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REVIEW

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The Emergence of Drug Transporter-Mediated Multidrug Resistance to Cancer Chemotherapy

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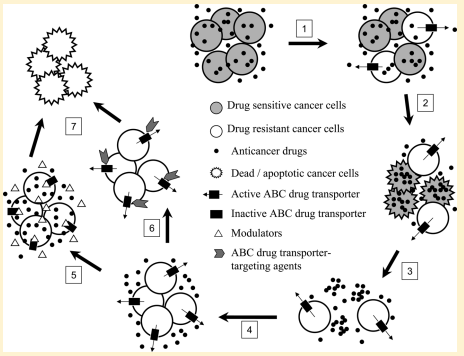
ABSTRACT: Chemotherapy is currently one of the most effective ways to treat metastatic cancers. However, of the various mechanisms that are involved in conferring resistance, upregulation of drug efflux ATP-binding cassette (ABC) transporters, such as P-glycoprotein (ABCB1), multidrug resistance protein 1 (ABCC1) and ABCG2, has become a major obstacle to cancer chemotherapy and seriously affects the clinical outcome. To date, at least 15 ABC drug transporters have been identified and characterized to transport and confer resistance to practically the entire spectrum of cancer drugs, causing multidrug resistance (MDR) in cancers. Unfortunately, despite decades of research, there is still no real solution to MDR. This review highlights some of the major findings, the roles and problems associated with MDR-linked ABC drug transporters in metastatic cancers and solid tumors, and the current strategies to improve the clinical outcome in cancer chemotherapy.



KEYWORDS: cancer, ABC transporter, multidrug resistance, tumor microenvironment

A. INTRODUCTION

Chemotherapy can precede other treatment modalities (neoadjuvant chemotherapy), can follow other treatments (adjuvant chemotherapy) or can be administered alone depending on stage of cancer to be treated . Unfortunately, a large number of patients will develop drug resistance during the course of treatment and will no longer be responsive to multiple anticancer drugs that are functionally and structurally unrelated, a phenomenon called "multidrug resistance" or MDR. This often leads to cancer relapse and eventually death of these patients. Therefore, the first step towards finding successful cancer therapy is to study the multiple drug-evading mechanisms cancer cells have developed or utilized during the course of drug therapy to survive. The detailed mechanisms are somewhat complex and have been described fully in several recent in-depth reviews.[1−3] Many of these mechanisms are in response to the damages caused by anticancer agents while some mechanisms utilize endogenous proteins to prevent anticancer drugs from entering the cells, either by reducing drug uptake systems or enhancing transporter-mediated drug efflux. Although these independent mechanisms can work separately, they are more often interlinked and work synergistically.[4] This review focuses on the emergence and the significant clinical impact of ATP-binding cassette (ABC) transporters on cancer MDR. The emphasis will be on the three major ABC drug transporters associated with unfavorable clinical outcome, P-glycoprotein (Pgp), multidrug resistance protein 1 (MRP1) and ABCG2; however, we will also discuss several other MDR-linked ABC transporters that have emerged more recently. Moreover, we will discuss the potential role of ABC transporters in solid tumor chemoresistance, the current strategies and future perspectives regarding transporter-mediated cancer MDR.

B. MECHANISMS OF CANCER DRUG RESISTANCE

Essentially, successful cancer chemotherapy is dependent on two major factors: the (1) inherent patient factor and the (2) adaptation cancer cell factor. The "inherent patient factors" are the variations in individuals that affect the delivery of sufficient anticancer drugs to cancer cells. These variations include ample absorption and distribution of anticancer drugs within the patient's body without excessive drug metabolic inactivation or elimination. Ideally, the level of a particular drug at the site of a tumor should reach therapeutic levels without causing significant adverse effects. The development of new delivery systems or strategies should improve the pharmacokinetics and pharmacodynamics of a particular anticancer drug, and hopefully the therapeutic outcome as well.[5] The "adaptation cancer cell factors", on the other hand, are dependent on how cancer cells respond to the types of drugs administered. The response varies by the tissue of origin and by the intrinsic expression of a variety

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Table 1. Timeline of Major Discoveries of MDR-Linked Human ABC Transporters ABCB1 (Pgp), ABCC1 (MRP1) and ABCG2

