

# Drug resistance in breast cancer

## Review Article

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### Summary

Resistance to cytotoxic chemotherapy is the main cause of therapeutic failure and death in women suffering on breast carcinoma. Commonly, patients refractory to chemotherapeutic treatment regimens show resistance to multiple antineoplastic agents of different structure and mode of action, i.e. the cancerous breast tissue exhibits a multidrug resistance (MDR) phenotype. Clinical MDR of breast cancer is likely to be multifactorial and heterogenous. Several mechanisms have been identified to play a role in MDR, e.g. overexpression of various members of the superfamily of ABC (adenosine triphosphate binding cassette)-transporters have been shown to be associated with MDR in solid tumors including breast cancer. Besides the classical MDR transporter P-glycoprotein (P-gp) additional ABC-transporters such as MRP1 or BCRP have been analyzed concerning their role in clinical MDR of breast cancer. Moreover, "upstream" factors like transcription factors regulating the gene activity of ABC-transporter encoding genes, such as the Y-box transcription factor YB-1 were demonstrated to play a role in MDR of mammary carcinoma. However, since the available data are contradictorily, hitherto the clinical significance of these and various other molecules on breast cancer remains unclear. This review will discuss the current state of knowledge of MDR-associated factors and their impact on clinical MDR in breast carcinoma.

### I. Introduction

Breast cancer is the most frequent form of cancer and the leading cause of death among females in the Western world, where, despite of radical mastectomy approximately one third of affected women die (Kelsey and Berkowitz, 1988). Around one of 10 Western women will develop breast cancer at some time in their lifetime. Although chemotherapy improves survival rates in the adjuvant setting, around 50% of all treated patients will relapse (Harris et al., 1993). The major reason for therapeutic failure is the development of resistance against anticancer agents used. Under clinical circumstances it is unknown whether drug-resistant mammary carcinoma cells occur as a result of the pressure of antineoplastic agents, or if they were already present in the tumor at the start of the chemotherapeutic treatment that they survive.

Recent pharmacological treatment regimens of breast cancer include (i) conventional chemotherapy on the basis of cytotoxic anticancer drugs, (ii) in steroid-hormone receptor-positive patients an endocrine therapy, e.g. the use of adjuvant tamoxifen in estrogen receptor (ER)-positive tumors (Osborne, 1998), and (iii) an immunological-basing therapy, e.g. in proto-oncogene

HER-2/*neu*-positive neoplasms of the breast, the use of the monoclonal antibody trastuzumab directed against that oncoprotein (Hortobagyi, 2001; Vogel et al., 2002). For the majority of patients, the necessary treatment will probably be a combination of these pharmacological treatment options. However, here the mechanisms of drug resistance against classical cytotoxic compounds used against breast cancer will be discussed; endocrine and immunological therapy will not be within the scope of this mini overview.

Biological resistance mechanisms of solid tumors against cytotoxic antitumor agents can be distinguished in (i) pharmacokinetic resistance, and (ii) cellular resistance. Important factors of pharmacokinetics include low dose metabolic inactivation, the location of tumor deposits in so-called pharmacological sanctuaries, e.g. compartments behind the blood-brain barrier, and poor penetration of drugs through the interstitial tumor tissue. However, within this mini-review merely the cellular drug resistance mechanisms in breast cancer will be discussed.

Traditional chemotherapy protocols for the treatment of advanced breast cancer consisted of cyclophosphamide, methotrexate, 5-fluorouracil, prednisone, and vincristine combinations (Harris et al., 2000). Later on, anthracycline-

based chemotherapy has gradually become standard in the treatment of advanced breast cancer. Doxorubicin and its analogue epirubicin are considered as highly active anthracyclines, that are commonly used in combinations with 5-fluorouracil and cyclophosphamide. Although, breast cancer is often considered as one of the more drug-sensitive solid tumors, all initially responsive cancers relapse and develop drug resistance, in the case of resistance against a broad spectrum of structurally unrelated drugs with different mode of action a multidrug resistance (MDR).

