ABC-transporters: implications on drug resistance from microorganisms to human cancers

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Abstract

Resistance to chemotherapy is a common clinical problem in patients with infectious diseases as well as in patients with cancer. During treatment of infections or malignant tumors, the drug targets of prokaryotic or eukaryotic microorganisms and neoplastic cells are often found to be refractory to a variety of drugs that have different structures and functions. This phenomenon has been termed multidrug resistance (MDR). The mechanisms leading to MDR are frequently caused by trans-membrane xenobiotic transport molecules belonging to the superfamily of ATP-binding cassette (ABC) transporters. There is an urgent need to understand the structure-function relationships of these efflux pumps that underlie their transport mechanism and drug selectivity. This knowledge may allow the rational design of new drugs that can inhibit or circumvent the activity of these MDR transport molecules. Furthermore, the development of such chemosensitizing agents would help us learn more about the physiological functions and substrates of these pump proteins. This review will discuss the current state of knowledge of the functional and structural similarities among ABC-transporters in prokaryotic and eukaryotic cells and their impact on MDR.

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1. Introduction

Cellular resistance to anti-microbial agents or anti-neoplastic drugs is the major reason why treatment of infectious diseases or malignant tumors may fail. Pathogenic microorganisms as well as human cancer cells have developed various biological mechanisms that provide a defense against cytotoxic attack by chemotherapeutics. Ordinarily, resistance is specific to a single class of anti-microbial or anticancer drugs. One of the best studied example of specific bacterial resistance to penicillins, cephalosporins, and other related β-lactam antibiotics is that promoted by plasmid encoded β-lactamases [1]. Microorganisms as well as human cancer cells may also exhibit a cross-resistant phenotype against several unrelated drugs that differ widely with respect to molecular structure and target specificity. This phenomenon has been termed multidrug resistance (MDR). So far, different main types of MDR phenotypes have been described. ‘Classical’ MDR phenotype, first described for cancer cells, is mediated by a plasma membrane-spanning multidrug transport protein, P-glycoprotein (P-gp) [2]. This phenotype is characterized by the presence of cross resistance to a well defined spectrum of drugs as well as by specific agents that reverse resistance—termed as chemosensitizers or MDR modulators (Table 1). A large number of different multidrug transporter proteins have since been identified in cancer cells [3] as well as in pathogenic microorganisms [4].

It is important to note that cancer cells can exhibit an MDR phenotype by mechanisms other than those involving P-gp. Alterations in signal-transduction pathways can negate the activity of drugs that accelerate programmed cell death [5]. The classical example for such a defective cellular pathway is a mutated, functional inactive p53 that is no longer able to trigger apoptosis after detection of DNA damage caused by given agents [6]. Cellular repair enzymes, such as DNA repair systems, can also mediate a MDR phenotype [7]. However, in the case of the DNA-mismatch repair (MMR) system, a loss of MMR enzyme activity leads to a drug-resistant phenotype [8]. This phenomenon

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Table 1

Selected drugs of the ‘classical’ MDR spectrum and ‘classical’ MDR modulators

<table>
<thead>
<tr>
<th>Drugs transported by P-gp</th>
<th>‘Classical’ MDR modulators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthracyclines: daunorubicin, doxorubicin</td>
<td>Calcium channel blockers: verapamil, nifedipine, azidopine, dihydropyridines</td>
</tr>
<tr>
<td>Vinca alkaloids: vinblastine, vincristine, vindeine</td>
<td>Immunosuppressants and derivatives: cyclosporin A, FK506, PSC833</td>
</tr>
<tr>
<td>Epipodophyllotoxines: etoposide, teniposide</td>
<td>Antiarrhythmics: quinine, quinidine, amiodarone</td>
</tr>
<tr>
<td>Antibiotics: actinomycin D, daunomycin, mitomycin C, Taxanes: paclitaxel</td>
<td>Antibiotics: hydrophobic dactinomycin, mitomycin C, teniposide</td>
</tr>
<tr>
<td>Others: colchicine, topotecan, STI571, valinomycin, puromycin, eteine</td>
<td>Epipodophyllotoxines: etoposide, teniposide</td>
</tr>
<tr>
<td>Many other hydrophobic amphipathic drugs and derivatives</td>
<td>Steroid hormones and derivatives: progesterone, tamoxifen</td>
</tr>
<tr>
<td>HIV protease inhibitors: sequinavir, indinavir, retanavir</td>
<td>Others: GF120918, LY335979, VX710, XR9576, R101933, LY335979, OC1440935</td>
</tr>
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may be caused by a decreased MMR-dependent stimulation of cellular pathways triggering apoptosis after recognition of DNA-damage by the MMR system. Activation of xenobiotics detoxifying enzyme systems, such as glutathion-S-transferases (GST), also play an important role in the development of MDR in human malignancies [9]. MDR cancer cells have been shown to isolate anticancer drugs in vesicular compartments and extrude them via exocytosis [10] or reduce drug-uptake by a reduction of endocytosis [11].