year	major discovery
1968	discovery of MDR phenomenon in mouse cell lines[183]
1973	demonstration of direct drug efflux leading to drug resistance in tumor cell lines[7]
1974	correlation of colchicine resistance to reduced colchicine uptake in colchicine-selected CHO cell lines[184]
1974	demonstration that MDR caused by a energy dependent mechanism[185]
1975	isolation of revertants showing reversion of MDR phenotype[186]
1976	demonstration of Pgp surface expression in MDR cell lines[8]
1978	demonstration of MDR being a dominant phenotype[187]
1979	purification of Pgp from MDR CHO cell lines[10]
1979	isolation of MDR human cancer cell lines[9]
1981	demonstration that MDR can be reversed by verapamil[14]
1982	demonstration of DNA-mediated transfer of MDR[188]
1983	linking Pgp surface expression to MDR phenomenon using Western blot analysis[189]
1984	correlation of MDR to amplification of specific DNA sequences in drug-selected CHO cell lines[190]
1985	detection of Pgp in clinical samples associating Pgp as MDR marker[21]
1985	amplification of Pgp genes in MDR mammalian cell lines[16]
1985	isolation of monoclonal antibodies to Pgp[191]
1985	the term "ATP-binding cassette" superfamily defined[192]
1986	cloning of first human MDR sequences and the detection of amplified *MDR1* and *MDR2* genes in human cancer cell[17]
1986	demonstration of amplified MDR1 expression leads to increased drug resistance in human cell lines[18]
1986	isolation and characterization of DNA sequences amplified in MDR hamster cells[193]
1986	cloning of full-length MDR1 gene; demonstration of its structure and homology to bacterial ATP-dependent transport proteins[194,195]
1986	MDR1 encodes Pgp[20]
1986	full-length MDR1 cDNA confers MDR[196]
1987	demonstration of MDR mRNA expression pattern in human tumors and tissues[19]
1987	detection of MDR1 mainly expressed in the liver[26]
1987	cellular localization of Pgp in normal tissues[27]
1987	identification of MDR1 promoter[197]
1988	demonstration of Pgp substrate specificity altered by spontaneous mutations in MDR1[198]
1988	demonstration of a retrovirus carrying MDR1 can confer MDR and polarized expression of Pgp in MDCK cells[199]
1989	demonstration of Pgp highly expressed in brain capillaries[28,200]
1989	first report of leukotriene D4 (LTD4) receptor antagonist MK-571; later used as an MRP-specific inhibitor[201]
1990	demonstration of Pgp ATPase activity[29]
1990	reverse-transcription analysis of MDR1 showing widespread MDR1 expression in human cancers[202]
1990	introduction of hydrophobic vacuum cleaner model[203]
1991	demonstration of Pgp expression as a predictor of the therapeutic outcome for neuroblastoma[204]
1991	demonstration of a non-Pgp ABC transporter-mediated transport of doxorubicin and mitoxantrone from MDR human breast cancer cell line[205]
1992	MRP1 identified and cloned[42]
1992	demonstration of Pgp expression and activity causing the "side population" in human hematopoietic stem cells[206]
1992	demonstration of substrate-stimulatable ATP hydrolysis of reconstituted Pgp[30]
1992	demonstration of important Pgp function at the blood−brain barrier[32]
1992	retroviral transfer of MDR1 into bone marrow cells conferred drug resistance *in vivo*[207]
1993	induction of MDR in human cells by transient exposure to different chemotherapeutic drugs[208]
1994	blood−brain barriers of MDR1 knock out mouse were defected and resulted in altered drug sensitivity[33]
1994	MRP1 detected using monoclonal antibodies[209]
1994	ATP-dependent transport of glutathione S-conjugates by MRP1[48]
1994	MRP1 linked to MDR in cancer[53]
1994	MRP1-mediated LTD4, LTC4 and LTE4 transport is inhibited by MK-571[49]
1995	demonstration of Pgp affecting antiepileptic drug therapy[34]
1996	demonstration of clinical application of MDR reversing agent[35]
1996	discovery of MRP1 tissue distribution[52]
1996	association of MRP1 upregulation with MDR in selected cancer cell lines[54]
1996	high-level MRP1 expression detected in breast carcinomas[62]
1996	demonstration of MRP1-mediated glutathione-dependent drug transport[50]
1996	demonstration of MRP1-mediated transport of anionic conjugates of steroid hormones[51]

Table 1. Continued

year	major discovery
1997	discovery of multiple drug-binding sites on Pgp[31]
1997	demonstration of Pgp not being essential in metabolism in the condition where xenotoxins are absent[210]
1997	demonstration of oral bioavailability of amphipathic drugs (such as paclitaxel) is limited by Pgp[36]
1997	demonstration of drug-stimulatable ATP hydrolysis of reconstituted MRP1[43]
1998	Pgp inhibited apoptosis in lymphoid cells that is independent of its function[211]
1998	additional N-terminal TMD of MRP1 is not required for LTC_4 transport[44]
1998	detection of MRP1 at the oropharyngeal mucosal layer and the testicular tubules[212]
1998	detection of MRP1 overexpressed in various forms of lung cancer[61]
1998	discovery of breast cancer resistance protein BCRP (ABCG2) in MCF-7/AdrVp cells[67]
1998	discovery of MDR-linked ABCP (ABCG2)[68]
1999	association of tuberous sclerosis with MDR1 expression and drug-resistant epilepsy[213]
1999	molecular cloning of ABCG2 cDNA[214]
2000	demonstration of the requirement of ATP hydrolysis for both the transport and the reset of human Pgp[215]
2000	demonstration of vanadate-sensitive MRP1 ATP hydrolysis[45]
2000	detection of MRP1 at human placenta[65]
2000	discovery of the protective role of MRP1 in the human CNS[66]
2000	demonstration of reduced drug accumulation in ABCG2-expressing MDR MCF7 breast cancer cells[71]
2000	association of MDR with MXR (ABCG2) that is localized in the plasma membrane[70]
2000	detection of high ABCG2 expression in blast cells from AML patients[72]
2000	demonstration of fetal protection by ABCG2[216]
2000	demonstration of ABCG2-mediated transport inhibited by fumitremorgin C (FTC)[217]
2001	demonstration of survival advantage in AML patients treating with drugs and MDR inhibitor in clinical trials[37]
2001	identification of MRP1 polymorphism[218]
2001	demonstration of mice lacking MRP1 being more resistant to bacterial lung infection[219]
2001	discovery of ABCG2 substrate specificity being altered by mutations in the ABCG2 gene[220]
2001	identification of ABCG2 as a molecular determinant of the "side population" in stem cells[86]
2001	detection of subcellular localization and distribution of ABCG2 in normal human tissues[221]
2002	demonstration of ABCG2 homodimer formation that is essential for MDR[222]
2002	demonstration of arginine-482 in ABCG2 as a mutation hot spot[223]
2002	detection of elevated ABCG2 expression in relapsed or refractory AML[81]
2002	demonstration of the role of ABCG2 in "side population" stem cells in mice and hematopoietic cells[224]
2002	overexpression of ABCG2 in hematopoietic stem cells effluxes Hoechst 33342[225]
2002	demonstration of ABCG2-mediated transport of pheophorbide-a[226]
2002	detection of ABCG2 localized in microvessel endothelium of human brain[227]
2002	detection of ABCG2 localized on the surface of the chorionic villi in placenta[228]
2003	association of MDR in epilepsy with a polymorphism in MDR1[38]
2003	association of ABCG2 expression to flavopiridol resistance in AML patients[229]
2004	identification of an estrogen response element in the ABCG2 gene[230]
2004	demonstration of functional ABCG2 exists in homotetramer form[231]
2004	demonstration of ABCG2 function in cellular folate homeostasis[232]
2004	association of ABCG2 expression with survival advantage during hypoxia conditions[233]
2004	prediction of MDR1 substrates, inhibitors and agents that are preferentially cytotoxic to MDR cells[234]
2004	demonstration of verapamil-induced apoptosis through MRP1-mediated extrusion of GSH[235]
2004	demonstration of altered substrate specificity and transport activity of MRP1 caused by mutations of charged amino acids in MRP1[46]
2005	demonstration of single amino acid replacement of ABCG2 at position 482 altering the substrate specificity of ABCG2[77]
2005	demonstration of ABCG2-mediated transport of a tyrosine kinase inhibitor, imatinib mesylate[236]
2005	association of ABCG2 expression with homeostasis of endogenous porphyrins[237]
2005	demonstration of ABCG2-mediated transport of primary bile acids and unconjugated sterols[238]
2006	association of MRP1 function with nitrogen monoxide-mediated iron release from cells[239]
2006	demonstration of MRP1-mediated transport of sphingosin-1-phosphate from mast cells[240]
2006	purification and structural analysis of ABCG2 as a tetramer of dimers[69]
2007	demonstration of a silent polymorphism in the MDR1 gene can affect substrate specificity[241]
2007	implication of ABCG2-mediated transport of vitamin K3[242]
2008	identification of residues responsible for the asymmetric function of NBDs of MRP1[47]