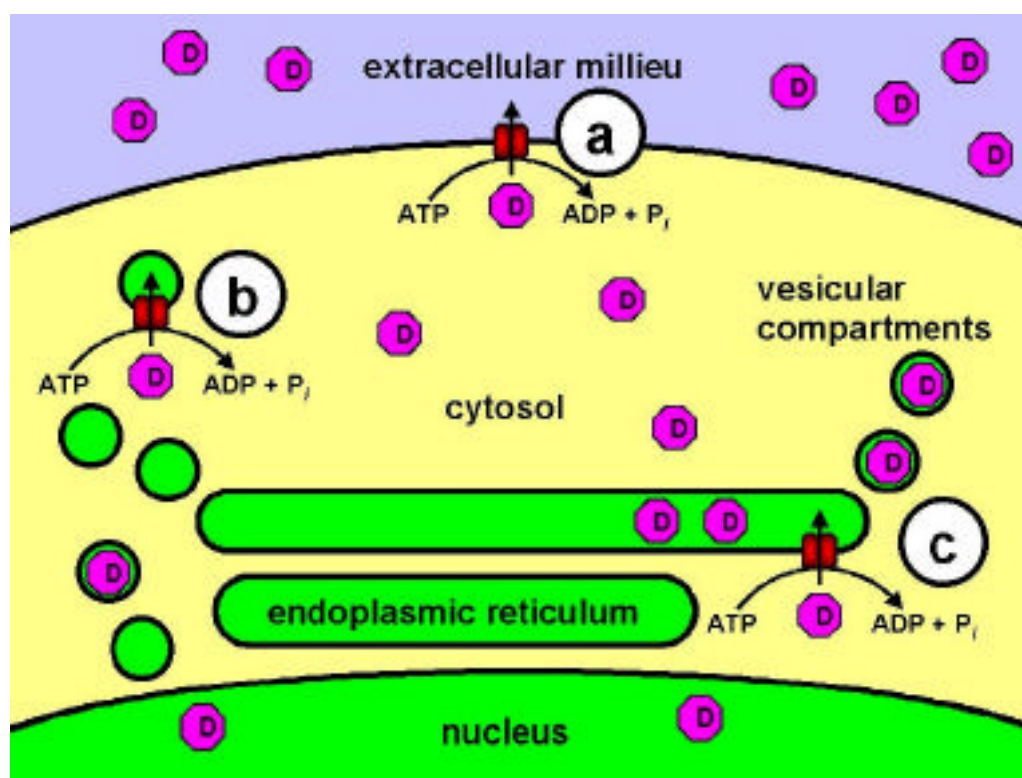
## II. The multidrug resistance (MDR) phenotype in breast cancer

The original concept of MDR was introduced into the scientific literature in 1970 (Biedler et al., 1970). The multidrug-resistant phenotype is frequently characterized by a cross-resistance to drugs to which the tumor has not been exposed previously. Such a MDR phenotype can be intrinsic (primary) or acquired (secondary). The development of a MDR in advanced breast cancer is primarily responsible for the failure of current treatment regimens (Trock et al., 1997). Despite comprehensive knowledge on in vitro mechanisms of MDR, the precise nature of the in vivo drug-resistant phenotype in breast cancer remains unclear.

At least two types of MDR can be distinguished on the basis of different mechanisms: (i) the so-called “classical” or P-glycoprotein (P-gp)-depending MDR, and (ii) the “atypical” or non-P-gp-depending MDR. The most extensively studied mechanism of drug resistance is the “classical” MDR phenotype characterized by a typical cross resistance pattern against natural product-derived anticancer agents, such as anthracyclines (doxorubicin and epirubicin are among the most effective cytotoxic drugs used in the treatment of breast cancer), epipodophyllotoxines, *Vinca* alkaloids, or taxanes, and the reversibility by the calcium channel inhibitor verapamil and cyclosporin A derivatives. The underlying mechanism conferring this “classical” MDR phenotype is the cellular overproduction of a 170-kDa, membrane-spanning P-gp (P-170, PGY1, MDR1, ABCB1) (Ling et al., 1997), member of the superfamily of ABC (adenosine triphosphate binding cassette)-transporters (Lage, 2003).

## III. Human ABC-transporters

ABC-transporters act as energy-dependent drug efflux pumps, thereby decreasing the accumulation of cytotoxic agents in the intracellular milieu (Figure. 1). ABC-transporter proteins are defined by the presence of a highly conserved approximately 215 amino acids consensus sequence designated as ABC, ABC domain,



**Figure 1.** Schematic diagram that shows various possibilities of mechanistic action of ABC-transporters mediating drug resistance in breast cancer. (a) ABC-transporters are predominantly localized to the cytoplasm membrane. In an ATP-dependent manner the drugs will be extruded from the cell by the transporter proteins. (b) On the other side, it is also possible that ABC-transporters pump activity contributes to vesicular compartmentation of cytotoxic drugs, or (c) that ABC-transporters facilitate phase II drug metabolism by carrying xenobiotic substances into the lumen of the endoplasmic reticulum. D, anticancer drug.