3. ABC-transporters

ABC-transporter proteins have to be distinguished from ABC-proteins. Both types of proteins are defined by the presence of a highly conserved approximately 215 amino acids consensus sequence designated as ABC, ABC domain, ABC-ATPase domain, or nucleotide-binding domain (NBD). The domain contains two short peptide motifs, a glycine-rich Walker A- and a hydrophobic Walker B motif [16], both involved in ATP binding and commonly present in all nucleotide-binding proteins. A third consensus sequence is named ABC signature [17] and is unique in ABC domains. ABC-containing proteins couple the phosphate bond energy of ATP hydrolysis to many cellular processes and are not necessarily restricted to transport functions. However, the proper meaning of the term ABC-transporter protein, also designated as traffic ATPase or permease for import systems, is satisfied when the ABC-protein is, in addition, associated with a hydrophobic, membrane-embedded transmembrane domain (TMD) usually composed of at least six transmembrane (TM) α-helices. TMDs are also designated as membrane spanning domains (MSD). The TMDs are believed to determine the specificity for the substrate molecules transported by the ABC-transporter protein. The minimal structural requirement for a biological active ABC-transporter...
seems to be two TMDs and two ABCs [TMD-NBD]2. In ‘full-transporters’, this structural arrangement may be formed by a single polypeptide chain and in multi-protein complexes by more than one polypeptide chain (Fig. 2). Most of the prokaryotic genes encoding ABC-transporters are organized in operons that contain ABC domains and TMDs as separate subunits requiring assembly as a biologically active transporter. In some ABC-transporter encoding genes the different domains are already fused into higher structural units, e.g. in so-called ‘half-transporters’ [TMD-NBD] or ‘full-transporters’ [TMD-NBD]2. The organization of ABC-transporter encoding genes in eukaryotic organisms also shows wide variation. Nevertheless, the different domain combinations are commonly distributed in one or two genes encoding ‘half- or full-transporters’.

4. ABC-transporters in bacterial drug resistance

Although most prokaryotic drug transporters belong to the class of secondary active transporters in particular drug–proton exchange systems [14], several drug transporting systems utilize the energy of ATP hydrolysis to drive drug extrusion and belong to the ABC-transporter family (Table 2). Most drug transporters mediate single drug resistance (SDR) in Gram-positive bacteria. SDR transporters are specific for a single or a group of closely related drugs, such as Ardl from Streptomyces capreolus that confers resistance to the aminonucleoside antibiotic A201A [18], the Streptomyces logisporoflavus transporter TnrB [19] that mediates SDR to polyether–iophore antibiotic tetronasin, or the plasmid-encoded Staphylococcus epidermidis MsrA protein. MsrA that consists of two NBDs and mediates active efflux of the macrolide antibiotic erythromycin from MsrA encoding plasmid transformed cells of Staphylococcus aureus [20]. Because antibiotic resistance of some important human pathogens, such as S. aureus or S. epidermidis is mediated by ABC-transporters, the inhibition of ABC transporter function in these organisms has been a subject of intensive study.

Since bacteria that synthesize antibiotic active substances must also be protected from the toxic effects of these compounds, it was not surprising that Streptomyces peuceticus the producer of the clinical commonly used anticancer anthracyclines, daunorubicin and doxorubicin, expresses the SDR transporter DrrAB that confers resistance to both cytotoxic secondary metabolites [21].