Table 1. Continued

year	major discovery
2009	analysis of Pgp X-ray crystal structures[41]
2010	association of MRP1 with plasma concentration of vitamin B12[243]
2010	association of blood−brain barrier Pgp with brain amyloid-beta level in Alzheimer's disease[39]
2010	implication of a protective role of ABCG2 in Alzheimer's neuroinflammatory response[244]
2010	purification and structural analysis of substrate-free and substrate-bound conformations of ABCG2[245]

of key regulatory genes in the patient. These regulatory or drug resistance genes are often altered upon drug treatment, causing variations in drug sensitivity.[3] Acquired drug resistance is recognized as one of the major problems contributing to the failure of cancer chemotherapy. Though a majority of cancer cells are intrinsically resistant to xenobiotics, many have acquired drug resistance during one or multiple courses of chemotherapy.[4] The mechanisms of cancer drug resistance can be generalized into the following 6 types: (1) reduced/loss/alteration of specific drug target, (2) enhanced drug metabolism, (3) enhanced cellular repair mechanisms, (4) reduced drug uptake, (5) enhanced drug efflux and (6) drug compartmentalization. Cancer cells that adapted the first three mechanisms are often resistant to a group of drugs that are similar either in structure or function. In contrast, the latter three mechanisms directly alter the drug accumulation within cancer cells, leading to resistance to a variety of drugs that are structurally and functionally independent, also known as MDR.[6] Collectively, anticancer drugs are ineffective if the intracellular drug concentration is significantly reduced and/or regulatory pathways such as induction of apoptosis, cell cycle arrest and DNA damage are altered, as detailed in a recent in-depth review.[3] In spite of the presence of multiple mechanisms of resistance, an energy-dependent drug transport system is perhaps the most efficient and common cause for acquired resistance.

C. ATP-BINDING CASSETTE TRANSPORTER-MEDIATED DRUG RESISTANCE

Transporter-mediated drug efflux provides cells the first line of defense against xenobiotics. It is the most direct and effective way to reduce intracellular drug concentration in normal and cancer cells. One of the earliest reports of drug resistance mediated by an energy-dependent outward transport was described by Dano et al. in 1973. It was discovered that daunomycin was actively effluxed out of drug resistant tumor cells, and this transport could be competitively inhibited by other anticancer agents.[7] Consequently, the first human ABC drug transporter, P-glycoprotein, was identified and characterized by various groups a few years later (Table 1).[8−10] In general, cancer cells will respond to initial chemotherapy; after that, a considerable number of patients will relapse with MDR form of cancers. It is thought that one of the ABC drug transporters, such as Pgp, MRP1 or ABCG2, becomes upregulated in some cancer cells during chemotherapy, causing insensitivity to drugs (Figure 1). Another possibility is that a small percentage of cancer cells have intrinsically higher levels of ABC drug transporters, allowing them to survive the initial chemotherapy and resulting in the MDR form of cancer.[4] The discovery of human ABC transporter proteins that utilize energy derived from ATP to mediate drug transport has changed the perspective we have on drug resistance and modern chemotherapy. To date, genes for 48 ABC proteins have been identified in the human genome and are subdivided into seven families (ABC A−G), based on structural and sequence similarities. ABC transporters are membrane proteins from the ABC protein superfamily consisting of both transmembrane domains (TMDs), which form substrate-binding pockets, and distinctive nucleotide-binding domains (NBDs), which generate energy from ATP hydrolysis to actively transport a variety of compounds across biological membranes.[11] From the total of 48 human ABC transporters identified, at least 20 members are associated with known human diseases/disorders, including Dubin−Johnson syndrome (ABCC2), pseudoxanthoma elasticum (ABCC6) and cystic fibrosis (ABCC7).[12] Several members are transporters capable of actively effluxing a wide range of anticancer drugs and, essentially, reducing intracellular drug concentration and eventually conferring cross-resistance to varieties of chemotherapeutics drugs, resulting in MDR. Among them, P-glycoprotein (Pgp or ABCB1), multidrug resistance protein 1 (MRP1 or ABCC1) and ABCG2 (MXR or BCRP) are most frequently associated with the development of transporter-mediated MDR in cancer chemotherapy.[13] The sequence of major discoveries related to these three ABC drug transporters is listed in Table 1.

