug resistance protein 2 (cMOAT) is expressed predominantly on the canalicular membrane of hepatocytes and released into the bile (Paulusma et al 1997) A mutation in human MRP2 has been detected in Dubin Johnson syndrome, a pathology characterized by a defect in hepatic multispecific organic anion transport (Buchler et al 1996)

Multidrug resistance protein1 (MRP1)

The human multidrug resistance protein MRP1 is a 190 kDa membrane glycoprotein that causes resistance of human tumor cells to various anticancer drugs (Cole et al 1992, Cole and Deeley 1993, Grant et al 1994, Zaman et al 1994) This type of resistance involves the extrusion of these drugs out of the cell Using monoclonal antibodies specific against different domains of MRP the localization of MRP in a panel of normal human tissues and malignant tumors was determined (reviewed in Flens et al 1997) Whereas in malignant tumors strong plasma membrane MRP staining was frequently observed, in normal human tissues MRP staining was predominantly cytoplasmic MRP was detected in several types of epithelia, muscle cells, and macrophages The presence of MRP in epithelia may have a connection with an excretory function and MRP can play role in protecting the organism against xenobiotics

The multidrug resistance-associated protein 1 gene was first isolated after molecular cloning from H69AR human small cell lung cancer cell line (Cole et al 1992, reviewed in Deeley and Cole 1997) *Mrp1* gene encodes the previously described GS-X (ATP-dependent glutathione S-conjugate export pump) Studies using plasma membrane vesicles prepared from MRP1-overproducing cell lines

demonstrated increased ATP-dependent, high-affinity transport activities for cysteinyl leukotrienes (LTC_4, Jedlitschky et al. 1994; Leier et al. 1994; Müller et al. 1994). MRP1 also confers resistance to a spectrum of natural product chemotherapeutic agents, which include the *Vinca* alkaloids, the anthracyclines, and the epipodophyllotoxins. However, it has not been possible to demonstrate direct MRP-mediated, ATP-dependent transport of vincristine and aflatoxin B_1. This was found only in the presence of physiological concentrations of GSH (Keppler et al. 1996; Barnouin et al. 1998; Loe et al. 1998; Renes et al. 1999). MRP1 can act as a primary active transporter of a wide range of organic, anionic conjugates, some of which are physiological substrates (Ishikawa et al. 1996; Loe et al. 1997). These include a structurally diverse array of glutathione, glucuronide, and sulfate conjugates with LTC_4, and also anionic conjugates of bile and steroid hormones (Loe et al. 1996). In addition, the MRP1 is responsible for the release of oxidized glutathione (GSSG) from cells. This active efflux of GSSG is considered to be an important mechanism that maintains the reduced status of intracellular thiols under oxidative stress (Leier et al. 1996). All these facts indicate, that MRP1 besides other, should cooperate with glutathion S-transferases and other enzymes of glutathion detoxification systems as exporter of cysteinyl drug conjugates (i.e., final product of glutathion detoxification system). This assumption is fully consistent with the finding of Morrow et al. (1998) that GST (isoenzyme A1-1) is acting synergically with MRP1 in protection of MCF7 (breast carcinoma cells) against antineoplastic drug.

Structure of MRP1

Cole et al. (1992), Cole and Deeley (1993) and Stride et al. (1997) assumed from primary structure and hydropathy analyses of MRP1 that this protein contains two MSDs (MSD1, MSD2), those linked with intracellular parts associated with NBD1 oriented to C termini (Figure 1A). Hipfner et al. (1997) corrected the above structure with modification that this contains two NH_2-proximal MSDs (MSD1 and MSD2) and one COOH-proximal MSD (MSD3). MSD2 and MSD3 represent the structures corresponding to MSD1 and MSD2 in the previous model. Like other members of the ABC-superfamily, the nucleotid binding domains (NBDs) of MRP are preceded by a polytopic membrane spanning domain (MSD) that may contain as many as six transmembrane (TM) helices. However, MRP also contains a third, NH_2-proximal MSD1. Topology predictions derived from various protein structure algorithms indicate that this region, MSD1, could span the membrane four to six times. NH_2 terminus of MRP is extracellular, thus it appears most likely that MSD1 spans the membrane five times (Figure 1B).