The first genuine ATP-dependent prokaryotic MDR transporter LmrA was identified in Lactococcus lactis [22]. The 65 kDa transporter protein consists of 590 amino acid-residues, a N-terminal TMD with six TM α-helices, and a C-terminal NBD. Thus, LmrA is a ‘half-transporter’ that is homologous to members of the P-gp containing human subfamily B (MDR/TAP) of ABC-transporters, most notably the classical MDR transporter P-gp itself. LmrA and each half of P-gp share 34% identical amino acids-residues and 16% of conservative amino acids substitutions [23]. Reconstitution studies using mutant NBDs of LmrA suggested that LmrA is functionally active as a homodimeric complex [24]. The pharmacological characteristics of LmrA are very similar to P-gp, and include the spectrum of substrates
Important antineoplastic drugs such as anthracyclines, e.g. daunorubicin and doxorubicin, and vinca alkaloids, e.g. vincristine and vinblastine, as well as cytotoxic agents such as colchicine, ethidium bromide, valinomycin, Hoechst 33342, or rhodamine 123 are part of this spectrum [26]. Moreover, chemosensitizers or MDR modulators such as verapamil, or cyclosporin A that reverse of P-gp-mediated MDR, also act as potent inhibitors of LmrA [25]. Transformation of the hypersensitive Escherichia coli strain CS1562 by a LmrA encoding plasmid has resulted in an increased resistance to 17 out of 21 clinically most used antibiotics belonging the aminoglycoside, lincosamide, macrolide, quinolone, streptogramins, tetracyclines, chloramphenicol broad spectrum family of antibiotics [27]. In addition, a slightly enhanced resistance to certain β-lactam antibiotics has been observed. As described for several ABC-transporters, including P-gp [28,29], these membrane-spanning proteins can translocate phospholipids. LmrA catalyzes the ATP-dependent transport of fluorescent phosphatidylethanolamine, but not of fluorescent phosphatidylcholine [30]. These observations demonstrate that LmrA may be involved in the transport of specific lipids or lipid-linked precursors in L. lactis. Taken together, these data demonstrate the remarkable broad substrate specificity of LmrA.

A structural homologue to L. lactis LmrA is the plasmid encoded ABC-transporter HorA of Lactobacillus brevis [31]. HorA mediates resistance to hop (Humulus lupulus L.) compounds, i.e. iso-α-acids, that give beer a bitter taste and exert bacteriostatic effects on most Gram-positive bacteria due to their ability to dissipate the proton motive force [32]. Furthermore, HorA confers resistance to the structurally unrelated compounds novobiocin, ethidium bromide, and Hoechst 33342 [31]. Of major importance is that HorA is encoded by a plasmid. Thus, HorA may be exchanged between pathogenic microorganisms and may be responsible for acquired clinical MDR of bacteria.

Organisms low in the scale of evolution such as archaeabacteria express ATP-dependent MDR-transporter systems. The halophilic archaeon Haloferax volcanii
contains a drug efflux system that mediates the transport of anthracyclines, vinca alkaloids, ethidium bromide, monensin, and rhodamine 123 [33,34]. Moreover, this ATP-dependent drug efflux system can be inhibited by calcium channel antagonists verapamil or nifedipine which are also potent inhibitors of P-gp, and have therefore been termed to act as ‘classical MDR modulators’. These observations collectively indicate the presence of a P-gp- respective LmrA homologous drug extrusion protein in Haloferax volcanii. However, the gene encoding this ABC-like MDR transporter remains to be cloned.

Surprisingly no biological active SDR or MDR ABC-transporter has yet been identified in Gram-negative bacteria. Although sequence homology searches identified a few putative drug transporters in Gram-negative bacteria, no experimental evidence demonstrated any drug transport by these ABC-transporters [35]. For example, the L. lactis LmrA homologue MsbA in E. coli, involved in the transport of lipids to the inner surface of the outer membrane [36], does not show any drug-related functions. Although the E. coli genome [37] encodes 57 ABC-transporters that occupy almost 5% of the genome [38], and represent the largest family of proteins in E. coli, the question of why ABC-transporter-based drug-transporting systems are absent in Gram-negative bacteria in view of the relatively frequent occurrence of such ABC-transporters in these organisms is not yet clear.