P-Glycoprotein (Pgp). Prior to the discovery of the first ABC transporter, the MDR phenomenon had been observed and studied by investigators in drug-selected tumor cell lines. It was understood that reduced cellular drug accumulation is caused by an undetermined energy-dependent, carrier-mediated mechanism. But it was not until 1976 that Juliano and Ling discovered a 170 kDa cell membrane glycoprotein named P-glycoprotein, and established its link to the MDR phenotype.[8] Subsequent studies confirmed that P-glycoprotein is an energy-dependent drug pump that is overexpressed on the surface of many MDR cancer cells (Table 1). Moreover, one of the major findings at that time was that MDR caused by Pgp can be reversed by compounds such as verapamil.[14] Almost 30 years later, research on inhibitor-based chemosensitization and on the identification of new inhibitors of ABC transporters is currently ongoing.[15] With the advances in biochemistry and molecular biology technologies, the cellular localization, relative protein expression level, the structure, the function, molecular pathways and genetic polymorphisms of Pgp have all been extensively characterized over the past 3 decades (Table 1). Most studies in the 1980s focused on understanding the mechanism for the MDR phenotype in different human cell lines,[16−20] but it was not until 1985 that Pgp was linked to clinical MDR for the first time.[21] Over the years, a large number of cancer treating drugs have been identified as substrates of Pgp, including *Vinca* alkaloids, taxanes, etoposide, teniposide, colchicines, actinomycin D, camptothecins,[6] imatinib mesylate,[22] saquinavir,[23] methotrexate[24] and mitoxantrone.[25] The cellular localization of Pgp in normal tissues was mapped in the late 1980s,[26] including the discovery of higher Pgp protein expression levels in the liver[27] and brain capillaries,[28] which further demonstrated the physiological function of Pgp. In the 1990s, numerous crucial functional and mechanistic studies were performed on Pgp, including the measurement of Pgp ATPase

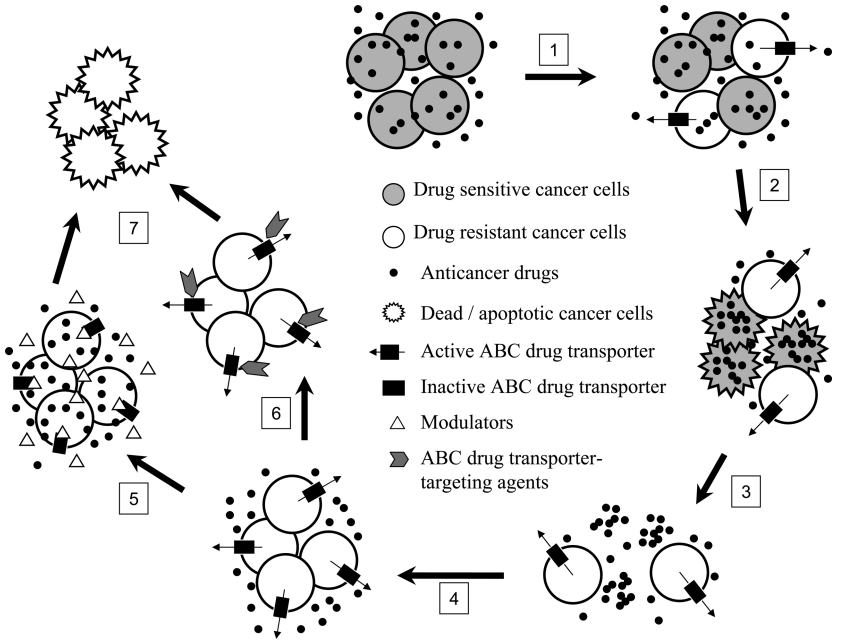

Figure 1. Proposed regimen against drug sensitive and ABC transporter-positive MDR cancer cells. (1) ABC drug transporter(s) overexpressing cancer cells (or cancer stem cells) arise by selection/induction/mutation pathways during the course of chemotherapy. (2) ABC drug transporter mediates active drug efflux in MDR cancer cells, whereas sensitive cancer cells accumulate more drugs, which results in cell death. (3) Only MDR cancer cells can survive the full course of chemotherapy. (4) Cancer relapse caused by proliferation of MDR cancer cells. At this stage, MDR cancer cells can be treated with one of the following regimens: (5) MDR cancer cells can be treated with anticancer drugs in the presence of modulators that inhibit the function and/or expression and/or localization of the ABC drug transporters.[15] (6) On the other hand, Pgp-positive MDR cancer cells can also be treated with agents that selectively target and induce cell death in Pgp-overexpressing MDR cancer cells.[182] (7) Elimination of all cancer cells.

activity[29,30] and Pgp drug-binding sites,[31] the demonstration of Pgp function at the blood−brain barrier,[32−34] and the potential use of reversing agents to resensitize Pgp-mediated MDR.[35] Pgp is now accepted as a factor for limiting oral bioavailability of anticancer drugs[36] and affects therapeutic outcome in patients,[37] prevents drug penetration through the blood−brain barrier[33] and potentially affects chemotherapy of patients with either epilepsy[38] or Alzheimer's disease.[39] Structurally, Pgp is considered as a typical "full transporter" composed of 2 transmembrane domains, each linked to an ATP-binding domain. The binding of substrates and nucleotides to respective binding sites and how ATP hydrolysis is driving the transporting mechanism have been carefully investigated by various groups using combinations of biochemical, pharmacological and mutational studies.[40] But it was not until more recently that Aller et al. confirmed and revealed more detailed structural information on Pgp from its X-ray crystal structures.[41]