Substrate specificity of MRP1

Although MRP1 is able to confer resistance to drugs which are P-glycoprotein (PGP) (MDR1) substrates, the substrate specificity of MRP1 (Table 1) seems to be different from that of PGP (MDR1). Transport studies with membrane vesicles isolated from MRP1 overexpressing cells, either *in vitro* selected or transfected,

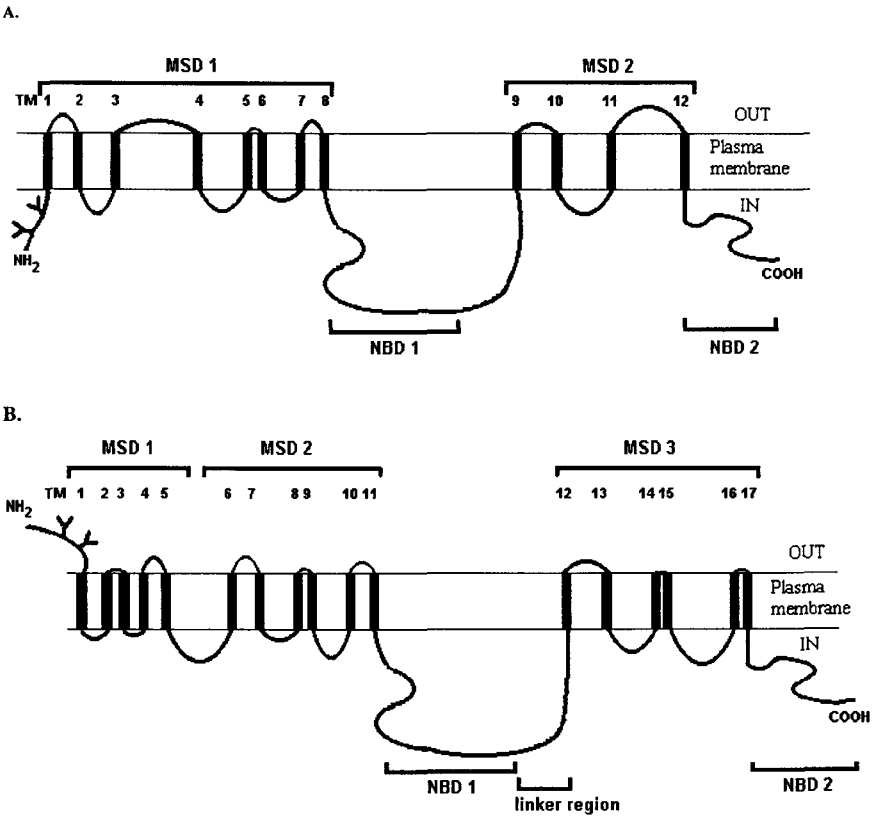

Figure 1. Models of multidrug resistance protein (MRP) membrane topology. **A.** first model of MRP contained two hydrophobic membrane spanning domains (MSD1 and MSD2) with 8 and 4 transmembrane helices (TM) and hydrophobic nucleotide-binding domains (NBDs), **B.** corrected model of MRP; this model contains three MSDs (MSD1, MSD2, MSD3) with 5, 6, and 6 TMs.

revealed that MRP1 is a transporter of multivalent organic anions, preferentially glutathione S-conjugates (GS-conjugates, Müller et al. 1994; Jedlitschky et al. 1996; Loe et al. 1996, 1997; Barnouin et al. 1998) but also of sulphate conjugates (Jedlitschky et al. 1996) and glucuronides (Jedlitschky et al. 1996; Loe et al. 1996). Also oxidized glutathione (GSSG), complexes of reduced glutathione (GSH) with arsenite (Zaman and Pardini 1995) and unmodified compounds in the presence of GSH (Loe et al. 1996, 1997) are MRP1 substrates. In view of its substrate specificity and the ubiquitous expression of MRP1 in human tissues (Zaman et al. 1993) and blood cells (Burger et al. 1994), the putative physiological role of MRP1 seems to be cellular extrusion of metabolites of GSH-dependent detoxification reactions (Müller et al. 1996).