5. ABC-transporters in drug resistance of fungi

In fungi, two major classes of xenobiotics transporters are involved in drug resistance, the family of primary active ABC-transporters and the family of proton-motive force driven MFS-transporters. In contrast to prokaryotic microorganisms, in fungi the ABC-transporters comprise the largest number of membrane-spanning efflux pumps [13]. Thus, it was not surprising that the first described ABC-transporter homologous to P-gp from a non-mammalian system was that from S. cerevisiae. This transporter consisting of 1290 amino acid-residues, is designated as Ste6p and is encoded by the STE6 gene [39]. Ste6p exhibiting a [TMD-NBD]2 configuration is physiologically involved in the transport of the mating a-factor pheromone, but it has no role in drug resistance. However, from at least 29 putative ABC-transporter encoding genes in S. cerevisiae [40] 5 genes encode ABC-transporters, Pdr5p, Pdr12p, Snq2p, Ycf1p, and Yor1p, mediating a MDR phenotype when present in multiple copies. Thus, overexpression of the Ste6p and P-gp homologous transmembrane transporter Pdr5p results in resistance to a large number of chemically unrelated compounds, including several classes of clinically important antimycotics, antibiotics, anticancer drugs, mycotoxins, agricultural fungicides, and herbicides [41–43]. Pdr12p can confer resistance to food preservatives such as sorbic, benzoic, and propionic acid [44,45]. Snq2p confers hyper-resistance to the mutagens 4-nitroquinoline-N-oxide and triaziquone, as well as to the chemicals sulphonmethuron methyl and phenanthroline when present in multiple copies [46]. Ycf1p, and Yor1p exhibit a broad substrate specificity with partial overlapping to that of Pdr5p [47–50]. Since null mutants of the Pdr5p, Pdr12p, Snq2p, Ycf1p, and Yor1p encoding genes are viable and no drug resistance-independent phenotype could be clarified, the physiological roles of these MDR-associated ABC-transporters in S. cerevisiae remains to be defined.

In other yeast species, such as the fission yeast Schizosaccharomyces pombe, further ABC-transporters have been identified. Sequencing of the S. pombe genome revealed at least six members from the family of ABC-transporters [51]; for two of these it could be demonstrated to play a role in MDR. Enhanced expression of the 1362 amino acids containing P-gp homologue Pmd1 encoded by pmd1+ gene confers resistance to the antimeytotic agent leptomycin B, as well as to various other cytotoxic compounds [52]. The second ABC-transporter involved in MDR in S. pombe is Bfr1 consisting of 1530 amino acid-residues. The Bfr1 encoding gene bfr1+ or hba2 was identified due to its ability to mediate resistance to brefeldin A when over-expressed [53,54]. Moreover, Bfr1 confers resistance to various other compounds, including actinimycin, cerulenin, and cytochalasin B, and it is not essential for cell growth or mating.

Candida albicans, isolated in more than 50% of fungal infection cases [55], has become an increasing clinical problem in the past decades. The increase in fungal, especially C. albicans infections is considered to be a consequence of the growing number of immuno-compromised patients, e.g. organ transplant -, cancer -, of HIV patients. As a consequence of the application of antymycotic agents, drug resistance has become problematic for the treatment of candidiasis [56]. In C. albicans five genes encoding members of family of ABC-transporters have been identified, CDR1, CDR2, CDR3, CDR4, and CDR5. Two gene products of these genes, Cdr1p and Cdr2p, have been demonstrated to play a major role in drug resistance. Cdr1p, consisting of 1501 amino acids, confers resistance to the clinically important azole antymycotics fluconazole and miconazole, as well as to allylamines, morpholines, and several other drugs [57,58]. The 1499 amino acids containing ABC-transporter Cdr2p, encoded by the CDR2 gene, exhibits 84% identity with Cdr1p and confers resistance to azole antifungal agents, other antymycotic agents, such as terbinafine and amorolfine, and to a variety of metabolic inhibitors, when over-expressed [58].
6. ABC-transporters in parasites