Multidrug Resistance-Associated Protein 1 (MRP1). For a while, it was believed that P-glycoprotein was the only ABC transporter to mediate MDR in cancer. It was not until 1992 that Cole and colleagues identified the second major ABC drug transporter named "multidrug resistance-associated protein 1" (MRP1 or ABCC1) in the MDR human small cell lung cancer H69AR cell line.[42] Unlike Pgp, MRP1 has an additional NH_2-terminal transmembrane region and an apparent molecular mass of 180−190 kDa. Due to the lack of structural information from X-ray crystallography on MRP1, the structural and mechanical information is limited to various biochemical, pharmacological and mutational studies.[43−47] Similar to Pgp, MRP1 has a broad substrate specificity that is capable of handling structurally diverse substrates, including LTD4, glutathione, glutathione conjugates, glucuronides, sulfates and drug conjugates.[48−51] Although low levels of MRP1 are ubiquitously expressed throughout the body,[52] the overexpression of MRP1 does lead to the MDR phenotype in multiple drug-selected cancer cell lines.[53,54] Moreover, MRP1 confers resistance to many anticancer agents including etoposide, anthracyclines and *Vinca* alkaloids,[55] imatinib mesylate,[56] saquinavir,[57] methotrexate,[58] mitoxantrone,[59] irinotecan and SN-38.[60] In the clinic, higher or elevated levels of MRP1 are detected in many cancer types, including lung, non-small cell lung cancer (NSCLC),[61] breast and prostate cancer,[62] and MRP1 is associated with accelerated relapse in breast cancer.[63,64] Moreover, the protective role of MRP1 in human placenta and CNS was also suggested.[65,66]

ABCG2. ABCG2 (also known as breast cancer resistance protein, BCRP; placenta-specific ABC transporter, ABCP; and mitoxantrone resistance protein, MXR) was discovered at the same time by different research groups in 1998.[67,68] In contrast to the four core domains of Pgp, ABCG2 is a "half transporter" consisting of two core domains (one ATP-binding domain followed by one transmembrane domain) that dimerize in a reverse orientation to form a functional unit.[69] In 2000, ABCG2 was mainly found localized in the plasma membrane[70] and linked to reduced drug accumulation and MDR.[70−72] Its physiological and pharmacological importance has been clearly demonstrated by its ability to transport clinical drugs and physiological substrates (such as porphyrins and sterols) from cells, its impact on drug bioavailability, and its role in protecting cells or tissues from xenobiotics.[73,74] Moreover, the overexpression of ABCG2 in cancer cells causes drug resistance to anticancer agents such as etoposide,[75] docetaxel and paclitaxel,[76] saquinavir,[57] mitoxantrone, topotecan, CPT-11, SN-38, methotrexate, flavopiridol,

anthracyclines and many tyrosine kinase inhibitors.[74] On the other hand, it is important to note that most of the studies on ABCG2 are performed on cell-based *in vitro* assays and that the role of ABCG2 in clinical settings is not as well-defined. Studies using ABCG2-overexpressing cell lines showed that the substrate specificity (and resistance) of ABCG2 is altered by one amino acid variation at position 482. Though the wild-type residue at this position is arginine, it can be replaced by others such as glycine or threonine, dependent on drug selection regiment, which results in different drug resistant profiles.[77] In addition to the single mutation at R482, mutations at other residues, including E446, N557 and H630, can also alter the substrate specificity of ABCG2.[78]

As mentioned earlier, unequivocal proof linking ABCG2-overexpression to clinical MDR in cancer is still lacking. However, studies do show strong correlations of high ABCG2 expression with tumor drug resistance and relapse, especially in patients with advanced non-small cell lung cancer or acute myeloid leukemia (AML).[79] For instance, Steinbach et al. showed elevated ABCG2 levels in relapsed childhood AML.[80] In the same year, Van Den Heuvel-Eibrink et al. also found higher ABCG2 expression in relapsed AML samples.[81] Additionally, tyrosine kinase inhibitors (TKIs) such as imatinib, nilotinib, dasatinib and lapatinib for the treatment of leukemia are also excellent substrates of ABCG2.[82,83] The clinical impact of ABCG2 on TKI based chemotherapy is currently under evaluation.[84] In addition to MDR, ABCG2 is considered to play a protective role in cancer stem cells (CSCs). The cancer stem cell hypothesis is based on the presence of rare cancer cells with the indefinite potential for self-renewal that drives tumorigenesis, similar to distinctive properties of stem cells,[85] which will be discussed in detail later. Briefly, stem cells were initially characterized by the "side population" phenotype described by Zhou et al. in 2001 (Table 1), which is caused by ABCG2-mediated efflux of Hoechst 33342 dye.[86] Thereafter, ABCG2 expression was found in a wide range of human stem cells, including human hematopoietic stem cells,[87] human limbal epithelial stem cells,[88] human neural stem progenitor cells[89] and human embryonic stem cells,[90] thus ABCG2 is considered as one of the biomarkers for stem cells as well as for CSCs. Likewise, it was shown that the overexpression of ABCG2 caused by drug selection regimens can contribute to "stem cell-like" properties in MDR breast cancer cell lines.[91] Some scientists also believe that CSCs may have a critical role in tumorigenesis, development, metastasis and relapse.[92] The exact role of ABCG2 in CSCs remains to be determined.