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 (Walker et al., 1982), both involved in ATP binding and commonly present in all nucleotide-binding proteins. A third consensus sequence is named ABC signature (Hyde et al., 1990) 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, 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) -helices. 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]<sub>2</sub>. In “full-transporters”, this structural arrangement may be formed by a single polypeptide chain and in multiprotein complexes by more than one polypeptide chain. The organization of human ABC-transporter encoding genes are commonly distributed in one gene encoding a “full-transporters” [TMD-NBD]<sub>2</sub> or two genes encoding subunits of heteromeric “half-transporters” [TMD-NBD] (Figure. 2).

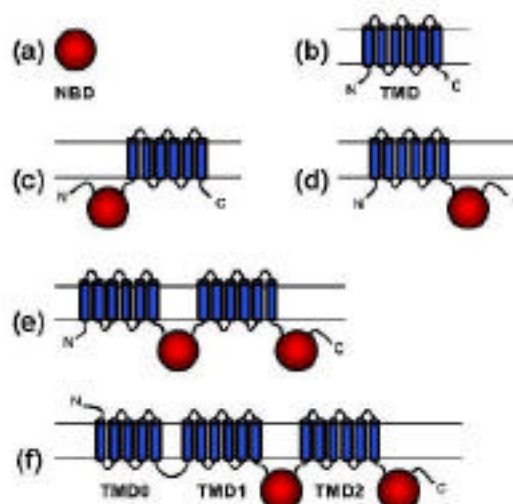
Since completion of the human genome sequence (Lander et al., 2001; Venter et al., 2001), 48 different ABC-transporters have been identified and were divided by their phylogenetic characteristics into 7 subfamilies, ABCA, ABCB, ABCC, ABCD, ABCE, ABCF, and ABCG (Dean et al., 2001). Besides P-gp mediating 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 in human cancers (Table 1).

### A. P-gp (ABCB1)

The 170 kDa P-gp represents the first purified (Riordan et al., 1979) human ABC-transporter protein and is the best characterized molecule involved in MDR (Ling et al., 1997). Structurally, this *mdr1* gene encoded transporter consists of 1280 amino acids residues forming a [TMD-NBT]<sub>2</sub> configuration. Very early studies of MDR demonstrated frequently expression of P-gp in breast cancer (e.g. Sugawara et al., 1988; Goldstein et al., 1989; Ro et al., 1990; Gerlach et al., 1987; Wallner et al., 1991; Verrelle et al., 1991; Keith et al., 1990; Merkel et al., 1989; Sanfillippo et al., 1991; Wishart et al., 1990; Schneider et al., 1989). These early studies were limited by small sample size, the retrospective character, differences in detection methods and therewith the enormous discrepancies in results. In these studies the percentage of P-gp-positive breast cancer samples varied between 0% and 85%. However, a meta-analysis of 31 studies performed by Trock et al. (1997) revealed that 41% of breast cancers expressed P-gp, the frequency of detectable expression increased after therapy, and the P-gp expression was associated with a higher likelihood of

treatment failure. Likewise this meta-analysis confirmed the considerable heterogeneity among the studies. The P-gp incidence in these 31 studies ranged from 0% to 80%. As shown in Table 2, also in very recent studies of P-gp expression these discrepancies persist anymore. Even when using the same monoclonal anti-P-gp antibody JSB-1, the detection rate ranged from 0% to 71% (Yang et al., 1999; Faneyte et al., 2001).

The putative reasons for the enormous discrepancies in P-gp detection were already discussed extensively in the mid 1990s (Beck et al., 1996). The problems designing a study providing improved P-gp expression data can be summarized as follows: (i) methods using P-gp on protein level as well as on mRNA level using whole tumor specimens can not differentiate from adjacent normal epithelial cells, stroma cells and tumor cells; (ii) Western blotting analyzes for P-gp protein expression and Northern blotting analyzes for *mdr1* mRNA expression are not sensitive enough to detect low levels in clinical samples; (iii) many polymerase chain reaction (PCR)-based assays for detection of the *mdr1*-specific mRNA fail to take into account the fact that quantitation of PCR amplification