Table 1. Substrates of MRP1

SUBSTRATES	REFERENCES
leukotriene C_4	Leier et al 1994, Loe et al 1996, Stride et al 1997
leukotriene D_4, E_4	Leier et al 1994, Jedlitschky et al 1996
N-acetyl-leukotriene E_4, S-Glutathionyl 2,4-dinitrobenzene, 6α-Glucuronosyl hyodeoxycholate, Glucuronosyl etoposide, 3α-Sulfatolithocholyltaurine	Jedlitschky et al 1996
S-Glutathionyl Aflatoxin B1, Aflatoxin B_1 + reduced glutathione	Loe et al 1997
S-Glutathionyl Prostaglandin A_2	Evers et al 1997
S-Glutathionyl ethacrynic acid	Zaman et al 1996
S-Glutathionyl N-ethylmaleimide	Bakos et al 1998
Chlorambucil	Barnouin et al 1998
Bilirubin	Jedlitschky et al 1997
Melphalan	Jedlitschky et al 1996, Barnouin et al 1998
Glutathione disulfide	Leier et al 1996
17β-Glucuronosyl estradiol	Jedlitschky et al 1996, Loe et al 1996, Stride et al 1997
Folate, Fluo-3	Keppler et al 1998, 1999
Methotrexate	Hooijberg et al 1999
p-Aminohippurate	Leier et al 1999
Vincristine + reduced glutathione	Loe et al 1996, 1998, Stride et al 1997, Renes et al 1999
Daunorubicin + reduced glutathione	Renes et al 1999

There is no direct evidence for conjugation of GSH to drugs to which MRP1 confers resistance (Zaman and Pardini 1995; O'Brien and Tew 1996). There are indications that MRP1 mediates GSH-transport (Zaman and Pardini 1995; Rappa et al 1997), and may act as a co-transporter for GSH and the drugs. Thus, GSH may be a low affinity substrate for MRP1. From experiments using the vanadate-trapping technique it has been suggested that GSH as well as anticancer drugs directly interacts with MRP1 (Taguchi et al 1997). Transport of anionic MRP1-substrates such as GSH- and glucuronide-conjugates are inhibited by hydrophobic (cationic) *vinca* alkaloids (Müller et al. 1994; Loe et al 1996) and anthracyclines (Loe et al 1996). One hypothesis explaining these results is that MRP1 may contain two binding sites: one for hydrophobic compounds and one for hydrophilic compounds. This would allow a similar binding of GSH and the hydrophobic drug

as well as binding of hydrophobic compounds conjugated to GSH, glucuronate or sulphate

Renes et al (1999) found that MRP1 transports vincristine and daunorubicin in an ATP- and GSH-dependent manner in comparison with PGP They also found that N-(4',4'-azo-n-pentyl)-21-deoxy-ajmalinium (APDA), substance that is a substrate for PGP, is not a substrate for MRP1 A possible explanation for the inability of MRP to bind and transport these chemotherapeutic agents, and a clue to one of the proteins potential physiological role, was provided by the demonstration that MRP in inside-out membrane vesicles can act as a high-affinity, primary active transporter of the cysteinyl leukotriene, LTC_4 (Jedlitschky et al 1994, Loe et al 1996) MRP can also actively transport a variety of other GSH-conjugated xenobiotics, including the GSH conjugates of the activated forms of the potent carcinogen aflatoxin B1 (Loe et al 1997) These latter findings suggest that MRP may have a protective role in chemical carcinogenesis Consideration of the molecular structure of LTC_4 prompted the suggestion that MRP transports anionic conjugates of the drugs rather than the unmodified drugs themselves It was also demonstrated that MRP could transport unmodified vincristine and aflatoxin B1 into membrane vesicles in an ATP-dependent manner but only in the presence of GSH (Loe et al 1996, 1997) In addition to LTC_4, it has been shown that other endogenously formed organic anion conjugates previously proposed as putative physiological substrates of P-glycoprotein, such as 17b-estradiol, (17-b-D-glucuronide), bilirubin glucuronides, and some sulfated bile salts, are actively transported by MRP *in vitro* (Loe et al 1996, Jedlitschky et al 1996, 1997) However, although MRP may be an efficient transporter of certain glucuronides, glucuronate itself, unlike GSH, does not stimulate the active transport of unmodified xeno- or endobiotics Another endobiotic shown to be actively transported by MRP *in vitro* is oxidized GSH or GSSG (Leier et al 1996) The identification of this compound as a physiological substrate raises the possible role of MRP in cellular defenses against oxidative stress and perhaps also in the maintenance of intracellular redox potential