Drug resistance has become a major impediment to the treatment of diseases caused by protozoan parasites. Malaria, caused by the parasite *Plasmodium*, is the most prevalent tropical disease and is estimated to affect 300–500 million people and cause 2–3 million deaths annually. Of the four *Plasmodium* species that infect man, *Plasmodium falciparum* causes profound pathology and an overwhelming parasitemia resulting in high mortality rates in untreated cases. More than 41% of the world’s population is at risk for this infection that is due to the rapid spread of drug-resistant protozoans [59]. Although mutations in the gene encoding the transmembrane transporter protein PfCRT are associated with resistance of *P. falciparum* to the primary antimalaria agent chloroquine [60], ABC-transporters are good candidates for drug resistance mediating factors. PfCRT belongs to a family of putative transporters or channels consisting of ten transmembrane segments, that are not associated with a typical sequence or other recognizable features such as ATP binding motifs. Sequencing of the *P. falciparum* genome revealed fewer members of the family of ABC-transporters in *P. falciparum* than in *S. cerevisiae* [61]. However, at least three ABC-transporter encoding genes have been identified in *P. falciparum*, two P-gp homologues Phg1 [62,63] and Phg2 [64], and PfGCN20 [65]. The pfdm1 gene on chromosome 5 encoding Phg1 was originally identified to be amplified in chloroquine-resistant isolates [62]. Further studies demonstrated that the 162 kDa ABC-transporter Phg1 is localized at the parasite vacuole, the site of action of chloroquine [66], but subsequent analyses failed to correlate the over-expression of the pfdm1 gene with resistance to chloroquine [66]. Additional studies, e.g. linkage analyzes, strongly suggest that additional factors are required for chloroquine resistance [67,68]. However, the activity of Phg1 can be inhibited by verapamil and transfection experiments using different alleles of the Phg1 encoding cDNA demonstrated that mutations in Phg1 can confer resistance to the clinical important antimalarial agents mefloquine, quinine and halofantrine. Moreover, the same mutations influence parasite resistance towards chloroquine in a strain-specific manner and the level of sensitivity to the structurally unrelated compound artemisinin [69]. Transfection experiments of Phg1 in CHO cells suggested that Phg1 may function physiologically as a regulator of digestive vacuole pH in concert with an H+-ATPase either as a chloride channel or as indirect regulator of chloride channels [70]. Although, the available data are confusing and conflicting, it appears most likely that the chloroquine-resistant phenotype in *P. falciparum* is a complex event mediated by multiple genes with an important contribution of the ABC-transporter Phg1. There has been no data to support the function of the 110 kDa gene product of the pfmdr2 gene Pgh2, and the 95 kDa PfGCN20 transporter encoded by the pfgn20 gene, in drug resistance of *P. falciparum*.

Among parasitic infections, leishmaniasis has increased dramatically and has become the second leading cause of death worldwide [71]. Chemotherapy remains the only effective way to control infections caused by the intracellular *Leishmania* species. Drugs for the treatment of leishmaniasis are pentavalent antimonials in the form of glucantime and pentostam [72], but these antimony-based and alternative metal-based drugs are not very efficient due to their toxicity and the increased appearance of drug resistance [73]. Although, the *Leishmania*, i.e. *Leishmania major*, genome project has not been completed, several ABC-transporters have been identified and found to be involved in drug resistance of *Leishmania* species [74]. It was reported that the gene pgpA encoding the ABC-transporter PGPA is frequently amplified in metal-resistant *Leishmania tarentolae* [75]. Transfection experiments clearly demonstrated that PGPA contributes to resistance against antimony- and arsenic-based antiprotozoan drugs [76–78]. Since the level of drug resistance conferred by PGPA differed depending in which *Leishmania* species the PGPA encoding cDNA was transfected, it led to the suggestion that PGPA requires additional factors for conferring high levels of drug resistance. The availability of these so far unknown factors may differ in various *Leishmania* species. PGPA, together with PGBP, PGPC, PGPD, and PGPE represents a subfamily of ABC-transporters with homology to the human subfamily C (CFTR/MRP subfamily) [79]. Transfection experiments failed to demonstrate a role in drug resistance for any of the additional four ABC-transporters, indicating that PGPA is the only member of this subfamily involved in drug resistance. In several *Leishmania* species the ABC-transporter MDR1, a homologue to the human P-gp was characterized [80–84]. Transfection experiments using the *Leishmania* MDR1 encoding cDNA indicated that this ABC-transporter can cause a multidrug-resistant phenotype directed against anticancer agents of the human classical MDR spectrum. Although, these drugs are not used in the treatment of leishmaniasis, *Leishmania* MDR1 may extrude cytotoxic compounds from the cell and be required for detoxification.