Other MDR-Linked ABC Transporters. In addition to Pgp (ABCB1), MRP1 (ABCC1) and ABCG2, at least 12 other ABC transporters are currently linked to MDR or can cause reduced intracellular drug accumulation. These ABC transporters, ABCA2,[93] ABCA3,[94] ABCB4,[95] ABCB5,[96] ABCB11,[97] ABCC2,[76] ABCC3,[98] ABCC4,[99] ABCC5,[99] ABCC6,[100] ABCC10,[101] and ABCC11,[102] are being studied extensively at present by various groups. In terms of the ABCC family, several other members are also known to cause cancer drug resistance. MRP2 (ABCC2), for instance, was discovered as a conjugate efflux pump with similar substrate specificity to MRP1, but is localized to the apical membrane of many types of polarized cells.[103] High levels of MRP2 in cancer cells cause chemoresistance to vinblastine,[103] vincristine,[75] irinotecan (CPT-11),[104] SN-38,[104] doxorubicin,[75] epirubicin,[75] etoposide,[75] arsenite,[105] mitoxantrone,[103] methotrexate,[58] and saquinavir.[57] In contrast to MRP1, MRP2 also transports topotecan,[106] cisplatin,[75] docetaxel and paclitaxel.[76] Several other MRPs have also been well-characterized over the years. MRP4 (ABCC4)-mediated transport of the nucleoside phosphonate analogue antiretroviral drug 9-(2-phosphonylmethoxyethyl)adenine (PMEA) was first reported by Schuetz et al. in 1999.[107] Since then, more chemotherapeutics were identified as transport substrates of MRP4, including methotrexate,[108] azidothymidine,[109] and camptothecins (topotecan, irinotecan and SN-38)[110] as well as thiopurines (6-mercaptopurine and 6-thioguanine).[111] Similar to MRP4, MRP5 (ABCC5) was reported to transport antiviral drugs such as PMEA,[112] anticancer agents methotrexate,[113] azidothymidine[109] and thiopurines (6-mercaptopurine, 6-thioguanine and 5-fluorouracil).[114] Moreover, MRP5 is also associated with resistance to cisplatin[114] and the deoxycytidine analogue gemcitabine.[115] Less is known about MRP8 (ABCC11), which is structurally similar to MRP4 and MRP5. It has been shown to confer resistance to 5′-fluorouracil, 5′-fluoro-2′-deoxyuridine, 5′-fluoro-5′-deoxyuridine, 2′,3′-dideoxycytidine and PMEA.[116] In 2004, MRP7 (ABCC10) was discovered by Hopper-Borge et al. to confer drug resistance to docetaxel, paclitaxel, vincristine, vinblastine,[101] SN-38, daunorubicin, etoposide, cytarabine and gemcitabine, as well as antiviral agents 2′-3′-dideoxycytidine and PMEA,[117] and later associated with drug resistance to the antimitotic drug vinorelbine in non-small cell lung cancer.[118]

In contrast to ABC transporter-mediated drug efflux, intracellular drug sequestration by ABCB5 or ABCA3[94,120−122] can potentially bring about resistance to chemotherapeutics such as cisplatin and daunorubicin[94,119,94,120−122] ABCB5 was initially discovered by Frank et al. to mediate rhodamine transport in progenitor cells[123] and to cause resistance to doxorubicin in human melanoma cells,[96] where it was also identified as a marker of melanoma stem cells.[124] On the other hand, high levels of ABCA3 were first observed in specimens from patients with acute myeloid leukemia (AML),[125] who showed higher resistance to doxorubicin.[121] Further studies confirmed that ABCA3-mediated subcellular lysosomal imatinib sequestration conferred MDR in leukemia cells.[126] Nevertheless, the characterization and the clinical relevance of these transporters mentioned in this section are still ongoing.

In addition to passive diffusion, many drugs enter cells via facilitated transport[127] mediated by carriers such as members of the solute carrier (SLC) family.[128] For example, carriers such as SLC22A1, SLC22A2 and SLC22A3 were suggested to mediate uptake of platinum-based drugs,[129] whereas SLC22A4 and SLC01B3 were reported to uptake doxorubicin[130] and paclitaxel,[131] respectively. Genetic polymorphisms or decreased expression of these uptake transporters results in reduced accumulation of certain drugs.[130,132] Another example of reduced drug uptake occurs in cisplatin resistance. Cisplatin is frequently used to treat many types of solid tumors, including head and neck cancer and ovarian cancer. Although cisplatin is commonly used as chemotherapy, the mechanisms of how it enters and accumulates in cells or how cells confer resistance to it are still unclear.[133] In 1995, Shen et al. established MDR cell lines from human hepatoma and KB adenocarcinoma cells that are highly resistant to cisplatin.[134] Since there is no clear evidence linking any energy-dependent pumps to cisplatin resistance, a likely explanation is that cisplatin enters cells via endocytosis and that reduced endocytosis will cause decreased cisplatin accumulation.[135,136] More recently, not only more ABC drug transporters have been discovered but other energy-dependent efflux pumps have also been identified. For instance, copper transporting protein

ATP7A and ATP7B were shown to mediate resistance to agents such as cisplatin.[129,137] Likewise, the Ral-binding, GTPase-activating protein RLIP76/RALBP1 is also capable of mediating drug resistance by transporting glutathione conjugates of doxorubicin, vincristine, mitomycin-c and melphalan out of cells.[138−140]