Several studies have demonstrated that human immunodeficiency virus (HIV) proteinase inhibitors (PIs) are substrates of the multidrug transporter P-glycoprotein (PGP) (Kim et al 1998, Lee et al 1998, Washington et al 1998) In addition to previous observations, Srinivas et al (1998) demonstrated for the first time that HIV PIs interact also with MRP1, another multidrug transporter protein

P-glycoprotein (PGP)

P-glycoprotein (PGP) is a 170-kDa plasma membrane protein that is most often involved in the multidrug resistance phenomenon (responsible for failure of many human cancer chemotherapies) PGP was first detected as a surface phosphoglycoprotein overexpressed in cultured cells with developed multidrug resistance (Juliano and Ling 1976) and was subsequently cloned from mouse, and human cells (Chen et al 1986, Gros et al 1986) PGP is expected to work as an ATP-driven efflux pump, transporting through the plasma membrane an unusually broad but well defined

spectrum of structurally unrelated cytotoxic drugs, including the *Vinca* alkaloids, anthracyclines, epipodophyllotoxins, and taxanes (Biedler and Riehm 1970, Endicott and Ling 1989, Ford 1995, reviewed in Gottesman et al 1995) The clinical relevance of the transport by P-glycoprotein has been suggested by several studies Strong PGP expression has been discussed as a negative prognostic marker for chemotherapy as shown in studies of breast and ovarian carcinomas (Veneroni et al 1994), osteosarcoma (Baldini et al 1995), Ewing's sarcoma (Roessner et al 1993), neuroblastoma (Chan et al 1996), and some hematological malignancies such as acute myeloid leukemia (Samdani et al 1996)

PGP overexpression may be involved also in etiology of HIV-1 infection, because inhibition of virus production by MDR1 transporter was recently observed (Lee et al 2000) On the other hand, inhibitors of HIV-1 proteases (like ritonavir, saquinavir and indinavir) represent substrates for PGP encoding by *mdr1* gene (see below) Expression of *mdr1* in CD4+ T-lymphocytes, the major target for HIV-1 infection, has also been reported (Bommhardt et al 1994, Lucia et al 1995, Bining and Miller 1997) HIV-1 protease inhibitors ritonavir, saquinavir, and indinavir (effective in inhibiting HIV-1 replication) were found to be recognized by PGP, and their effectiveness in inhibiting HIV-1 is reduced in *mdr1*-expressing cells (Lee et al 1998) Recently Lee et al (2000) found that the overexpression of PGP in cells reduces the susceptibility of CD4+ human cells to HIV-1, probably by affecting viral fusion as well as downstream events This represents the first observation that the expression of a multimembrane-spanning protein inhibits HIV-1 infection High PGP expression reduces uptake of HIV-1 protease inhibitors, so although PGP-expressing cells may be relatively resistant to HIV-1 infection, once these cells are infected it may be more difficult to eradicate the virus

Localization of PGP in normal tissue and possible physiological functions for PGP