The protozoan *Entamoeba histolytica* infects 500 million people, causes 50 million cases of dysentery or liver abscesses, and kills 100,000 humans each year worldwide [85]. Treatment of amoebiasis is primarily by amoebicides, using mainly metronidazole and emetine. Since differences in drug susceptibility have been reported in *E. histolytica* isolates as well as laboratory strains, it was suggested that the parasite can develop a MDR phenotype in its human host [86,87]. Evidence was provided that verapamil-sensitive ABC-transporters
may be involved in resistance against amoebicides in drug-resistant parasites [88,89]. Presently, six P-gp-like genes, EhPgp1–6, have been cloned and characterized [90]. Apparently, four of these genes, EhPgp1, EhPgp2, EhPgp5, and EhPgp6, are transcribed in drug-resistant mutants of *E. histolytica* where only the expression of the EhPgp1 and EhPgp2 genes correlates with the drug resistance level in the trophozoites.

### 7. ABC-transporters in human cancers

In the Western world cancer is the second largest cause of mortality, after cardiovascular disease [91]. The phenomenon of MDR in cancer cells, the simultaneous cross resistance of tumor cells to structurally and functionally unrelated drugs, is as old as the chemotherapeutic treatment of human malignancies. The original concept of MDR was introduced into the scientific literature in 1970 [92]. At least two types of MDR can be distinguished on the basis of different mechanisms, the so-called ‘classical’ or P-gp-depending MDR, and the ‘atypical’ or non-Pgp-depending MDR. The ‘classical’ MDR phenotype is characterized by a typical cross resistance pattern against natural-product anticancer agents, such as vinca alkaloids, anthracyclines, or taxanes, and the reversibility by verapamil and cyclosporin A derivatives (Table 1). The ABC-transporter P-gp was originally isolated from the plasma membranes of Chinese hamster ovary cells displaying the ‘classical’ MDR phenotype in 1976 [93]. P-gp was purified in 1979 [94], found to be encoded by MDR1 or ABCB1 gene [95], and also found to be over-expressed in a large number of multidrug-resistant human and mammalian cells [96,97]. Structurally, the human 170 kDa P-gp consists of 1280 amino acids residues forming a [TMD-NBT]2 configuration. In various cancer types, including acute myeloid leukaemia, various childhood tumors, and local-regionally advanced breast cancer, over-expression of the P-gp encoding MDR1 gene has been found to correlate with poor outcome in patients treated with chemotherapy [98–103]. These data indicate that P-gp-mediated MDR is the cause of poor treatment outcome in P-gp-positive tumors.

Since completion of the human genome sequence [104,105], 48 different ABC-transporters have been identified and divided by their phylogenetic characteristics into 7 subfamilies, ABCA, ABCB, ABCC, ABCD, ABCE, ABCF, and ABCG [106]. Besides the MDR1 gene encoded P-gp mediating the ‘classical’ MDR phenotype, ABC-transporters have important roles in ‘atypical’ forms of MDR and at least 12 human ABC-transporters are associated with drug transport (Table 3).

The second major ABC-transporter involved in MDR of human cancers was described in 1992 [107]. This 190 kDa ABC-transporter was found to be over-expressed in a doxorubicin-selected lung cancer cell line and originally named MDR-associated Protein, MRP. Due to the identification of various homologous proteins to MRP...
The cystic fibrosis trans-membrane conductance regulator CFTR [109], the MRPs constitute the 12 transporter containing human ABC-transporter subfamily C. MRP1 shares only between 34 and 58% sequence identity with the other MRPs (MRP2-MRP6), but the overall membrane topology is similar in all members of subfamily C. In addition to the [TM-D-NBT]2 configuration of P-gp, MRP1 has an additional TMD0 domain consisting of 5 TM α-helices attached to the N-terminal forming a [TMD0(TMD-NBT)2] configuration. Anticancer drug substrates for MRP1 include anthracyclines, vinca alkaloids, epipodophyllotoxins, and methotrexate, whereas the physiological substrates appear to be glutathione-, glucuronide-, and sulfate-conjugated compounds [110]. Although, MRP1 expression was analyzed in different human cancers, e.g. breast cancer [111] or ovarian cancer [112], studies have both confirmed and rejected a correlation between clinical outcome and expression. Thus, the role of MRP1 in clinical MDR remains to be elucidated.