D. THE EMERGENCE OF ABC TRANSPORTERS IN SOLID TUMORS

The ineffective use of chemotherapy to treat solid tumors is multifactorial. Solid tumors are heterogeneous and structurally complex containing different kinds of cells. Most early studies focused mainly on the transformation of normal cells into cancer cells; however, little time was spent studying the various components presented within a solid tumor, including fibroblasts, endothelial cells, immune cells, connective tissue and the extracellular matrix.[141] These components, collectively called the "stroma", are crucial in tumor initiation, progression and drug resistance. The actual composition of the tumor stroma can be highly variable and is often altered as the tumor progresses. Differences can be seen among patients and even within different areas of the same tumor. Moreover, tumor stroma cells can secrete factors to induce signaling events involved in tumor progression and drug resistance, known as "environment-mediated drug resistance". This resistance transiently protects the tumor cells from chemotherapeutic agent-induced apoptosis.[142] The mechanisms of environment-mediated drug resistance can be subdivided into two categories as described in a recent in-depth review:[143] (1) soluble factor-mediated drug resistance, which is induced by cytokines, chemokines and growth factors secreted by stroma cells; and (2) cell adhesion-mediated drug resistance, which is mediated by the adhesion of tumor cell integrins to stroma fibroblasts or to components of the extracellular matrix, such as fibronectin, laminin and collagen. Both of these forms of environment-mediated drug resistance can induce signaling events involved in the activation of survival, cell cycle arrest and antiapoptotic pathways that further affect the sensitivity of tumors to drug treatment.[143] In addition to the two well-studied environment-mediated drug resistances mentioned above, several reports suggested that cell survival may also be associated with the emergence of ABC transporters. Although the exact mechanism is still undetermined, studies have demonstrated that Pgp inhibition[144,145] or knock down of MRP1,[146,147] MRP4[148] or ABCG2[149] can promote cell cycle arrest and apoptosis, as well as inhibit cell proliferation in human leukemia cells, vascular smooth muscle cells, human neuroblastoma cell lines, colon cancer cell lines and retinal progenitor cells in *in vitro* and mouse models. However, the detailed mechanisms of ABC transporter-mediated cell survival, proliferation and apoptosis remained unknown until more recently, when cytokines, sex hormones and growth factors were shown to regulate the expression and function of ABC transporters in normal tissues.[150−153] It was therefore hypothesized that cell adhesion or the factors produced by tumor stroma cells are capable of regulating ABC transporter expression and functional activity. For example, *in vitro* treatment of primary placental trophoblast cells with tumor necrosis factor-α (TNF-α) or interleukin (IL)-1β reduced the expression and function of apical Pgp and ABCG2 while IL-6 increased the expression of the basolateral ABCB4. On the other hand, epidermal growth factor (EGF) and insulin-like growth factor II increased the expression and functional activity of ABCG2. Similarly, estradiol or progesterone also stimulated Pgp, ABCG2 and ABCB4 expression and increased their functional activities.[151] More importantly, several pro-inflammatory cytokines were shown to regulate ABC transporter expression and further cause drug resistance in several cancer cell lines and their xenografts.[154] Although direct evidence from *in vivo* tumor microenvironment studies is still lacking, these findings suggest that the contribution of tumor stroma to ABC transporter expression and functional activity may be particularly important for drug resistance.

Clinical evidence has shown that tumor hypoxia is an independent prognostic indicator of unfavorable clinical outcome in cancer patients and is correlated with increased tumor chemoresistance.[155] The chemoresistance is attributed to intrinsic cellular mechanisms such as the lack of oxygen for antitumor drugs to act, DNA overreplication, increased genetic instability, the antiproliferative effect of hypoxia[156] and mostly the increase in ABC drug transporters[157,158] and increases in gene transcription induced by hypoxia inducible factors (HIFs). Earlier work on HIFs and ABC transporters demonstrated that HIF-1α facilitates the transcription of the MDR1 due to functional hypoxia responsive element that exists in MDR1's promoter.[158] Although it is still unclear whether this hypoxia responsive element is also present in the promoters of all other ABC transporters, it would not be surprising if HIF-1α plays an important role in the hypoxia-induced upregulation of ABC transporter gene expression. In addition to HIFs, reactive oxygen species (ROS) is another crucial mediator of hypoxia-induced ABC transporter expression. Hypoxia and reoxygenation are distinctive stimuli capable of stimulating ROS formation. Hypoxia stimulates ROS formation from mitochondria[159] and xanthine oxidase,[160] whereas reoxygenation induces NADPH oxidase-derived ROS formation.[161] Studies have shown that these mechanisms to generate ROS are both necessary and sufficient to stabilize and activate HIF-1,[162] most probably via modulation of prolyl hydroxylase (PHD) activity.[163] Moreover, ROS also plays a crucial role in modifying gene expression through regulating the activity of other transcription factors, such as activator protein-1 (AP-1)[164] and nuclear factor kappa B (NF-κB).[165] Although the exact impact of tumor microenvironment-generated ROS on the regulation of ABC transporters in solid tumors is still not completely understood, reports show that ROS affects both the expression and functional activity of ABC transporters while other studies contradict this. For example, cells treated with H_2O_2 can induce a significant increase in MDR1 expression and Pgp function.[166,167] ROS may contribute to upregulated HIF-1 or NF-κB activation and induce MDR1 expression through functional HIF-1α or NF-κB transcriptional binding sites on MDR1's promoter.[168,169] In contrast, a study in 2001 indicated that ROS is involved as a second messenger in receptor tyrosine kinase signaling pathways and may act as a negative regulator of Pgp expression. Evidently, higher Pgp expression was found in tumor spheroids with a high percentage of quiescent cells, elevated glutathione levels, reduced ROS levels and minor activity of the mitogen-activated kinase (MAPK) members such as c-Jun amino-terminal kinase (JNK), extracellular signal-regulated kinase ERK1, 2 and p38.[169] In order to clarify this controversy and to determine if ROS can also mediate other ABC transporters, further studies are required.

Overall, hypoxic regions in tumors are likely to have a reduced supply of nutrients such as glucose and essential amino acids, causing tumor cells to switch their energy metabolism to glycolysis.[141] In hypoxic conditions, glycolysis is an easier metabolic pathway to produce ATP for tumor survival, and it is also a more

efficient pathway for the production of CO_2 and carbonic acid. However, decreased clearance of these acidic metabolic products leads to low interstitial pH, termed "tumor acidity", which can influence drug resistance by decreasing the uptake of some chemotherapeutic drugs through tumor acidity-mediated chemical changes or inhibition of active transport.[143] For example, studies revealed that cells exposed to an acidic extracellular environment (pH 6.6) can increase Pgp-mediated drug efflux and further decrease the cytotoxicity of chemotherapeutic drugs.[170,171] It was proposed that Pgp activity is increased by low extracellular pH as a result of lowered intracellular calcium levels and inhibition of PKC.[171] However, additional experiments are required to test this hypothesis and to determine the mechanism of tumor acidity on ABC transporter functional activity.