Although PGP is expressed normally in many different tissues, the physiological functions and molecular mechanisms of the protein actions remain under active investigation Based on expression in epithelia of the intestine, kidney, liver, and endothelial cells of the blood-brain barrier, a role for PGP in membrane transport that may secure several physiological processes is suggested These processes can involve distribution of substrates, effectors and hormones or prevention against toxins and xenobiotics Due to wide tissue distribution of PGP it seems that this protein is a part of transport systems in cells and plays an important role in normal and pathological physiology of cells In epithelial cells of the lower gastrointestinal tract (jejunum, ileum, and colon), high levels of PGP are located only on the mucosal surface of these tissues This suggests a function of PGP in prevention of substrates uptake and perhaps in facilitation of excretion across the mucosa of the gastrointestinal tract (Thiebaut et al 1987) In kidney and liver, PGP is present on the brush border and biliary face, respectively, of proximal tubule cells and hepatocytes (Cordon-Cardo et al 1990) This distribution is consistent with a role of PGP in excretion of xenobiotics and endogenous toxins into the urine and bile Interestingly, the localization of PGP on the luminal surface of capillary endothelial

cells in the brain is consistent with a role of PGP in forming of the blood-brain barrier (Thiebaut et al. 1989; Tsuji et al. 1993). PGP is expressed also in placenta (MacFarland et al. 1994), which suggests its role in protection of fetus from toxic xenobiotics in the initial part of pregnancy (van Kalken et al. 1992).

PGP is also expressed in hematopoetic stem cells, natural killer cells, antigen-presenting dendritic cells, and T and B lymphocytes (Klimecki et al. 1994; Randolph et al. 1998). Recent work by Johnstone et al. (1999) and others have demonstrated that functional PGP might play a role in regulating programmed cell death and differentiation (Los et al. 1997; Robinson et al. 1997; Smyth et al. 1998). There are two hypotheses to define the method of apoptosis inhibition by PGP, but this is still a theoretical model (reviewed in Johnstone et al. 2000).

Localization of PGP in steroid-secreting glands suggests that PGP might be involved in secretion of steroids, or in protection of steroid-secreting cell plasma membranes from the toxic effects of high steroid concentrations. Consistent with this assumption are findings that progesterone is a PGP inhibitor (Yang et al. 1989) and other steroids, especially corticosterone, are transported by epithelial monolayers expressing PGP (Ueda et al. 1992).

Some studies suggest that PGP may act as a flippase for phospholipids and this flippase activity is ATP-dependent (van Helvoort et al. 1996). Recently, PGP has been shown to regulate the translocation of a wide variety of short chain analogs of phospholipids from the inner to outer leaflet of the plasma membrane. Moreover, the function of human *mdr3* as a flippase for native phosphatidylcholine and sphingomyelin was also suggested (Bosch et al. 1997; Bezombes et al. 1998).

Lange and Steck (1994) have shown that esterification of plasma membrane cholesterol in rat hepatoma cells is inhibited rapidly by treatment with a wide variety of amphiphilic compounds and most of these compounds were nonspecific inhibitors of PGP. Recent studies with cells derived from tissues that normally express PGP suggest a possible indirect role of PGP in facilitating cholesterol trafficking, associated with enhanced esterification of plasma membrane cholesterol (Debry et al. 1997; Luker et al. 1999; reviewed in Johnstone et al. 2000). The cholesterol substrate for esterification originates in the plasma membrane and must be transported to the endoplasmic reticulum for esterification. Debry et al. (1997) have assumed that this transport may be secured by MDR1 PGP.

PGP might also act as an ion channel. It now appears that *mdr1* in humans (Bond et al. 1998; Vanoye et al. 1999) and *mdr1a* in mice can regulate the activity of volume-activated chloride channels, but not *mdr1b* (Valverde et al. 1996; Bond et al. 1998). Chloride channel regulation is inhibited by phosphorylation of PGP by protein kinase C (PKC) (reviewed in Johnstone et al. 2000).