**Figure 2.** Models for the predicted domain arrangements of human ABC-transporter proteins involved in anticancer drug resistance. (a) Nucleotide binding domain [NBD] containing a Walker A and a Walker B motif, and the ABC signature. (b) Transmembrane domain [TMD] consisting of six transmembrane (TM) -helices. Probably, the TMDs are forming a pore structure in the membrane. (c) [NBT-TMD] configuration, e.g. ABC8 (White, ABCG1), BCRP (ABCG2). (d) [TMD-NBT] configuration, e.g. TAP1 (ABCB2), and TAP2 (ABCG3). (e) [TMD-NBT]<sub>2</sub> configuration, e.g. MDR1 (ABCB1), MRP4 (ABCC4), MRP5 (ABCC5), MRP7 (ABCC7). (f) [TMD<sub>0</sub>(TMD-NBT)<sub>2</sub>] configuration, e.g. MRP1 (ABCC1), MRP2 (ABCC2), MRP3 (ABCC3), MRP6 (ABCC6). The upper parts of the topological models represent the extracellular orientation or the lumen of a cellular compartment, such as the endoplasmic reticulum, Golgi apparatus, peroxisome, or mitochondrion, whereas the bottom represents the intracellular, cytosolic compartment. It is notable that the topological models are highly schematic, and that “half-transporters” (c, d) have to assemble in a homo- or heterodimeric structure to form a biological active transporter molecule.

products is most accurate in the exponential phase of the reaction; (iv) PCR-based detection protocols may exhibit a much higher sensitivity than alternative methods including immunohistochemistry (IHC); (v) although IHC has the advantage that cancer cells can be distinguished from contaminating cells, problems arise in the quantification of the P-gp expression level; (vi) it is difficult to detect P-gp in formalin-fixed tumor tissue and differences in fixation techniques may contribute to the variability of the data, even when the same antibody was used; (vii) the commonly used anti-P-gp antibodies exhibit experimental difficulties, e.g. C219 crossreacts with the human MDR3-transporter protein, that has not been demonstrated to confer MDR, and also shows cross reaction with myosin, or MRK16 that is highly specific for P-gp, but may have heterogeneous staining even in control cell lines; (viii) disagreements whether breast cancer cells should be scored as Pgp-positive if cytoplasm is stained but membrane staining cannot be identified. Breast cancer studies requiring P-gp-specific membrane staining often report a far lower frequency of P-gp expression (Faneyte et al., 2001). All of these problems are not specific for P-gp; they obtain alike for all other ABC-transporters and alternative drug resistance-mediating factors.

Correlation of P-gp expression in breast cancer and clinical drug resistance was investigated in several studies. In locally advanced breast cancer P-gp expression has been demonstrated to increase as a result of chemotherapy. Chevillard et al. (1996) reported that the P-gp incidence increased from 14% to 43%; Chung et al. (1997) found an increasing P-gp incidence from 26% to 57%. Although the meta-analysis of P-gp expression studies in breast cancer by Trock et al. (1997) concluded that women with P-gp-positive tumors were more likely to experience chemotherapy failure, several recent studies have not been able to confirm a significant influence of P-gp expression on response rate or overall survival (Linn et al., 1997; Wang et al., 1997; Honkoop et al., 1998). Thus, the impact of P-gp expression on clinical outcome of breast cancer patients still remains open.

## B. MRP1 (ABCC1)

The second major ABC-transporter involved in MDR of human cancers was first described in 1992 (Cole et al., 1992). 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 (Borst et al., 2000), it is now designated as MRP1 or ABCC1. In addition to the [TMD-NBT]<sub>2</sub> configuration of P-gp, MRP1 has an additional TMD<sub>0</sub> domain consisting of 5 TM<sub>0</sub>-helices attached to the N-terminal forming a [TMD<sub>0</sub>(TMD-NBT)<sub>2</sub>] configuration. Anticancer drug substrates for MRP1 include anthracyclines and methotrexate commonly used for treatment of breast cancer, and *Vinca* alkaloids, and epipodophyllotoxins, (Jedlitschky et al., 1996). Since MRP1 is expressed ubiquitously in normal human tissues, it is not surprising detecting MRP1 expression in neoplastic tissue including breast cancer. Finding of the

MRP1 encoding mRNA in 100% of breast cancer specimens by RT-PCR at expression levels comparable with normal tissues reinforces this point (Filipits et al., 1996; Dexter et al., 1998; Burger et al., 2003). An immunohistochemical study finding 34 % of breast cancer samples positive for MRP1 expression reported a correlation between MRP1 expression and relapse-free