Within solid tumors, one important characteristic of cancer stem cells is their high expression of ABC transporters, such as ABCG2 and Pgp, compared with their differentiated progeny.[172] It is plausible that high levels of ABC transporters provide cancer stem cells with enhanced protection from cytotoxic agents enabling a potential stem cell-driven relapse after chemotherapy (Figure 1, step 3). However, the precise mechanism and the reason why high levels of ABC transporters existed in cancer stem cells remained unclear. In solid tumors, cancer stem cells reside within specific anatomical locations also referred to as "niches". These niches provide a unique microenvironment where stem cells are maintained in an undifferentiated state and their commitment to lineage-specific differentiation is tightly regulated. As suggested by current observations, cancer stem cells may exist in two types of niches: (1) the hypoxic niche, located distally from the functional blood vessels, and (2) the perivascular niche.[173] Although we are still at the very early stages of understanding the mechanism by which these niches regulate the maintenance or differentiation of cancer stem cells, at least three mechanisms by which tumor hypoxia may regulate the maintenance and the differentiation of cancer stem cells have been proposed. First, hypoxia directly prevents cancer stem cells from undergoing differentiation or promotes the dedifferentiation of cancer cells to produce stem cell-like cancer cells. Second, hypoxia inhibits differentiation of immature stromal cells and maintains them in an undifferentiated state. Third, hypoxia induces expression of paracrine factors such as chemokines in stromal cells that facilitate homing of cancer stem cells in the niche.[174−176] Evidence from an increasing number of studies suggests that HIFs may modulate these mechanisms involved in the maintenance or differentiation of cancer stem cells.[176,177]

At present, it is not yet completely understood whether the presence of ABC transporters has a fundamental role in the cancer stem cell phenotype or if it occurs as a result of other genetic changes induced by the tumor microenvironment. However, many groups are currently working on answering this particular question. For example, a recent study demonstrated that the overexpression of CD133 in rat C6 glioma cells leads to significant upregulation of Pgp/ABCB1 with a corresponding increase in activity and the reluctance to undergo apoptosis from camptothecin and doxorubicin via a HIF-1 independent mechanism.[178] It is known that the Oct4-TCL1-AKT pathway acts on embryonic stem cells and cancer stem cells in cell proliferation through inhibition of apoptosis. However, a recent report highlights another role of this pathway demonstrating that Oct4 mediates chemotherapeutic drug resistance in liver cancer cells via activation of ABCG2.[179] Therefore, a similar pathway may contribute to the regulatory mechanism of ABC transporter expression in cancer stem cells. Interestingly, these cancer stem cell genes or pathways have been shown to be upregulated by hypoxia via HIFs' direct or indirect signaling pathways. In all, although the exact mechanism of hypoxic niche-mediated ABC transporter regulation in cancer stem cells remains to be determined, these studies clearly demonstrate that hypoxia plays an important role for cancer stem cells in acquiring or maintaining the high expression and function of ABC transporters in solid tumors.

E. STRATEGIES TARGETING ABC DRUG TRANSPORTERS

It is unrealistic to eliminate or deactivate any of the ABC transporters permanently due to their important physiological and pharmacological roles in the human body.[4] However, MDR in cancer caused by the overexpression of ABC drug transporters can be modulated transiently by various means, including direct inhibition, gene silencing, transcriptional regulation and drug encapsulation.[180] Among them, selective inhibition or modulation of the ABC protein function and/or expression with a wide range of nontoxic modulators should be, in principle, direct and straightforward.[15] In 1981, the calcium channel blocker verapamil was the first "chemosensitizer" reported to reverse MDR *in vitro*.[14] Inhibitors or chemosensitizers directly compete with transported drugs at the substrate-binding sites, thus blocking the function of the ABC drug transporter in transporter-positive MDR cancer cells and maintaining the intracellular cancer drug concentration in MDR cancer cells to a level that is comparable to transporter-negative drug sensitive cancer cells (Figure 1). In spite of this, there is still no clinically applicable inhibitor of ABC transporters to date. The reason behind unsuccessful clinical trials is complex, but it is largely due to unfavorable toxicity of inhibitors. It is not surprising since the first generation inhibitors are clinical medicines that were not designed to target ABC transporters; therefore higher concentrations were used, resulting in more severe side effects. However, the second and third generation inhibitors (including XR9576, Biricodar and LY335979) were designed based on the data collected from previous studies, making them more potent, selective and less toxic. In addition, the so-called "fourth generation" modulators are also currently being actively explored. They are compounds isolated from natural sources, which provide additional diversity of chemical scaffolds that can be used to construct new classes of modulators.[15,181] In addition to the "conventional inhibitors", there is a class of compounds that can cause collateral sensitivity or induce preferential cytotoxicity to transporter-positive MDR cancer cells (Figure 1, step 6). Though the mechanism is still unknown, the potential of utilizing this unique property to resensitize transporter-positive MDR tumors is currently being evaluated.[182]

F. CONCLUSIONS

It is apparent now that MDR in cancer is caused by multiple mechanisms that operate either independently or in unison. Overexpression of drug transporters is just one of the many ways that cancer cells have adapted in order to survive the diversity of agents used in cancer chemotherapy. It has been over 30 years since the discovery of Pgp in 1976, yet there is still no simple solution to deal with MDR in cancer. The complexity and the

identification of new MDR-linked ABC transporters have made the problem even more challenging. On the other hand, based on the new discoveries and advancements made on the identification, biological characterization and structural analysis of MDR-linked ABC transporters over the years, we are one step closer to understanding clinical MDR in cancer.

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