MRP2 or cMOAT, has been shown to be the bilirubin glucuronide transporter at the canalicular membrane of the hepatocyte [113]. MRP2 originally was found to be over-expressed in cisplatin-resistant cancer cells [114]. Moreover, transfection experiments demonstrated that MRP2 can confer resistance against anthracyclines, vinca alkaloids, epipodophyllotoxins, camptothecins, and methotrexate [115]. Despite clear identification of the role of MRP2 in normal physiology and by several in vitro studies demonstrating a role in drug resistance, convincing evidence for a role in clinical MDR has not yet been forthcoming.

Transfection experiments have shown that over-expression of MRP3 conferred resistance against vinca alkaloids, epipodophyllotoxins, and methotrexate [116,117]. MRP4 was shown to confer resistance against nucleotide-based antiviral drugs as well as methotrexate [118–120].

In addition, transfection studies demonstrated that MRP5 is able to mediate resistance against thiopurine anticaner drugs 6-mercaptopurine and thioguanine and the anti-HIV drug 9-(2-phosphonylmethoxyethyl)adenine [121]. Finally, there is no indication that MRP6 is associated with any form of drug resistance.

The long sought mitoxantrone transporter was identified by 3 independent studies. This ABC-transporter was designated as ‘breast cancer resistance protein’ (BCRP) [122], ‘mitoxantrone resistance-associated protein’ (MXR) [123], or ‘placenta-specific ABC gene’ [124]. The 72 kDa ABC-transporter is a so-called ‘half-transporter’ with a [NBD-TMD] configuration that probably forms dimers to produce an active transport complex [125]. The very high expression of BCRP in normal placenta suggests that this transport molecule may have a putative physiological role in maintaining the placental barrier. Moreover, BCRP was found to be over-expressed in various drug-resistant cancer cell lines selected for resistance to mitoxantrone, doxorubicin or topotecan. Investigations of the clinical relevance are at a very early stage and have yielded contradictory data. Thus, whereas BCRP is not consistently up-regulated in relapsed/refractory acute myeloid leukemia (AML) [126], alternative studies revealed that expression of BCRP is associated with clinical resistant disease in AML of the elderly [127] and childhood AML [128].

The remaining human ABC-transporters that were demonstrated to be able to transport drugs, exhibit only a weak correlation between expression and drug-resistant phenotype. Thus, over-expression of ABC2 contributes to estramustine resistance [129] and that over-expression of both sub-units of the dimeric ‘transporter associated with antigen presentation’ (TAP), TAP1 and TAP2, results in increased resistance against mitoxantrone or etoposide [130,131].

8. Conclusion

ABC-transporters are believed to date back more than 3 billion years in evolutionary time and are distributed in all three kingdoms of living organisms [132]. These transport proteins play important physiological roles in the transport of different molecules through biological membrane structures. Moreover, from bacterial cells over fungi cells and protozoan cells to human cancer cells this superfamily of transporter proteins is of fundamental relevance for the phenomenon of drug resistance. The activity of ABC-transporters represent a basic biological strategy to defend living cells against the cytotoxic attack of xenobiotics. However, the characterization of the detailed mechanisms causing drug resistance, as well as in pathogenic prokaryotic and eukaryotic microorganisms, and human cancer cells, is of fundamental importance for the development of therapeutic strategies to prevent or circumvent resistance to treatment. By understanding the exact mechanisms it will be possible to design improved patient-tailored combinations of chemotherapy and selection of the most effective chemosensitizing agents.

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[108], it is now designated as MRP1. Together with the cystic fibrosis trans-membrane conductance regulator CFTR [109], the MRPs constitute the 12 transporter containing human ABC-transporter subfamily C. MRP1 shares only between 34 and 58% sequence
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