**Table 1:** Recent expression analyzes of drug resistance-mediating factors in breast cancer

Study	n	Expression [%]	Method
<b><u>P-gp</u></b>			
<b><u>(ABCB1)</u></b>			
Burger et al., 2003	59	4 high; 54 low	RT-PCR
Faneyte et al., 2001	140	71 (cytoplasm)	IHC
Arnal et al., 2000	30	100 low	RT-PCR
Yang et al., 1999	40	92-100	RT-PCR
Dexter et al., 1998	106	0	IHC
Linn et al., 1997	33	39	RT-PCR
Linn et al., 1997	31	6	IHC
Linn et al., 1997	31	100 low	RT-PCR
Linn et al., 1997	40	64 before CT; 57 after CT	RT-PCR
Filipits et al., 1996	134	60	RT-PCR
	63	9 strong; 48 weak	IHC
<b><u>MRP1</u></b>			
<b><u>(ABCC1)</u></b>			
Burger et al., 2003	59	27 high; 31 low	RT-PCR
Dexter et al., 1998	31	100	IHC
Linn et al., 1997	31	100 low	RT-PCR
Linn et al., 1997	40	20 before CT; 56 after CT	RT-PCR
Nooter et al., 1997	259	34	IHC
Filipits et al., 1996	134	100	RT-PCR
	63	24 strong; 76 weak	IHC
<b><u>MRP2</u></b>			
<b><u>(ABCC2)</u></b>			
Burger et al., 2003	56	23 high; 32 low	RT-PCR
<b><u>BCRP</u></b>			
<b><u>(ABCG2)</u></b>			
Faneyte et al., 2002	52	100 (variable levels)	RT-PCR
Burger et al., 2003	59	0	IHC
	59	29 high; 71 low	RT-PCR
<b><u>YB-1</u></b>			
Janz et al., 2002	83	76	IHC
Saji et al., 2003	63	100	IHC
<b><u>MVP (LRP)</u></b>			
Burger et al., 2003	59	17 high; 41 low	RT-PCR
Pohl et al., 1999	99	21 high; 47 intermediate; 20 low	IHC
Linn et al., 1997	40	71 before CT; 69 after CT	RT-PCR

IHC, immunohistochemistry; RT-PCR, reverse transcriptase polymerase chain reaction; CT, chemotherapy.

**Table 2:** Human ABC-transporters associated with drug resistance

ABC-transporter		Physiological substrates	Drugs	References
Common names	HUGO nomenclature			
ABCA2	ABCA2	steroids	estramustine	Laing et al., 1998; Vulevic et al., 2001
P-gp, P-170, MDR1, PGY1	ABCB1	phospholipids, neutral and cationic organic compounds	anthracyclines, Vinca alkaloids, epipodophyllotoxins, taxanes, antibiotics, and many others	Ling, 1997; Ueda et al., 1999
TAP1	ABCB2	peptides	mitoxantrone, epipodophyllotoxins	Izquierdo et al., 1996a; Lage et al., 2001
TAP2	ABCB3	peptides	mitoxantrone, epipodophyllotoxins	Izquierdo et al., 1996a; Lage et al., 2001
MDR3, PGY3	ABCB4	phosphatidylcholine	paclitaxel, Vinca alkaloids	Ruetz et al., 1994; Gottesman et al., 2002
BSEP, SPGP, ABC16, PGY4	ABCB11	bile salts	paclitaxel	Childs et al., 1998; Gerloff et al., 1998
MRP, MRP1	ABCC1	glutathion-, and other conjugates, organic anions, leukotrienes	anthracyclines, Vinca alkaloids, epipodophyllotoxins, methotrexate	Cole et al., 1992; Jedlitschky et al., 1996; Borst et al., 2000;
MRP2, cMOAT	ABCC2	glutathion-, and other conjugates, organic anions, leukotriene C <sub>4</sub>	platin-drugs, anthracyclines, Vinca alkaloids, epipodophyllotoxins, camptothecins, methotrexate	Taniguchi et al., 1996; Cui et al., 1999; König et al., 1999
MRP3, MOAT-D, MLP2	ABCC3	glucuronides, bile salts, peptides	Vinca alkaloids, epipodophyllotoxins, methotrexate	de Jong et al., 2001; Kool et al., 1999; Zeng et al., 1999
MRP4, MOAT-B	ABCC4	organic anions	nucleotide analogs, methotrexate	Schuetz et al., 1999; Chen et al., 2001; Chen et al., 2002
MRP5, MOAT-C	ABCC5	organic anions, cyclic nucleotides	nucleotide analogs	Jedlitschky et al. 2000; Wijnholds et al., 2000
BCRP, MXR, ABCP	ABCG2	prazosin	mitoxantrone, anthracyclines, camptothecins, topotecan	Doyle et al., 1998; Allikmets et al., 1998; Miyake et al., 1999; Lage and Diemel, 2000

survival (Nooter et al., 1997), whereas a RT-PCR-based study reported that MRP1 expression merely correlated with progression-free survival in patients treated with anthracycline-based therapy regime (5-fluorouracil, adriamycin/epirubicin, and cyclophosphamide), but not in patients treated without anthracyclines (cyclophosphamide, methotrexate, 5-fluorouracil) (Burger et al., 2003). Thus, the role of MRP1 in clinical MDR of mammary carcinoma remains to be elucidated.