Genes encoding PGP

PGP genes from hamster, mouse and human have been cloned and sequenced, but PGP homologues have been identified also in several other animal species. PGP is encoded by a small multigene family (*mdr* class *1*, *2* and *3*). All three isoforms are present in rodents, while in humans only two isoforms *1* and *3* are expressed (Childs

and Ling 1994) There are also differencies in the localization of PGP genes Human genes are closely linked on chromosome 7, mice genes are smaller and are located on the fifth chromosome, and hamster genes are on the twentieth chromosome Products of these genes have the largest homology for ATP-binding sites and for the first and the second intracytoplasmic loop in both halves of the molecule

Transfection studies have demonstrated that the *mdr1* and *mdr2* isoforms de crease intracellular concentrations of a wide variety of structurally diverse chemotherapeutic agents, resulting in MDR On the other hand, the closely related *mdr3* is not associated with resistance to drugs (Ruetz and Gros 1994) *Mdr3* isoform can behave as phosphatidylcholine (PC) translocase, or flippase, responsible for efflux of this phospholipid into the bile *Mdr1* has been also reported to regulate the translocation of a range of short-chain phospholipid analogs (Bosch et al 1997) and endogenous phospholipids such as sphingomyelin (Bezombes et al 1998)

The genes encoding MRP and PGP are evolutionary very distant The *mrp* gene is more closely related to the cystic fibrosis gene, *CFTR*, whereas the *mdr1* gene is evolutionary more related to the bacterial *hemolysin B* gene than to the mammalian *mrp* gene (Ling 1997) It is also not clear whether MRP can recognize drugs directly (similar to PGP), or whether additional modification, such as conjugation of the drug, is required

Structure of PGP

PGP encoded by the *mdr1* gene has 1 280 amino acids organized in two tandem repeats of 610 amino acids, joined by a linker region of 60 amino acids (Chen et al 1986) Each repeat has an N-terminal hydrophobic domain containing six putative membrane-spanning α helices followed by a hydrophilic domain containing a cytoplasmic nucleotide-binding domain (Fig 2) with characteristic Walker motifs A and B (Kast et al 1996) Each nucleotide-binding domain has been shown to efficiently bind ATP (and its analogs) and hydrolyze ATP (Baubichon-Cortay et

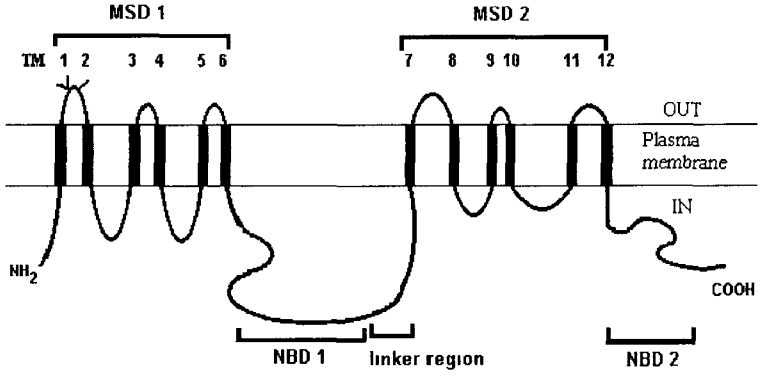

Figure 2. Topological model of P-glycoprotein This model contains two membrane spanning domains (MSDs) with six and six transmembrane helices (TMs) and two nucleotide-binding domains (NBDs) with ATP-binding sites and Walker A and B motifs (not shown)

al 1994, Sharma and Rose 1995, Dayan et al 1996) There has been considerable effort to understand the role of various domains in the mechanism of transport by PGP Both halves of PGP can be expressed as separate polypeptides, or they may be fused together in one of several arrangements, but substrate-stimulated ATPase activity was detected only when the two halves were expressed simultaneously (Loo and Clarke 1994a) It is also clear that interaction between the ATP-binding sites and the drug binding domains is essential for drug transport ATP-binding sites are essential because inactivation of either site by mutagenesis or chemical modification inhibits substrate stimulated ATPase activity (Azzaria et al 1989, Urbatsch et al 1995, Loo and Clarke 1995) However, it is clear, that the next major breakthrough in understanding the mechanism of action of human PGP will occur with the generation of high-resolution two-dimensional and three-dimensional structures