### C. MRP2 (ABCC2)

MRP2 (cMOAT / ABCC2) exhibiting a [TMD<sub>0</sub>(TMD-NBT)<sub>2</sub>] configuration, has been shown to be the bilirubin glucuronide transporter at the canalicular membrane of the hepatocyte (König et al., 1999). MRP2 originally was found to be over-expressed in cisplatin-resistant cancer cells (Taniguchi et al., 1996). Moreover, transfection experiments demonstrated that MRP2 can confer resistance to the clinically important substance class of anthracyclines and methotrexate, as well as to platinum containing drugs, *Vinca* alkaloids, epipodophyllotoxins, and camptothecins (Cui et al., 1999). Thus, MRP2 may

have a role in clinical MDR of breast cancer treated with anthracycline- or methotrexate-containing chemotherapeutic regimens. However, there are only sporadic data available concerning to MRP2 expression in breast cancer. So far, a RT-PCR-basing study demonstrated no correlation between clinical outcome and MRP2 mRNA expression level (Burger et al., 2003). These preliminary data suggest that the importance of MRP2 in breast cancer remains uncertain and that further studies are necessary to clarify the role of MRP2 in drug-resistant phenotypes of mammary carcinoma.

### D. Other MRPs (ABCC3 – ABCC6)

Transfection experiments have shown that overexpression of MRP3 conferred resistance against *Vinca* alkaloids, epipodophyllotoxins, and methotrexate (Kool et al., 1999; Zeng et al., 1999). MRP4 was shown to confer resistance against nucleotide-based antiviral drugs as well as methotrexate (Schuetz et al., 1999; Chen et al., 2001; Chen et al., 2002). In addition, transfection studies demonstrated that MRP5 is able to mediate resistance against thiopurine anticancer drugs 6-mercaptopurine and

thioguanine and the anti-HIV drug 9-(2-phosphonylmethoxyethyl)adenine (Wijnholds et al., 2000). Finally, there is no indication that MRP6 is associated with any form of drug resistance. However, so far there are no data available demonstrating an expression of these MRPs in breast cancer.

### E. BCRP (ABCG2)

Recently, the long sought mitoxantrone transporter was identified by 3 independent studies nearly contemporaneously. Since this ABC-transporter was identified in a breast cancer-derived cell line, it was designated as “breast cancer resistance protein” (BCRP) (Doyle et al., 1998). Alternative designations are “mitoxantrone resistance-associated protein” (MXR) (Miyake et al., 1999), “placenta-specific ABC gene” (Allikmets et al., 1999) or ABCG2 according to the suggestions of the HUGO, Human Gene Nomenclature Committee. 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 (Lage and Dietel, 2000).

Up to the present, detection of BCRP in clinical samples of breast carcinoma was performed in three different studies using immunohistochemistry and/or RT-PCR. The first study analyzed samples of 43 breast cancer patients by RT-PCR and found no correlation of BCRP mRNA expression and relapse or prognosis (Kanazaki et al., 2001). Faneyte et al. (2002) analyzed 52 breast cancer samples (25 primary breast carcinomas and 27 patients who received preoperative anthracycline-based chemotherapy) by RT-PCR and found widely varied BCRP mRNA expression levels, whereby no difference in BCRP mRNA expression between anthracycline-naïve and treated tumor samples could be detected. Applying immunohistochemistry, BCRP was detected in normal breast epithelium and vessels but not in neoplastic cells. As a consequence, BCRP expression level was not associated with a decreased response or survival time. On the contrary, an alternative RT-PCR-based study analyzed 59 primary breast cancer specimens of patients who received either anthracycline-based (5-fluorouracil, adriamycin/epirubicin, cyclophosphamide) chemotherapy or a cyclophosphamide, methotrexate, 5-fluorouracil consisting regime as first-line systemic treatment after diagnosis of advanced disease. In the anthracycline-treated subgroup of patients, this study demonstrated a correlation between BCRP mRNA expression level and progression-free survival (Burger et al., 2003). However, no correlation between the BCRP mRNA expression level and post-relapse overall survival was found. In conclusion, these preliminary data suggest that BCRP expression may have some predictive value for clinical outcome, but the role of BCRP in clinical drug-resistant breast cancer has to be investigated much more in detail.

### F. Other ABC-transporters

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 (Laing et al., 1998) 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 (Izquierdo et al., 1996a; Lage et al., 2001). However, so far there are no data available that these ABC-transport proteins play any role in drug resistance of breast cancer.

### IV. YB-1

YB-1, a member of the DNA-binding protein family, was initially reported as a transcription factor which interacts with the so-called Y-box - an inverted CCAAT box - region of the promoter of MHC class II genes (Didier et al. 1988). In vitro experiments using multidrug-resistant MCF-7 breast carcinoma cells demonstrated that nuclear localization of this transcription factor regulates the transcriptional activity of the P-gp encoding *mdr1* gene (Ohga et al., 1996). Immunohistochemistry demonstrated that in 27 out of 27 samples of untreated primary breast cancers, YB-1 was expressed in the cytoplasm although it was not detectable in normal surrounding breast tissue. In a subgroup of breast tumors (9 of 27), however, YB-1 was also localized in the nucleus and, in these cases, high levels of P-gp were present (Bargou et al., 1997). The data suggest that nuclear localization of YB-1 is associated with expression of P-gp and as a result with a MDR phenotype in breast carcinoma.

There are contradictory data concerning the clinical relevance of nuclear YB-1 protein expression in breast cancer. An immunohistochemical study with 83 samples of breast cancer patients (41 patients treated with different chemotherapeutic regimens and 42 patients without any postoperative chemotherapy) reported that high YB-1 expression in neoplastic tissue and surrounding benign epithelial cells was significantly associated with poor patient outcome (Janz et al., 2002). In patients, who received postoperative chemotherapy, the 5-year relapse rate was 66% in patients with high YB-1 expression. In contrast, in patients with low YB-1 expression level, no relapse has been observed within that time. These data clearly suggest that YB-1 protein expression indicates clinical drug resistance in breast cancer and has prognostic and predictive significance. In marked contrast to these observations, an alternative immunohistochemical study using samples of 63 breast carcinoma specimens concluded that nuclear expression of YB-1 (and P-gp expression) may not be a useful prognostic marker in breast carcinoma (Saji et al., 2003). However, patients in this study underwent mastectomy and the influence of YB-1 expression on chemotherapeutic responding rate - if there has been any chemotherapy - has not been analyzed. Thus, the impact of YB-1 expression on clinical drug resistance of mammary carcinoma remains a promising topic.

## V. Major vault protein (MVP)

Another MDR-associated factor included in many clinical studies is MVP, the “major vault protein” also known as LRP (“lung resistance protein”). MVP is an integral part of the vault complex that is found in the cytoplasm and in the nuclear membrane (Scheffer et al., 2000). Vaults are the largest ribonucleoprotein particles known so far (13 MDa); they are almost ubiquitously expressed at the highest levels in potentially toxin-exposed epithelia of the gastrointestinal tract and in macrophages (Izquierdo et al., 1996b). It has been reported that vaults are involved in the intracellular distribution of chemotherapeutic agents including anthracyclines (Dalton et al., 1999). Thought to mediate redistribution of anticancer drugs away from their targets in the nucleus, MVP expression may be coordinately regulated with ABC-transporters such as P-gp or MRP1 although direct evidence that this is the case is lacking. Clinical data indicate that MVP is often expressed in human malignancies and that the expression level may be associated with poor response to chemotherapeutic treatment in ovarian carcinoma and acute myelogenous leukemia (AML) (Dalton et al., 1999; Scheffer et al., 2000). Studies on MVP expression in breast cancer are limited. The available data showed by immunohistochemistry that MVP is frequently expressed in primary breast cancer, but its expression level was independent to response to chemotherapy or survival (Linn et al., 1997; Pohl et al., 1999). A recent study applying a RT-PCR-based MVP detection protocol reported that high expression level of MVP mRNA was found to be significantly associated with poor progression-free survival in anthracycline-treated patients but not in a subgroup of patients who received an chemotherapeutic regime without anthracyclines (Burger et al., 2003). In conclusion, MVP may have some predictive value for clinical outcome of breast carcinoma patients, but its role has to be confirmed in additional studies.

## V. Additional drug resistance mechanisms

Resistance to antineoplastic agents clinically applied for the treatment of breast carcinoma can also be mediated by additional mechanisms.