Substrates and modulators of PGP

Resistant tumors are found to be cross-resistant to a broad but well-defined spec trum of structurally unrelated cytotoxic drugs, including the *Vinca* alkaloids, an thracyclines, epipodophyllotoxins, and taxanes These compounds are chemically diverse and include not only anticancer drugs, but also therapeutic agents such as HIV-protease inhibitors (Table 2)

The ability of PGP to catch and transport the substances with this diversity of physico-chemical structures may be fulfilled only at existence of effective binding sites but with broad substrate specificity (Chen and Simon 2000) Recently we have described that the flexibility of drug molecule (i e possibility of substance to exist in various structural isoforms), hydrophobicity, molecular weight less than 1300 g/mol and acidobasic properties that enable the substance to exist at physiological pH in non-protonised form represent important features that predetermine the substance to be a substrate for PGP (Breier et al 2000)

Many pharmacological agents from diverse structural classes have been identified as PGP inhibitors (chemosensitizers) or MDR modulators (Table 3) The mechanism by which modulators inhibit PGP function is specific for single chemosensitizer Inhibition of drug transport could potentially result from the blockage of specific recognition of the substrate, binding of ATP, ATP hydrolysis, or coupling of ATP hydrolysis to translocation of the substrate Most reversing agents block drug transport by acting as competitive or noncompetitive inhibitors and by binding either to drug interaction sites or to other modulator binding sites, leading to allosteric changes

Several chemosensitizers were predicted to interact with the same structure of PGP molecule as a substrate and compete between each other (for calcium entry blockers see Kessels and Wilberding 1984) On the other hand, Pascaud et al (1998) described that PGP has distinct but interacting binding sites for cytotoxic drugs (vinblastine) and reversing agents (verapamil and several dihydropyridines) However, in this time it is not clear whether PGP really contains distinct binding sites for substrates and inhibitors, or common binding sites for both with complicated

Table 2. Substrates of PGP

SUBSTRATES	REFERENCES
Anticancer drugs *Vinca* alkaloids – vincristine, vinblastine Anthracyclines – doxorubicin, daunorubicin, epirubicin Epipodophyllotoxins – Etoposide, Teniposide, Verapamil, nifedipine, bepridil, nicardipine, niguldipine, nitrendipine, trifluoperazine, Actinomycin D, Mitomycin C Paclitaxel – taxol	Gottesman and Pastan 1988, Endicott and Ling 1989, Beck and Qian 1992, Gottesman and Pastan 1993, Simon and Schindler 1994
Cyclic and linear peptides Gramicidin D, Valinomycin and others*	Zhan et al 1997, Sharom et al 1998
Other cytotoxic agents Colchicine, Emetine, Ethidium bromide	Lee et al 1998, Polli et al 1999, Rao et al 1999, Hochman et al 2000
HIV-protease inhibitors Ritonavir > Saquinavir > Nelfinavir ≫ Indinavir	Shapiro et al 1997, Ueda et al 1992
Other compounds Hoechst 33342, Rhodamine 123, Calcein-AM, cortisol, aldosterone, dexamethasone, tetraphenylphosphonium (TPA+), triphenylmethylphosphonium (TPMP+), diphenyldimethylphosphonium (DDP+)	Gros et al 1992

*Sharom et al 1998 described a diverse group of linear or cyclic peptides (proteases inhibitors, membrane active toxins, membrane active antibiotics, immunosuppressant, ion selective ionophors, etc) as substrates of PGP

structure in which distinct parts responsible for binding different substances may exist The latter possibility may be deduced from paper of Loo and Clarke (1994b), where it has been shown that different aminoacids of TM6 segment (residues 330–351) of human P-glycoprotein play a role in resistance against different agents For example· replacement of Val338 by Ala resulted in enhanced resistance to colchicine and reduced relative resistance to vinblastine; replacement of Gly341 by Val conferred little resistance to colchicine or doxorubicin but resistance to vinblastine or actinomycin D was retained, replacement of Ala342 by Leu conferred resistance to all four drugs; replacement of Ser344 by Ala, Thr, Cys, or Tyr was unable to confer drug resistance; changes to Phe335 affected dissociation of vinblastine.