A mechanism that has been identified to contribute to drug resistance in cancer is mediated by a decreased activity of the nuclear enzyme DNA topoisomerase II (Topo II) (Danks et al., 1988). In mammalian cells two Topo II isoforms, the 170 kDa Topo II and the 180 kDa Topo II exist as homodimers. Drug resistance phenotypes due to decreased expression and activity of Topo II isoforms have been described for several drug-resistant cancer cell lines derived from various tissues including breast cancer cells (Sinha et al., 1988). One study analyzing specimens of 15 cases of breast carcinoma concluded that Topo II mRNA expression level might be a useful marker of clinical response to anthracycline treatment in breast cancer patients (Kim et al., 1991). However, these conclusions could not be confirmed by

others. On the contrary, these studies found no significant difference in Topo II mRNA levels in breast cancer patients between relapsed and nonrelapsed groups (Efferth et al., 1992; Ito et al., 1998).

Drug resistance can result from defective cellular signal transduction pathways leading to apoptosis. Defects may be the consequence of malignant transformation; e.g. in cancers with mutant or non-functional p53 (Lowe et al., 1993). Furthermore, cancer cells may acquire deficiencies in apoptotic pathways during exposure to anticancer drugs, such as alterations of ceramide levels (Liu et al., 2001) or alterations in the cell cycle machinery that regulate checkpoints and prevent initiation of programmed cell death.

MDR can also result from coordinately regulated detoxifying cellular systems, such as DNA repair pathways, e.g. enhanced activity of O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT) (Pegg et al., 1990) or alterations in the DNA mismatch repair (MMR) system (Lage and Dietel, 1999). Furthermore, activation of the system of the cytochrome P450 mixed-function oxidases can mediate a drug-resistant phenotype. A coordinate induction of P-gp and cytochrome P450 3A has been reported (Schuetz et al., 1996).

However, all those data have not been consistent, and studies using specimens of human breast cancer have not yet confirmed a link with clinical drug resistance (Symmans, 2001).

## VI. Conclusions

For overcoming therapy resistance of mammary carcinoma the mechanisms that are involved have to be elucidated. In the case of a monocausal drug resistance mechanism, such as the overexpression of an ABC-transporter, a disruption of drug extrusion results in a re-sensitization of tumor cells to treatment with antineoplastic agents, and therewith may allow a successful drug treatment of the multidrug-resistant cancer cells. Pharmacologically active drug resistance-reversing compounds are designated as MDR modulators or chemosensitizers. One obstacle in applying MDR modulators arises from their commonly occurring intrinsic toxicity at doses necessary to be active, e.g. heart failure, hypotension, hyperbilirubinemia, bone-marrow and neurological toxicity. Additionally, tumor cells can develop resistance against the applied chemosensitizers, a so-called tertiary resistance. However, with recognition of the problems of potency and pharmacokinetic interactions, so far the third-generation of MDR-modulators (e.g. XR-9576, R-101933, LY-335979, OC144-093) has been developed and was applied in the first clinical trials (Gottesman et al., 2002).

Although, several mechanisms have been identified to contribute to clinical drug-resistance of breast carcinoma, hitherto the problem of therapy resistance against anti cancer drugs was not vanquished. An important problem is the principle that neoplastic tissues including breast carcinoma cells are genetically heterogenous. Although this phenomenon occurs as a

result of uncontrolled cell growth in the cancerous tissue and favors clonal expansion, tumor cells that are exposed to anticancer drugs will be selected for their ability to survive and grow in the presence of antineoplastic agents. Thus, in any population of cancer cells that underwent chemotherapeutic treatment, more than one mechanism of drug resistance may be active. In other words, the clinical drug resistance of breast carcinoma probably represents a multifactorial multidrug resistance phenomenon. Hence, the needful strategy for overcoming drug resistance has to target various drug resistance mediating factors simultaneously, e.g. by the development of “multispecific” inhibitors, such as the acridinecarboxamide derivative GF-120918 that reverses P-gp-mediated MDR as well as BCRP-mediated resistance (de Bruin et al., 1999). Moreover, it may be a promisingly strategy to inhibit upstream factors of drug resistance-mediating mechanisms. One possible upstream factor represents the Y-box transcription factor YB-1. Besides the regulation of the P-gp encoding *mdr1* gene, in vitro data suggest that YB-1 can induce the activation of alternative MDR factors such as MRP1 (Stein et al., 2001). Thus, the identification of additional upstream factors regulating the activity of MDR-mediating genes in breast cancer is of urgent need.

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