On the other hand, it has been found that several substances like ATP analogues, N-ethylmaleimide or vanadate affect PGP transport activity due to blocking of its ATP binding sites (al-Shawi et al. 1994; Urbatsch et al. 1994). Similarly to these substances, pentoxifylline and its derivatives depress the PGP mediated MDR probably in this way (Breier et al. 1994; Štefanková et al. 1996; Drobná et al. 2000)

Table 3. Chemosensitizing compounds which reverse multidrug resistance

REVERSING COMPOUNDS	REFERENCES
calcium channel blockers verapamil, galopamil, flunarizine, diltiazem, nimodipine, nifedipine, azidopine	Tsuro 1981, Cano-Gauci and Riordan 1987, Tytgat et al 1988, Barančík et al 1994, Boháčová et al 2000
calmodulin antagonists trifluoperazine, chlorpromazine, thioridazine, perphenazine	Kessels and Wilberding 1984, Vendrik et al 1992, Barančík et al 1994, Boháčová et al 2000
steroids progesterone, tamoxifen, cortisol	Ramu et al 1984, Barančík et al 1994
cyclic peptides cyclosporin A, valinomycin	Twentyman 1988, Barančík et al 1994
local anesthetics cinchocaine, articaine, lidocaine	Barančík et al 1994, Boháčová et al 2000
xantines pentoxiphylline	Breier et al 1994, Štefanková et al 1996, Boháčová et al 2000
drug analogs quinidine, chloroquine, hydrophobic cephalosporins	Wingler 1996, Bray and Ward 1998, Berger et al 1999, Vezmar and Georges 2000

PGP mediated MDR may be also affected by modulation of regulatory processes. For example phorbol myristate acetate (direct activator of PKC) was found to elevate (Barančík et al. 1995) and bisindolylmaleimid (inhibitor of PKC) was found to depress (Boháčová et al. 1999) the PGP mediated MDR Recently we observed differences in expression and activity of some mitogen-activated protein kinases (Barančík et al 1999) Moreover, we have found that inhibition of mitogen activated protein kinase p38-MAPK regulates the PGP mediated MDR (Barančík et al. 1999, 2001).

Conclusion

Although a wealth of information on ABC transporters has been generated in recent years, some important questions remain open

We still do not know:

- how can PGP and MRP operate as a drug efflux pump for such variable substances;
- how is their activity regulated and what are the ways to suppress the activity effectively;
- what is the exact role of PGP and MRP in normal physiology;

- how is PGP and MRP overexpression regulated in neoplastic cells under chemotherapeutic treatment,
- which ways can be used to stop the PGP and MRP overexpression

It is clear that the members of the superfamily of ABC transporters in prokaryotes and eukaryotes are involved in the transport of a wide range of substrates Some ABC transporters can transport very large substrates, whereas other prefer rather small substrates Our results (Breier et al 2000) suggest that to be transportable by PGP the substrates should fulfil some criteria (hydrophobicity, structural flexibility, molecular weight not exceeding 1000 g/mol, uncharged at physiological pH) However, these results should be further verified more precisely

ATP binding and hydrolysis appear to be essential for the proper functioning of PGP including drug transport Inhibition of drug transport could be induced by the blockage of specific sites responsible for recognition of the drugs, binding of ATP, ATP hydrolysis, or coupling of ATP hydrolysis to translocation of the drug

It is evident that substances effectively inhibiting transport activities of PGP and MRP would open a way to fundamental improvements in chemotherapy of resistant tumors In the future better knowledge about structural features of this inhibition is needed for understanding the molecular mechanisms of depression of PGP and other ABC transporters activity

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